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and a combination of bioinformatic and cytogenetic approaches. The minimum number of inversions between members of the *A. gambiae* complex and the outgroup species *A. funestus* and *A. stephensi* was calculated using the Multiple Genome Rearrangements (MGR) and Sorting Permutation by Reversals and block-INterchanGes (SPRING) programs. The physical mapping of *A. merus* chromosomes identified molecular coordinates of the proximal 2Ro+ inversion breakpoint in *A. gambiae*. DNA probes from 2La+ and 2Ro+ inversion breakpoints of *A. gambiae* were mapped to the *A. stephensi* chromosomes. The results suggest that the *A. gambiae* complex shares the 2La and 2Ro arrangements with the outgroup species. Assuming monophyletic origin of the inversions, this study concludes that physical mapping of ingroup and outgroup species can be used for identifying inversion breakpoints and ancestral autosomal arrangements within species complexes. Molecular characterization of the breakpoints in both ingroup and outgroup species will provide a solid basis for reconstructing the inversion history in the *A. gambiae* complex.

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STRUCTURAL ORGANIZATION OF THE MALARIA MOSQUITO HETEROCHROMATIN

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The development of new genome-based vector control strategies requires detailed knowledge about the organization and function of the mosquito genome. Heterochromatin is a functionally important and rapidly evolving part of the chromosome. Anopheline mosquitoes represent an ideal system for studying the structure and evolution of the heterochromatin because of the presence of polytene chromosomes in several tissues and great variability of heterochromatin among species. Two different morphological types, condensed/dark granulated (α-) and diffuse/light granulated (β-) heterochromatin have been identified in polytene chromosomes of ovarian nurse cells in *Anopheles gambiae* and *A. stephensi.* Only diffuse β-type forms large visible attachments of chromosomes to the nuclear envelope. The goal of this study was to characterize the two types of heterochromatin using immunostaining and bioinformatic analysis. Immunostaining of chromosomes using antibodies against *Drosophila* Heterochromatin Protein 1 (HP1) and nuclear envelope protein lamin Dm_0 revealed co-localization of these proteins in most of the heterochromatic and euchromatic sites. The total number of sites was 128/158 in *A. gambiae* and 266/268 for in *A. stephensi* chromosomes for HP1/lamin, respectively. Surprisingly, an alternative pattern of protein localization has been detected between the species: both proteins were concentrated in all pericentromeric areas of *A. gambiae* but in internal chromosome regions of *A. stephensi*. No antibodies have been detected in pericentromeric α-heterochromatin of 2R, 3R and 3L chromosomal arms in *A. stephensi* and intercalary α-heterochromatin of *A. gambiae.* Gene density, A+T content, and repetitive element content were analyzed in the assembled part of the *A. gambiae* heterochromatin (13.3 Mb) and euchromatin (219 Mb) using Biomart, ATCONTENT and RepeatMasker programs. All heterochromatic regions had higher A+T content and five times lower gene density than euchromatin. Analysis of transposable elements and tandem repeats revealed the major difference in proportion of DNA transposons between the two types of heterochromatin. These findings suggest that α- and β-types of heterochromatin may have different function in the mosquito genome.

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CONTRASTING PATTERNS OF EVOLUTION IN FIVE CHROMOSOMAL INVERSIONS OF *ANOPHELES GAMBIAE*

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Chromosomal inversion frequencies in the malaria mosquito *Anopheles gambiae* exhibit nonrandom distribution with respect to environmental heterogeneities. Although these associations have been known for decades, there exists scant data on genetic variation in A. gambaie inversions making it difficult to infer how they might allow for ecological adaptation. By hybridizing genomic DNA from each of 25 wild-caught mosquitoes to high density oligonucleotide arrays, we obtained genome-wide maps of genetic differentiation between alternate arrangements of five common polymorphic inversions. Due to reduced recombination between alternate arrangements, loci captured by inversions tended to show more divergence than collinear loci. However, the degree of divergence varied considerably from inversion to inversion. Furthermore, genetic differentiation of captured loci was not uniform along the length of an inversion. For two inversions (2La and 2Ru) we identified small genomic clusters that had significantly higher divergence than all other regions in the same inversion. Targeted sequencing from ~30 loci in larger sample sizes and in two outgroups allowed us to determine diversity within rearranged regions, perform tests of neutrality, and date the origin of the inversions. Differing ages and sizes partially explained the contrasting patterns of molecular evolution observed between the five inversions. Sequencing in the highly diverged genomic clusters revealed fixed SNPs between alternate arrangements. Known or theorized recombination rates between inversion breakpoints and clusters show that this persistent association is highly unlikely under a scenario of neutral evolution. Based on this high resolution genetic analysis, we suggest mechanisms that could be maintaining A. gambiae inversion polymorphisms in natural populations and extend these results to form a qualitative model of inversion evolution that will be applicable to other organisms.

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DEMOGRAPHIC HISTORY AND MICRO-GEOGRAPHIC POPULATION GENETICS OF *ANOPHELES ALBIMANUS* IN CENTRAL AMERICA BASED ON MITOCHONDRIAL DNA *CO1* AND *CYT B* SEQUENCES

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Anopheles (Nyssorhynchus) albimanus is a malaria vector in Central America and northern South America. Previous research conducted on *An. albimanus* using microsatellite loci and sequences of the mtDNA *ND5* gene hypothesized that mountain ranges across western Panama and Costa Rica restrict gene flow between Central and South America. We analyzed partial sequences of the mitochondrial DNA *CO1* and *Cyt b* genes of *An. albimanus* from 13 localities in Costa Rica, Panama and northwestern Colombia. A minimum spanning network based on *CO1* gene sequences detected three groupings separated by 6 and 7 mutational steps; however, there was no evidence of geographical structure among haplotypes. High haplotype diversity and low nucleotide diversity, unimodal mismatch distribution, 4 of 6 neutrality tests and a star-like shape of the minimum spanning network demonstrate that *An. albimanus* is not at equilibrium, due to either an expansion or selective sweep. Mountain ranges in western Panama and Costa Rica seem to have restricted dispersal historically between the Pacific and Atlantic coasts; however, more recent mutational events in the network suggest higher levels of relatedness

among all populations. We have started obtaining sequences of the more conserved mtDNA *Cyt b* gene from the same localities to corroborate our *COI* findings and investigate earlier demographic history of this important malaria vector.

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CHROMOSOMAL PLASTICITY AND EVOLUTIONARY POTENTIAL IN THE MALARIA VECTOR *ANOPHELES GAMBIAE* SENSU STRICTO: INSIGHTS FROM THREE DECADES OF RARE PARACENTRIC INVERSIONS

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In the *Anopheles gambiae* complex, paracentric chromosomal inversions are non-randomly distributed along the complement: 18/31 common polymorphic inversions are on chromosome arm 2R, which represents about 30% of the complement. Moreover, in *An. gambiae* sensu stricto, 6/7 common polymorphic inversions occur on 2R and 1 on 2L. Most of these inversions are considered markers of ecological adaptation that increase the fitness of the carriers of alternative karyotypes in contrasting habitats. However, little is known about the evolutionary forces triggering the origin and allowing subsequent establishment of chromosomal inversions in field populations. Here, we present data on 82 previously undescribed rare chromosomal inversions (RCIs) recorded during extensive field sampling in 16 African countries over a 30 year period, which may shed light on the dynamics of chromosomal plasticity in *An. gambiae* s.s. and its adaptive potential. We analyzed breakpoint distribution, length, and geographic distribution of RCIs, and compared these measures to those of the common inversions. We found that RCIs, like common inversions, are disproportionately clustered on 2R, which may indicate that this arm is especially prone to breakages. However, contrasting patterns were observed between the geographic distribution of common inversions and RCIs. RCIs were equally frequent across biomes and on both sides of the Great Rift Valley (GRV), whereas common inversions predominated in arid ecological settings and west of the GRV. Because 17/82 (21%) RCIs were found repeatedly at very low frequencies-- at the same sampling location across different years and/or in different sampling locations-- we suggest that RCIs are early in the process of establishment and subject mainly to drift under unperturbed ecological conditions. Nevertheless, RCIs may represent an important reservoir of genetic variation for *An. gambiae* s.s. in response to environmental change, further testifying to the considerable evolutionary potential hidden within this pan-African malaria vector.

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WHAT IS THE IMPACT OF ARBOVIRAL INFECTION ON VECTOR LONGEVITY?

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The idea that arboviruses should evolve towards a benign relationship with their vectors was prevalent in the early history of medical entomology. The idea was based on the belief that virulence (i.e., negative effects on vector fitness) was necessarily detrimental to the virus because a virus that is harmful to its vector is harmful to itself. Reduction in vector longevity was an example of this view, because arboviruses are generally transmitted only after an extrinsic incubation period that is close to or exceeds the average lifetime of their vectors. In the 1980's, conflicting observations and theoretical developments on the evolution of host-pathogen interactions challenged this view by supporting the idea that increased levels of virulence could potentially be advantageous provided they concomitantly increased transmission. However, while a number of studies have reported experimental evidence for increased vector mortality due to arboviral infection, others have failed to detect any significant impact; a single study even provided evidence for enhanced vector longevity. Whether this variation in the outcome of studies relies on biological differences between particular vector-virus systems or simply reflects differences in experimental settings remains unclear. Focusing on mosquito-virus interactions, we performed a meta-analysis of published studies to (1) evaluate the overall magnitude and statistical significance (across systems and studies) of the effect of arboviral infection on vector longevity, and (2) identify characteristics of individual studies (e.g., infection method, viral dose, environmental quality, etc.) influencing the magnitude of the observed effect. Our results unravel the biological and experimental factors underlying the impact of arboviral infection on vector longevity and help identify the evolutionary forces driving the virulence of arboviruses to their free-ranging vectors.

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THE EVOLUTION OF ANTI-MALARIAL IMMUNE GENES IN THE *ANOPHELES GAMBIAE* COMPLEX

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The goal of developing malaria resistant transgenic mosquitoes for malaria control has focused much attention on the immune system of *Anopheles gambiae*. Numerous genes are now known that affect the mosquito's ability to transmit *Plasmodium.* In order to narrow down these genes to those that specifically target *Plasmodium,* we have taken an approach that is based on the observation that malaria vectors and their *Plasmodium* parasite co-evolve. That is, we expect immune genes that specifically target *Plasmodium* to show signs of positive selection in those mosquitoes that transmit malaria, but not in closely related non-vector species. We have now investigated the evolution of 20 anti-malarial immune genes in six species of the *An. gambiae* complex, including both vector and non-vector species. Although we previously reported an instance of positive selection of *LRIM1* in the malaria vector *An. arabiensis,* the majority of the investigated anti-malarial immune genes show patterns of purifying selection. However, for TEP1 the two vector species *An. gambiae* and *An. arabiensis* carry two markedly divergent allele classes, indicating that this gene may be subject to balancing selection.

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ASSOCIATIONS BETWEEN URBAN STRUCTURE AND *AEDES AEGYPTI* LARVAL HABITATS IN PUNTARENAS, COSTA RICA

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Geospatial technologies have been increasingly applied to study vector-borne diseases, although their use in urban setting has been limited. In this study, high-resolution satellite imagery from QuickBird was analyzed to determine the relationships that urban structure, determined by tree

cover and built area, may have with the abundance of mosquito larval habitats and the *Aedes aegypti* container index in an urban area of Costa Rica. Two cross-sectional entomological field surveys were performed in the city of Puntarenas during the wet season of 2006 and the dry season of 2007. A geographical sampling method was used to select the areas to be surveyed: a grid (100 by 100 meters) was constructed and a stratified random sample of 34 cells (10%) was selected. All possible larval habitats were noted per cell, and mosquito larvae were identified. Two seasonal land cover maps were prepared using QuickBird multispectral imagery (2.4 m spatial resolution) with "water", "built", "tree", "grass/bare soil", and "paved" classes. The proportion of tree cover and built area was extracted for each of the cells, and regression models were analyzed for the number of larval habitats, *Ae. aegypti* container index, and pupae per person. In the wet season and when corrected by the number of locations evaluated in each cell, tree cover ($R^2 = 0.650$, $p<0.001$) and built area ($R^2 = 0.613$, $p<0.001$) were able to significantly explain the variation in total larval habitats. Larval habitats were positively associated with tree cover and negatively associated with built area, while the proportion of *Ae. aegypti* positive containers was negatively associated with tree cover. The significant regression models were used to create maps of larval habitat abundance in Puntarenas at the cell level. Results showed that the abundance of mosquito habitats in urban environments may be explained and predicted by using remotely sensed information. Areas within the urban environment with greater tree cover probably contain numerous *Ae. aegypti* and other mosquito larval habitats in the wet season and should be targeted for more efficient vector control.

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SURPRISES IN THE CLIMATE-MALARIA LINK IN THE AMAZON

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Global climatic changes are altering patterns of temperature and precipitation, potentially affecting regions of malaria transmission. Links between changing precipitation and malaria, however, are not well understood, although previous studies have made general predictions of increasing malaria with increasing precipitation. Here we find that the relationship between precipitation and malaria can change sign, depending on the underlying landscape: regions with few wetlands show a positive relationship between precipitation and malaria, while areas of high wetlands show a negative relationship. This result shows that the links between climate and malaria are more complex than previously believed, and must take into account regional ecological characteristics.

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EVALUATION OF A PCR-RFLP METHOD FOR IDENTIFICATION OF ANOPHELINE SPECIES FROM THE PACIFIC AND ATLANTIC COASTS OF COLOMBIA

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Many of the primary malaria vectors belong to complexes or groups which include several species with differences in their capacity to transmit malaria. Since their identification is complicated by cryptic morphology, the use of molecular methods may provide accurate identification of those species implicated in malaria transmission. We evaluated the applicability of an ITS2 based PCR-RFLP assay previously developed by our group, for accurate identification of anopheline species in two Colombian regions: the Pacific and the Atlantic Coast. We analyzed 203 specimens corresponding to four of the seven species included in the assay, which were identified using morphological characters: *Anopheles albimanus* (168/9575)*, Anopheles darlingi* (1/1)*, Anopheles rangeli* (6/6)*, Anopheles punctimacula* (6/6) and *Anopheles triannulatus* (22/1967)*.* The PCR-RFLP confirmed the identity of individual specimens of *An. albimanus, An. darlingi, An. punctimacula* and *An. triannulatus*, and helped to correct the species assignment of six specimens previously identified as *An. nuneztovari* to *An. rangeli*. Our results showed conserved restriction patterns for three of the species analyzed in both regions. Some *An. albimanus* specimens showed a 15bp difference in one restriction fragment caused by a mutation in one of the recognition sites of the enzyme; however, the pattern could still be easily assigned to *An. albimanus*. Therefore, this ITS2 PCR-RFLP proved to be a valuable assay for the accurate identification of anophelines in these two Colombian regions, which is essential for guiding vector control strategies.

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EVALUATING THE IMPACT OF ENVIRONMENTAL VARIABLES ON THE TRANSMISSION OF AMERICA CUTANEOUS LEISHMANIASIS IN RURAL COLOMBIA

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A large outbreak of American cutaneous leishmaniasis started in 2004 in the Municipality of Chaparral (Tolima, Colombia). The population was 46,090 in year 2005, and is divided between 142 *veredas* (townships or districts). The range was restricted between 1000 and 2000 m of elevation. Within this range, the *vereda*-level cumulative incidence of clinical cases ranged from 1 to 95%. In order to understand the environmental conditions favoring the outbreak, we studied climatic, coverage and topographic variables. These included elevation, coverage obtained from a supervised classification of Landsat and Aster satellite images (forest, shrubs, cultivation, pasture, and urban), and fourteen bioclimatic variables (www.worldclim.org). The spatial analysis was done at *vereda* level. The peak incidence of the disease was estimated to occur at a mean temperature of 20.6°C (95% CI 19.2-22.0°C). We found no significant changes in vegetation coverage, based on satellite images from 1989, 2002 and 2007; a period that includes the time of the outbreak. The coverage with forest or shrubs was associated with the disease, with incidence being 29% higher for each 10% increase in these types of vegetation.

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SPATIAL DISTRIBUTION OF MOSQUITO LARVAE AND THE POTENTIAL FOR TARGETED LARVAL CONTROL IN THE GAMBIA

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There is a growing interest in the scientific community for use of larval control as a tool for integrated vector management. Here the distribution of the aquatic stages of malaria vectors in rural Gambia was examined to assess the practicality of targeting larval control. Every accessible water body in a 400 km^2 area in rural Gambia was mapped and sampled for two consecutive years. Each water body was characterised by its distance to the edge of the alluvial plains, perimeter, habitat type, landcover type and the presence or absence of mosquito larvae assessed by standard dipping. Sampling was continuous in each site through the rainy and dry

seasons. During the rainy season, the peak period of malaria transmission, breeding sites were 70% more likely to have anopheline larvae in the floodplain of the Gambia River, than upland sites ($p < 0.001$). However, mosquitoes were found in all habitats, apart from moving water. Habitats most often colonised by anopheline larvae were the largest water bodies, situated near the landward edge of the floodplain, where culicine larvae were present. In the wet season 49% of sites had anophelines versus 19% in the dry season ($p < 0.001$). In conclusion, mosquitoes colonise a wide range of habitats, therefore ground larval control targeted at selected habitats is unlikely to be successful in extensively flooded habitats, characteristic of big river ecosystems. Nonetheless, larval control initiated at the end of the dry season and run throughout the rainy season could help reduce transmission.

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HABITAT SEGREGATION AND CHARACTERIZATION OF *ANOPHELES* LARVAE IN LOWLAND WESTERN KENYA

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Larval habitats of the anopheline vectors of human malaria form an integral part of transmission because they produce the adult female (i.e., vector) stage. In this study, conducted in a region holoendemic for *Plasmodium falciparum* malaria in lowland, western Kenya, the habitat characteristics and segregation of different species comprising the *Anopheles* community were studied. Two large scale sampling efforts in a 3.2 x 3.2 km area involved mapping with GPS of all encountered habitats and quantitative sampling with area samplers. Overall, a total of 1,198 habitats and 19,776 larvae in the wet season and 184 habitats and 582 larvae were sampled in the wet and dry seasons of 2006, respectively. In the wet season, ten species of *Anopheles* were found in the larval stage, with *An. gambiae* s.l. being most common and *An. arabiensis* the dominant form (78.5%) and *An. gambiae* s.s. relatively uncommon (21.5%) within that species complex. Larvae of these species generally occurred in ground water pools that were shallow, unshaded, had turbid water, contained little or no aquatic vegetation, and were located proximal to stream channels coursing through agricultural landscapes. Larval habitat characteristics and spatial distribution of the two sibling species in the *An. gambiae* complex were similar. Larval habitats of *An. gambiae* s.l were significantly different in form and location compared to other non-vector anophelines or to *An. funestus*. By contrast, larval habitats of 8 other anopheline species (predominantly *An. coustani*, *An. rufipes*, *An. squamosus*, and *An. maculipalpis*) were deeper, had more emergent vegetation, and were situated closer to streams. *Anopheles funestus* was very rare, having been collected only 44 times, and like *An. gambiae* s.s. its relative rarity likely reflects a long term decline owing to sustained use of ITNs. The results of this study provide essential information for habitat-based control programs that aim at targeting productive malaria vector habitats.

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LAND COVER ASSOCIATIONS OF IMMATURE *ANOPHELES* HABITATS IN A WESTERN KENYA LOWLAND ENDEMIC FOR MALARIA

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The distribution and abundance of larval *Anopheles* mosquitoes is strongly associated with landscape features such as topography, vegetation, and land use. These parameters regulate the geographical distribution of malaria vectors and thus the diseases with which they are associated. This study sought to take advantage of increasing knowledge of the environmental associations of *An. gambiae* and the available high resolution satellite data (IKONOS) to develop a generalized model of associating the location of larval habitats for *An. gambiae* s.l with land cover in Western Kenya. Additionally, the accuracy of IKONOS satellite imagery in detecting the presence of *An. gambiae* larval habitats was evaluated. Results demonstrated that land cover is significantly associated with the location of *An. gambiae* larval habitats. Agricultural and non-agricultural lands respectively, were positively and negatively associated with the presence of potential larval habitats. Agricultural lands represented 46% of the study area and accounted for 68% of potential habitats and 71% of *Anopheles* larvae samples. The numbers of potential larval habitats as well as agricultural activities decreased with increasing distance from the stream. The land cover composition structure was a consequence of a combination of land use and topographical factors. IKONOS image detected only a few of potential habitats larger than 4 X 4 m (IKONOS spatial resolution) but overlooked the smaller habitats which are not only the most productive but also the most frequent.

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ROLE OF A SERINE PROTEASE FROM *ANOPHELES GAMBIAE* IN *PLASMODIUM* DEVELOPMENT

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Studies on acsp30 (AY995188), an immune responsive serine protease from *Anopheles culicifacies*, the main vector for transmission of malaria in India, have been previously reported. African vector *Anopheles gambiae* serves as a well- studied mosquito model for elucidating the role of this serine protease in regulating *Plasmodium* development. We therefore studied a homologue of acsp30 in *An. gambiae* (agsp). Agsp transcript was induced upon, both *P. berghei* and *P. falciparum* infection. Double-stranded (ds) RNA-mediated knock down of Agsp in the susceptible G3 strain resulted in significant decrease in *P. berghei* and *P. falciparum* oocyst numbers compared to dsLacZ-injected controls. knockdown of Agsp in refractory L35 strain resulted in a dramatic reduction of melanized parasites and susceptible G3 mosquitoes depleted of CTL4 also resulted in such a decrease. No corresponding increase in live oocysts occurred, therefore indicating that this serine protease was not part of the melanization cascade. Serine protease cascades are tightly regulated by serine protease inhibitors or serpins (SRPNs) and knockdown of Agsp resulted in a specific reduction of SRPN6 transcript levels but not SRPN2 or SRPN10 revealing that Agsp is in the same cascade as SRPN6. Immunofluorescence microscopic studies are underway to investigate the phenotype obtained by Agsp knockdown.

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EFFECTS OF WEST NILE VIRUS DOSE ON SPATIOTEMPORAL MIDGUT INFECTION PATTERNS IN *CULEX PIPIENS QUINQUEFASCIATUS* SAY (DIPTERA: CULICIDAE)

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Culex pipiens quinquefasciatus were fed blood meals containing either 6.7 logs plaque-forming units (PFU)/mL or 4.8 logs PFU/mL of West Nile virus. Midguts and legs from five mosquitoes per dose were collected every other day from 0-12 days post-infection (dpi) to investigate spatiotemporal midgut infection and dissemination patterns. Midgut infection was assessed with immunofluorescent staining and virus dissemination to the legs was determined with qRT-PCR. Dissemination was calculated as the percentage of midgut infected mosquitoes with infected legs. Sixty percent of mosquitoes in the high-dose group had infected midguts as early as 2 dpi and 75% of mosquitoes with infected midguts had disseminated infections by 4 dpi. For the low-dose group, 20% of mosquitoes had infected midguts at 4 dpi and 100% of the midgut infected mosquitoes had disseminated infections by 8 dpi. The midgut infection rate at 4 dpi was significantly different between the high (75%) and low dose (20%) groups ($p<0.05$). The disseminated infection rate was also significantly different between doses at 4 dpi (75% high-dose and 0% low-dose) and 6 dpi (80% high-dose and 0% low-dose) ($p<0.05$). By 10 and 12 dpi, 100% of mosquitoes had infected midguts and 80% of mosquitoes with infected midguts had disseminated infections, suggesting the presence of a midgut escape barrier, regardless of initial virus dose. Our results indicate that virus dose had greatest effect on midgut infection and dissemination 4 and 6 dpi. We demonstrate variation in spatiotemporal patterns of midgut infection and dissemination between doses that may help explain variation in vector competence resulting from differences in the extrinsic incubation period of mosquito vectors.

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HIGH RATES OF FEEDING ON HUMANS IN THE GENERALIST BITER *AEDES ALBOPICTUS* IN ROME (ITALY)

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Although knowledge on the frequency of human-mosquito contact is essential for understanding the role of vector species in disease transmission to humans, no data are so far available on the feeding habits of *Aedes albopictus* in Italy or in other recently colonised temperate regions of Europe. During May-October 2006 and 2007, we exploited the sticky-trap developed by Facchinelli and colleagues to study *Ae. albopictus* host-feeding patterns in relation to different availability and abundance of putative hosts. The study was carried out in 4 sites within the Province of Rome, two of which (Site 1: "La Sapienza" University and Site 2: "Verano Cemetery") were located in urban settings, while the other two (Site 3: a horse-breeding farm/riding school and Site 4: a cattle-breeding farm) were located in rural settings in the town's outskirts. Blood meal origin was determined by direct ELISA on nitro-cellulose membrane, using anti-human, anti-dog, anti-cat, anti-bird, anti-rabbit, anti-bovine, anti-rat and anti-horse IgG antibodies. Cumulative collections yielded 519 *Ae. albopictus* blood-fed females, 58% of which produced results at the direct dot-ELISA, due to a limited amount and/or bad conservation of the blood in the specimens collected in the ST. The Human Blood Index was significantly different among sites, as follows: 96%, 79%, 55% and 23% in Sites 1, 2, 3 and 4, respectively ($Chi^2=87.8$; $df=3$; $p<0.001$). Interestingly, a relative high percentage of blood-meals was carried out on horses in Site 3 (47%), on bovines in Site 4 (55%) and on cats in Site 2 (27%), consistently with the fact that these animals are abundant hosts in these sites. The obtained results confirm the generalist feeding-behaviour shown for *Ae. albopictus* in its original range of distribution and highlights the high potential of this species as vector of human pathogens in urban areas of Italy, where both humans and the mosquito itself may reach very high density, suggesting that the recent Chikungunya epidemics in northern Italy may not remain an isolated episode.

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FREQUENCY OF MULTIPLE HUMAN BLOODMEALS TAKEN BY *ANOPHELES ARABIENSIS* MOSQUITOES IN MACHA, ZAMBIA

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The mosquito *Anopheles arabiensis* is the major vector of *Plasmodium falciparum* in the Macha region of southern Zambia. Spray catches for mosquitoes were performed three villages in Macha region during the 2005-2006 and 2006-2007 malaria seasons. DNA was extracted from blooded abdomens and PCR was used to determine which mosquitoes had fed on humans. Four human microsatellites were used to estimate the proportion of mosquitoes that had fed on more than one human host during the last gonotrophic cycle. Several statistical and computational methods were used to infer multiple feeding rates from the microsatellite data, including Monte Carlo simulations. The overall frequency of multiple human blood meals was 20% for both seasons. However, the multiple host feeding rate showed both temporal and spatial variation in the village areas sampled. Infection with *P. falciparum* did not affect the frequency of multiple blood feeding.

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CHARACTERIZATION OF WATER-HOLDING CONTAINERS AS MOSQUITO-HABITATS, AND DENGUE-PREVENTION COMMUNITY EDUCATION IN RURAL ECUADORIAN COMMUNITIES

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Dengue and Dengue Hemorrhagic Fever (DHF) are serious health concerns in South America. In recent years, there has been an alarming increase in cases of both classic Dengue and DHF in Ecuador. At present, prevention of transmission by control of the urban, container-inhabiting vector, *Aedes aegypti*, is essentially the only way to combat this disease. In June, 2007, as part of a tropical disease biology workshop, we visited rural communities in Ecuador and studied the nature and distribution of the containers which Ae. aegypti larvae and pupae inhabit. Container type, color, volume, access to sun light, presence of water, and presence of mosquito larvae/pupae were determined. Fifteen container types were recognized mainly on the basis of composition and size, the most common being plastic buckets, concrete/brick tanks, rubber tires, and large metal drums (20%, 11%, 9%, and 7% respectively; n = 136). The most productive (larvae and/or pupae present) containers were large metal drums (40% of mosquito-positive containers) and concrete/brick tanks (13%). Our results are consistent with those of other, more-extensive studies in the tropics, i.e. certain container types (e.g. large drums) are substantially more mosquito-productive than others. During summer 2008, we will build upon our findings from 2007 and will communicate the

results to community leaders, highlighting the potential risk of dengue/dengue hemorrhagic fever and providing information on the importance of container-mosquito habitat reduction in Dengue prevention. We will also distribute educational badges (especially to children) and posters to community members. Using a novel approach, each badge has been designed to provide information and poses a related question which is answered on another badge. We will also be interviewing community members in regard to mosquitoes and Dengue. The interview protocol has been designed to enable us to test the Health Belief Model, research which will help refine future community education approaches.

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MICROFILARIAL UPTAKE AND PENETRATION OF THE MIDGUT AMONG DIFFERENT MOSQUITO SPECIES FED SIMULTANEOUSLY ON THE SAME MICROFILAREMIC HOST

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We compared the number of microfilariae (mf) ingested and penetrating the midguts of mosquitoes fed concurrently on microfilaremic gerbils. We evaluated two mosquito-borne filarids (*Brugia malayi* and *B. pahangi*) and one tick-borne filarid (*Acanthocheilonema vitae*). The *Aedes* mosquitoes (*Ae. aegypti*, *Ae. taeniorhynchus* and *Ae. triseriatus*) ingested 10 times more *Brugia* mf than did co-feeding *Anopheles stephensi* and *Culex pipiens* mosquitoes. The quantities of *B. malayi* mf ingested by *Aedes* spp. were 5- to 9-times more than predicted based on the host mf at the time of feeding. There were no species differences in mf uptake when mosquitoes fed concurrently on an *A. vitae* mf gerbil and mf densities ingested were similar to that observed in host blood. *Brugia* mf penetrated the midguts of *Aedes* species more efficiently than those of *An. stephensi* or *Cx. pipiens*, whereas *A. vitae* mf did not penetrate the midguts of any mosquito species tested. To explain differences in mf uptake, we speculate that different mosquito species probe, cannulate and feed at different depths within the skin and that, during nocturnal migration out from alveolar capillaries, different mf species congregate at specific depths or "micro-zones" within the capillary beds of the skin. The specific micro-zone for a mf species may have been selected through evolution to correspond with the specific feeding depth of their arthropod vector. If true, then we surmise that *B. malayi* and *B. pahangi* mf within anesthetized gerbils inhabit the same micro-zone utilized by *Aedes* mosquitoes to obtain blood and is different from that utilized by *Cx. pipiens* or *An. stephensi*. Thus, *Aedes* 'concentrate' *Brugia* mf whereas *Cx. pipiens* and *An. stephensi* do not. In contrast, *A. vitae* mf (a tick-borne species) may congregate outside of the zone utilized by mosquitoes or, because argasid ticks do not cannulate vessels, *A. vitae* mf may disperse evenly throughout the vasculature. The ability of mf to penetrate the midgut may play a role in mf enhancement of arbovirus transmission, even in non-permissive species combinations.

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MODELING WEST NILE VIRUS TRANSMISSION AMONG BIRDS IN CONNECTICUT

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Despite the fact that West Nile virus (WNv) is now endemic in many areas of the US, its transmission dynamics in nature are still poorly understood. Consequently, endemic foci of infection and outbreaks of human disease cannot be predicted or prevented. In order to understand these dynamics, we must identify enzootic mosquito vectors and avian reservoir hosts, as well as their contact rates. In Connecticut, preferential feeding of *Culex pipiens* on American robins results in heterogeneous pathogen transmission, which can significantly affect the timing and intensity of WNv transmission. We present here a continuous-time model demonstrating that enzootic transmission of WNv is highly dependent upon variation in contact rates between the vector *Cx. pipiens* and their avian hosts. The model is parameterized with field data collected from 4 field sites in which we estimated vector and host abundance, as well as mosquito feeding preferences. Bird host abundance was estimated using point counts and mosquito abundance was estimated using data collected from surveillance traps. The mosquito feeding preference for a particular bird species was calculated by relating the proportion of blood-fed mosquitoes fed on a particular bird species to the abundance of that species at the site of the mosquito collection. Preliminary runs of the model show that, when *Cx. pipiens* preferentially feeds on robins over other birds in a community, robins act as super-spreaders, resulting in more frequent and intense WNv transmission, as opposed to a scenario where *Cx. pipiens* feed on all the available hosts in a community homogeneously. We also found that as host-specialization increases, transmission and infection among *Cx, pipiens* is more intense and occurs earlier in the season.

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VECTOR INCRIMINATION IN A HIGHLY ENDEMIC MALARIA LOCALITY OF CÓRDOBA, COLOMBIA

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In Colombia, 32% of the malaria cases reported during 2007 were from the department of Córdoba. To investigate potential vectors in Puerto Libertador, one of the municipalities with the highest malaria transmission in Cordoba, we collected mosquitoes monthly at La Bonga district, for two consecutive days (18:00-06:00 h) from July to December 2007. Natural infectivity by *Plasmodium falciparum*, *P. vivax* VK210 and VK247, were tested using ELISA and nested PCR. The predominant species were *Anopheles nuneztovari* s.l. (72%) and *An. darlingi* (21%); other species were present at <5% (*An. triannulatus* s.l., *An. punctimacula* and *An. oswaldoi*). *An. darlingi* was found naturally infected with *P. vivax* VK247, with an infectivity rate of 1.7% (IC 95%: 0.042-8.94). Biting activity of *An. darling* was detected throughout the night, in both intra- and peri-domestic areas. The results of this study incriminated *An. darlingi* as a vector in this area, but further investigations are warranted because *An. nuneztovari*, the most prevalent anopheline species, is considered primary vector in some regions of Colombia.

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FLUCTUATION IN WATER LEVEL OF LAKE VICTORIA AFFECTS ABUNDANCE OF *ANOPHELES FUNESTUS*

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The water level of Lake Victoria in 1998 was 1 m above its 10-year average from 1992 to 2001. Then the water level began to drop and was 1.4 m below the average in 2006. Although the water level rose considerably after heavy rainfall in late 2006, it has remained around 0.5 m below the average. As several breeding habitats occur on the lakeshore, this study examined whether the fluctuation in water level affected abundance of malaria vectors. Availability of breeding habitats and density

of adult mosquitoes were monitored biweekly in Suba district in western Kenya from late 2006. Locations and numbers of breeding habitats during the low lake water period were compared with the high water period in 1998 and 1999. The density of house resting mosquitoes was also compared. Several breeding habitats appeared on the narrow strip of land emerged along the lakeshore after the drop of lake water. There were more breeding habitats for Anopheles funestus during the high low lake water period compared with the high water period. Although the density of house resting mosquitoes was less compared with the high water period, the proportion of A. funestus to A. gambiae was higher. As the water level became stable after the increase in late 2006, the aquatic habitats in the newly emerged land became suitable for A. funestus, and its density in houses increased. The results suggested that fluctuation in water level affect abundance of A. funestus in villages near the lake.

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ADENOVIRUS 21 OUTBREAK AT THE COAST GUARD TRAINING CENTER IN CAPE MAY, NEW JERSEY

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Adenovirus is a ubiquitous, non-enveloped, double-stranded DNA virus that causes disease in man. Adenovirus is divided into 51 serotypes with most serotypes having specific organ tropism. In the military, febrile respiratory disease caused by adenovirus is endemic at all basic training sites. While serotypes 4 and 7 account for the majority of all acute respiratory diseases in military basic training recruits, other serotypes have caused epidemic disease at these training sites. A recent report found that adenovirus 21 (Ad21) has been increasing in prevalence in both military and civilian populations. An investigation of the Ad21 outbreak at the Coast Guard Training Center in Cape May, NJ was conducted on 27 September 2007. A proctored survey was administered to the recruit company of interest. A clinic chart review was conducted on all recruits from this company using a standardized data abstraction form. Additionally, a walk-through environmental assessment of the training center was performed. During the investigation environmental samples and recruit throat and serum specimens were taken. Pre-training serum specimens, drawn upon entry into service and stored at the Department of Defense Serum Repository, were also obtained for analysis by pairing with investigation specimens. An increase in febrile respiratory illness at this site was reported by NHRC starting in July 2007 and has persisted through the following spring. During this period of febrile respiratory illness there were no cases of lower respiratory disease or severe cases reported. The endemic adenovirus 4p of Cape May has been completely replaced by Ad21 as all culture-positive throat swabs that were processed during this period were Ad21. While complete lab results for the serum and throat specimens are pending, preliminary results show few recruits with detectable Ad21 antibodies prior to training and approximately 75% of recruits with a 4-fold increase in titer by week 7 of training. In conclusion, while this Ad21 outbreak has been prolonged, there has been no severe disease. Although Ad21 respiratory epidemics in the training environment have historically been uncommon, the increasing baseline prevalence of this serotype may lead to more frequent epidemics. Additionally, the adenovirus vaccine (Ad4/7) being developed by the Walter Reed Army Institute of Research may affect future adenovirus epidemics.

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VENTRICULAR DYSFUNCTION IN A PROBABLE MYOCARDIAL TUBERCULOSIS PEDIATRIC CASE

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Myocardial tuberculosis (MT) is a rare entity, mainly in children. The ventricular dysfunction associated with this process must be recognized and promptly treated. A pediatric case is described affecting a boy who developed a good resolution of his illness. A 10 year old child was admitted at the hospital presenting cough, fever, anorexic status and loss of weight. Diagnosis of tuberculosis (TB) was suspected. PPD measured 13mm. Leucocytosis and PCR of 22 mg/dl. Ziehl-Neelsen were negative for three sputum samples. Tuberculostatic treatment was initiated. High fever remained for the first days after the admission. Very bad progress was observed and suddenly he displayed respiratory distress and hypoventilation, heart failure rhythm and hepathomegaly. X-ray showed cardiomegaly. ECG: sinusal tachycardia. Cardiac ultrasound: biventricular dysfunction, ejection fraction 30%, mitral and tricuspid insufficiency and minimal pericardic effusion. AST/ALT elevation, throponins: 0.258 ng/ml and d-dimmers >1000ng/ml. Clinically better after tonic and diuretic treatment. Cardiac ultrasound was repeated with biventricular dysfunction and ventricular thrombosis (1.7x1.6cm) affecting the left apex. Cardiac resonance confirmed diagnosis. Thoracic TAC: hiliar lymph enlargement suggesting pulmonary TB diagnosis. Anticoagulant treatment was initiated with a good response and thrombus solved. Blood cultures were negative. PCR for virus with cardiac tropism: negative. Immunologic studies: normal. Lowenstein cultures: negative. Ventricular function resolved a few days later. The index case for TB was his soccer trainer and two other children diagnosed with TB a few weeks later. Although main causes of myocarditis are viral infections, adverse reactions of drugs and toxics, MT should be in the differential diagnosis. We describe a very rare pediatric case of MT with a thrombosis. Although myocardial biopsy confirms the diagnosis, the epidemiological context and the good response after treatment for TB was enough for us to decide MT was the most probable diagnosis in this case. Presumptive diagnosis includes clinical and cardiac ultrasound suggestive; serological tests do not obtain minimal sensibility and specificity for detecting virus. Heparin treatment is recommended in the acute phase for the resolution of thrombosis.

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MYCOBACTEREMIA IN RURAL THAILAND: INVASIVE SPECIES AND ANTIBIOTIC SUSCEPTIBILITY WITHIN AN IMMUNOCOMPROMISED POPULATION

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Bacteremia caused by mycobacteria is poorly described. In May 2005 an enhanced clinical microbiology system was established in Sa Kaeo, Thailand, a rural province along the eastern border with Cambodia. As part of this enhanced system, automated blood culture was added to detect invasive microbial pathogens such as mycobacteria from patients with pneumonia, sepsis, and other severe acute disease. Blood samples were collected as clinically indicated and processed using the BacT/Alert3D blood culture system with MB media. Alarm-positive bottles were examined by acid-fast staining with suspect mycobacteria subcultured on LJ slants and sent to the National Tuberculosis Reference Laboratory, Bangkok for identification by biochemical testing, drug susceptibility

analysis, and confirmation by GenProbe hybridization. From May 2005 - December 2007, 60 cases of culture-confirmed mycobacteremia were identified. *Mycobacterium tuberculosis* complex (MTB) accounted for 63.3% of isolates (38/60), *M. avium* complex (MAC) for 13.3% (8/60), and non-tuberculous mycobacteria (NTM) for 23.3% (14/60). Antibiotic susceptibility testing revealed that 100% of MAC and NTM were resistant to streptomycin while 34.2% of MTB (13/38) were resistant. For ethambutal, 100% of MAC and NTM and 5.6% of MTB (2/38) were resistant. For isoniazid, 100% of MAC and NTM and 21.1% of MTB (8/38) were resistant. For rifampicin, 100% of NTM were resistant while 87.5% of MAC (7/8) and 10.5% of MTB (4/38) were resistant. Three MTB isolates (7.9%) were multi-drug resistant (MDR) with 2 isolates resistant to all 4 first-line drugs. Further testing of the MDR isolates is ongoing. Thirty-six (60%) patients were male with a median age for men of 37 years (range 24-74). Fifty (83.3%) patients were confirmed as HIV positive; HIV status was not available or not determined for the remaining 10 (16.7%) patients. There were 9 known deaths - 2 from NTM and 7 from MTB. In conclusion, mycobacteremia is common in this setting, resulting in severe disease, and is frequently caused by NTM. Resistance to front-line drugs is widespread, complicating treatment. Blood culture with optimized media to support the recovery of mycobacteria is an important dtool in a setting with high HIV prevalence, permitting more rapid identification of active infections. The identification of MDR strains may foster the emergence of XDR-MTB without careful clinical management.

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EVIDENCE OF PRIMARY MDR RESISTANCE AMONG TUBERCULOSIS CASES IN PAPUA NEW GUINEA

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Tuberculosis (TB) is increasing at alarming rates in Papua New Guinea (PNG). WHO revealed an incidence of 104/100, 000 population in 2004, in a setting with only a 20% case-detection rate. To-date levels of TB drug resistance have never been investigated but are expected to be high. In a small hospital-based prospective study we investigated the prevalence of drug resistance among sputum-positive primary TB cases, with the specific objective of providing a basis for the formulation of future guideline for drug resistance monitoring and therapy approaches in hospital settings in PNG. From January 2006 to January 2007, 339 sputum isolates from suspected primary TB patients were studied at presentation at the routine TB clinic at the Modilon Hospital, Madang Province PNG. Of these 53% were AFB positive for acid-fast bacilli. Two hundred and forty samples were cultured using MGIT960 system of which 30.4% (73/240) showed positive growth. Drug resistance testing was successful in 64 isolates. Drug resistance was observed in 11 samples (17.2%), with 8 (12.5%) showing resistance to more than one drug. Resistance to Isoniazid (INH) was found in 10/11 (90.9%) of these isolates, followed by Streptomycin (STR, 9/11, 81.8%), Rifampicin (RIF, 5/11, 45.5%), with 2 cases (18.2%) each showing resistance PAS (Para-aminosalicylic acid) and Ethionamide (ETD), respectively. MDR TB (i.e. resistance to INH +RIF) was found in 5 cases (7.8%). Of these 3 showed resistance to at least 2 PNG second line drugs (1 STR and ETD; 1 STR and PAS; 1 STR and ETD and PAS). This study indicates the evidence of MDR TB drug resistance in PNG. Within PNG Madang province is considered at medium risk for TB. It is probable that similar or even higher rates of drug resistance are likely to be found in high risk areas of TB in PNG where HIV infection is much more common. Further studies on drug resistant TB in PNG are thus warranted.

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HIGH FREQUENCY OF ANTIBIOTIC RESISTANCE IN NASOPHARYNGEAL CARRIERS OF *STREPTOCOCCUS PNEUMONIAE* IN CHILDREN YOUNGER THAN 2 YEARS OF AGE IN LIMA, PERU

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Streptococcus pneumoniae resistant to penicillin and other antibiotics are an increasing clinical and public health problem in developing countries. Resistance levels are associated with overuse and misuse of antibiotics in children. The purpose of this study was to determine the carriage rate and current susceptibility patterns of *S. pneumoniae* in nasopharyngeal cultures of children younger than 2 years of age in Lima, Peru. Nasopharyngeal cultures were obtained from healthy children at the Pediatric Outpatient Clinics of 4 hospitals in Lima. Children 2 to 24 months of age who attended the clinic for a routine well child visit were cultured once. *S. pneumoniae* was isolated and disk diffusion was performed to determine resistance to 9 antibiotics. A total of 703 children (11.8m ± 6.6m) were evaluated during November 2007 and May 2008. The overall pneumococcal carrier rate was 28% (199/703); 27% (101/377) in children <1y and 30% (98/326) in children >1y. Resistance rates were as follows: cotrimoxazole 58% high-level resistance (HLR), penicillin 52% non-susceptible, erythromycin 33% HLR, tetracycline 33% HLR, clindamycin 13% HLR and chloramphenicol 10% HLR. Multidrug resistance (HLR >A3 antibiotics) was present in 40% of strains. There were no strains resistant to vancomycin, rifampin and levofloxacin. Resistant organisms were more common in older children (>1y) for penicillin (45% vs. 60%, $p<0.05$), cotrimoxazole (47% vs. 70%, $p<0.001$) and erythromycin (26% vs. 41%, $p<0.05$). Antibiotic use in the previous 3 months was very common in this population (46%). In conclusion, antibiotic resistance levels to penicillin and cotrimoxazole are high and have increased dramatically from previous reports in Lima. There is an urgent need for development and implementation of strategies to prevent and control the emergence and spread of resistant pneumococci.

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RESPIRATORY VIRUSES IN A PROSPECTIVE COMMUNITY-BASED PEDIATRIC COHORT IN NICARAGUA

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While viral respiratory diseases are not commonly thought of as a tropical disease problem, they do cause substantial morbidity and mortality in tropical countries. To investigate the distribution and burden of respiratory viruses in children in Managua, Nicaragua, we are performing a prospective community-based cohort study, embedded within an ongoing dengue cohort study of children 2-12 years of age. Nasal and throat swabs were collected from a random 20% of all cohort participants who presented for a medical appointment with influenza-like-illness, defined as a combination of fever or a history of fever with cough and/or sore

throat of 4 days or less duration. Samples were tested for influenza virus, respiratory syncytial virus (RSV), and parainfluenza virus (PIV) 1-3 by RT-PCR, and viral isolation was performed on all influenza RT-PCR-positive samples. A total of 3,285 children between the ages of 2 and 12 years old were followed for every primary care appointment from June 2007 through May 2008. To date, 680 swab samples have been collected and tested by RT-PCR for influenza virus A and B, RSV, and PIV1-3, of which 12% were positive for influenza virus (68 [85%] Influenza A and 12 [15%] Influenza B cases), 4% were positive for RSV, and 5% were positive for PIV. The estimated incidence of influenza in the cohort during this first year of the study was 21.1 cases per 100 person-years. A peak of influenza A/H3N2 was observed in June of 2007, and a peak of RSV activity occurred in November of 2007. The complete genomes of the influenza virus isolates are currently being sequenced to investigate virus evolution in the tropics and the relation between circulating viruses in tropical and temperate regions. All negative samples are undergoing further testing for a variety of viruses including rhinovirus, coronaviruses, human metapneumovirus, and adenovirus. This study is the first large-scale prospective community-based study examining respiratory viruses in Central America.

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A FAST THERAPEUTIC EFFICACY ASSAY WHICH DISCRIMINATES CIDAL AND STATIC ANTITUBERCULAR COMPOUNDS AGAINST *MYCOBACTERIUM TUBERCULOSIS* GROWING EXPONENTIALLY IN THE LUNGS OF MICE

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The development of new antitubercular drugs is a major global health challenge. In this connection, murine models of tuberculosis are a cornerstone for discovery of new antituberculars. Here, the development of specific assays for the different biological conditions that can be present in infected human beings is a requirement for an ordered drug discovery process. Thus, using a non-surgical intratracheal infection of C57BL/6 mice with *Mycobacterium tuberculosis* H37Rv Pasteur as a model of acute tuberculosis, we designed a fast assay to distinguish cidal patterns of efficacy against *M. tuberculosis* growing exponentially in the lungs of mice. We defined the parameters of the *in vivo* assay as a function of the size of the inoculum on the infection kinetics in the lungs. First, we established that treatment with antituberculars should start 24 h after infection. Then, we established the size of the inoculum and the duration of treatment in the assay to obtain at least 2 logs of increase in cfu with respect to the initial burden and at least 6 logs of total infectious burden at the time of sampling. According to these specifications, we established the assay as follows: Female C57BL/6 mice were infected by non-surgical intratracheal instillation of 10^5 viable CFU and randomly assigned to treatment groups (n = 5/group). We treated mice with compounds for 7 days. Finally, we sacrificed mice at day 9 after infection, aseptically extracted the lungs and, after homogenization, plated serial dilutions of homogenates to measure the CFU burden per whole organ. In these conditions, the infectious burden is 10^6-10^7 at day 9 after infection. We validated the one-week assay using the control compounds of known cidal-static properties. Isoniazid (25 mg/Kg, u.i.d., p.o.) and moxifloxacin (100 mg/Kg, u.i.d., p.o.) showed a marked cidal activity (typically > 4 logs) whereas linezolid (100 mg/Kg, u.i.d., p.o.) showed the maximum expected activity for a static compound (about 2 logs). Thus our results indicate that the assay described in this presentation allows the discrimination between cidal an static compounds *in vivo*.

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TUBERCULOSIS PRESENTING AS A CARCINOID TUMOR

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Gastrointestinal (GI) Tuberculosis (TB) is a major health problem in the underdeveloped countries and is the 6th frequent site of extra-pulmonary TB. There has been a resurgence of GI TB in the United States due to the influx of immigrants from high risk areas and AIDS. The pathogenesis is attributed to swallowing infected sputum, hematogenous spread from active pulmonary or miliary TB, ingestion of contaminated milk or food and contiguous spread from adjacent organs. Although any part of the GI tract can be involved the most common site is the ileocecal region due to the relative stasis and abundant lymphoid tissue. We present a case of ileal tuberculosis which presented as a carcinoid. A 31 year old male from the Philippines was referred to a gastroenterologist for anemia. At the time of presentation patient had complaints of diarrhea, right sided abdominal pain, flushing and weight loss. A colonoscopy revealed a cecal mass and a diagnosis of carcinoid was made for which he underwent a right hemi-colectomy. Due to persistent diarrhea post surgery he had a cat scan which showed colitis. Pathology of the resected specimen revealed necrotizing and non-necrotizing granulomatous inflammation with special stains demonstrating multiple acid fast bacilli (AFB). Chest radiography was normal but as pulmonary TB is seen in 20-25% of GI TB the patient was placed in respiratory isolation and was started on the 4 drug anti-TB regimen. The tuberculin skin test was positive. He was discharged after 3 negative sputum for AFB with results from the stool for AFB pending. In conclusion, surgeons can often miss infectious diseases since they are often misled by malignancies. In this particular case, the colon was not cultured and that was the reason why the stool was. The differential diagnosis of GI TB includes Actinomycosis, amebiasis, Yersinia enterocolitis, Crohn's disease, lymphoma and adenocarcinoma and it is very important to make this distinction especially in the treatment. For instance steroids used in the treatment of Crohn's disease can have a disastrous effect in GI TB. In conclusion the diagnosis of GI TB is to be based on a comprehensive approach that includes a thorough patient history, clinical, laboratory and radiologic findings. Although a definite diagnosis is based on a positive culture or histological examination of biopsy, barium enema, enteroclysis and computed tomography need to be used in the initial assessment of patients.

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H5N1 SURVEILLANCE IN RESIDENT, CAPTIVE, AND MIGRATORY BIRDS IN JAVA, INDONESIA

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The avian influenza virus H5N1 has been detected in captive birds across Asia, The Middle East and Europe and in migratory birds as far northwest as Scotland. To elucidate the role of migratory birds in an enzoonotic area, resident, captive and migratory birds were sampled at five sites in Java, Indonesia. Mist nets were used to trap birds and throat, cloacal and blood samples collected. Between October 2006 and September 2007, a total of 4278 birds comprising 27 species in 23 genera were sampled. The most commonly collected birds were the common sandpiper (17% of total), striated heron (8%), and the domestic chicken (55%). The prevalence of H5N1 antibodies in sampled birds was 5.3% (95% CI: 4.6 - 6%) with a range between 2.7% (1.6 - 3.8%) and 7.1% (5.4 - 8.7%) among the

five sites. A significantly higher percentage of captive birds, 16.1% (95% CI: 14 - 18.2%) showed antibody evidence of H5N1 exposure when compared to migratory (1.4%, 0.8 - 1.9%) or resident birds (1.1%, 0.5 - 1.6%). RNA was extracted from swabs and rRT-PCR conducted for the HA and M genes of H5N1. Seven resident birds (5 Muscovy and 2 mallard ducks) showed molecular evidence of H5N1 infection. These birds were alive and well when sampled suggesting mild, asymptomatic or unapparent disease. Following amplification, the HA, NA and M genes were sequenced from diagnostic samples and compared to sequences in Genbank. Phylogenetic analysis of the HA gene showed that the isolates were 97% similar to EU124153.1 A/chicken/West Java/Garut May 2006. While no known markers of neuraminidase inhibitor resistance were found within the NA gene, M segment analysis revealed the V27A mutation known to confer resistance to adamantanes. Our results demonstrate moderate serologic evidence of H5N1 infection in captive birds sampled in five sites in Java, Indonesia but limited infection in resident and migratory birds from the same regions. These data imply that even in a highly enzoonotic area the role of migratory birds in transmission of H5N1 to resident or captive birds is likely minor.

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DESCRIPTION OF FOUR ACUTE RESPIRATORY ILLNESS OUTBREAKS IN PERUVIAN MILITARY TRAINING UNITS - 2007

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Outbreaks of respiratory illness cause important morbidity and loss of productivity in military settings, especially those related to influenza. Furthermore, recruit populations can spread pathogens into the general population as occurred in the 1918 influenza pandemic. We describe four outbreaks of respiratory illness in Peruvian military training sites that occurred between February and March 2007 within a radius of 68.8 miles. Outbreaks were detected via an electronic surveillance system (Alerta). We defined acute respiratory illness (ARI) as any upper respiratory tract illness of less than 14 days. Outbreak investigations consisted in active surveillance of ARI cases. ARI cases with fever completed a clinical survey and provided nasal throat swabs for viral culture. The first outbreak occurred in epidemiological weeks (EW) 5-6. Attack rate in recruits was 8.1% (121/1500). Of 35 collected samples, three (8.6%) were influenza B positive. The second outbreak occurred during EW 6-9. Attack rate in recruits was 26.9% (73/271). Of 29 collected samples, three (10%) were influenza A; four (13.8%) were influenza B. The third outbreak occurred during EW 8-10. Attack rate in recruits was 16.7% (251/1500). Of nine collected samples, two (22%) were positive for influenza A and one (11%) for influenza B. The last outbreak occurred in EW 10-11. Attack rate in recruits was 40% (40/100). Of 37 collected samples, 31 (84%) were positive for influenza B. History of rhinorrhea, malaise and sore throat were associated with obtaining a positive culture for influenza ($p<0.05$). In comparison to officers, recruits had more risk for ARI (M-H RR 2.77, CI95%:2.30 - 3.40). In conclusion, outbreaks of acute respiratory illness in Peruvian military units showed high attack rates among recruits and were associated with influenza, particularly type B. Further investigation is needed to explore the mechanism and patterns of influenza transmission between nearby military units and the capability of spread this infection to the general population.

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SURVEILLANCE OF EMERGING DISEASE IN RESOURCE LIMITED SETTINGS

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The objective of this study was to develop an electronic disease surveillance system that will enhance disease reporting in resource limited settings in accordance with the 2005 International Health Regulations (IHR 2005). Changes in transportation and communication are making our world increasingly interconnected. While useful in many ways, this also facilitates rapid transmission of disease around the globe. IHR 2005 addressed this issue by requiring signatory countries to improve their ability for disease prevention, detection, and response, and to report all serious outbreak. Unfortunately rapid identification of outbreaks is very difficult in resource limited settings where surveillance is often paper-based. The goal of the project reported here is to develop and pilot rapid electronic data collection and disease detection methods suitable for resource limited countries. Two software applications were developed: Surveillance Tool for Analysis, Management, and Reporting data (STAMR); and Clinic Data Entry Software (CDES). STAMR is a desktop version of the web-based disease surveillance application ESSENCE. Like ESSENCE, STAMR reads case-level illness reports and flags significant increases in illness. The STAMR interface reads several different databases regardless of the variable structure. Rapid disease detection requires quick collection of disease counts from the lowest level of health care; something traditional paper surveillance cannot provide. CDES is a simple computer tool that creates an electronic database containing symptoms and/or diagnoses of health clinic patients. It replaces or supplements clinic patient log books creating a simple electronic medical record for each patient. Using this database CDES automatically creates and transmits reports to the local surveillance unit, via internet when possible. Local surveillance units can use STAMR on compiled clinic data to rapidly detect unusual increases in disease levels and respond to potential outbreaks. STAMR and CDES are being piloted in the Philippines. Initial results will be available in fall 2008. In conclusion, STAMR and CDES used together create a system that resource poor countries can use to improve the speed and validity of disease surveillance at the most local level. The current pilot test will help assess the utility and sustainability of these tools.

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A SYSTEMATIC REVIEW AND META-ANALYSIS OF TUBERCULOSIS INFECTION RISK IN DEPLOYED MILITARY PERSONNEL AND LONG-TERM CIVILIAN TRAVELERS

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Tuberculosis (TB) infection and transmission remains one of the greatest public health threats worldwide. However, there is a lack of quantifiable evidence of the TB infection risk to long-term travelers and deployed military personnel. There are also differing opinions in the travel medicine community on the risk to long-term travelers of TB infection. The purpose of this study is to quantify the risk of TB infection in long-term travelers and deployed military service members. We initially performed a systematic literature review on TB infection risks in travelers and deployed military members. We narrowed down 344 studies to four which were appropriate for our study. We also obtained unpublished U.S. and foreign military data. Combined, we had a total of nine study estimates on which we conducted random effects meta-analyses, and multiple sensitivity analyses using STATA v.10. The cumulative incidence rate for all nine studies in our initial model is 2.03 (1.73, 2.34). After excluding overly influential and heterogeneous studies, the Cumulative Incidence risk

estimate adjusted to 2.30 (2.06, 2.55). Meta-analyses using incidence density produced estimates approximately one percentage point or more greater than corresponding cumulative incidence estimates. The incidence density scatter plot showed markedly decreasing infection risk over an increasing range of travel durations. In contrast, the cumulative incidence plot showed a flat or only slightly decreasing risk. We found only a half-percentage risk difference between military-only and civilian-only studies (2.20% [1.87, 2.54] vs. 2.76% [1.25, 4.27]). We believe that the risk of TB infection to long-term civilian travelers and deployed military personnel approximates 2.30%. The risk stays relatively constant or slightly decreases over a range of travel durations. Military personnel have differences from civilian populations that may affect their relative risks. However, considering the heterogeneous nature of activities civilian travelers and deployed military engage in, their relative risks closely approximate.

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PREVENTING NIPAH VIRUS INFECTION: INTERVENTIONS TO INTERRUPT BATS ACCESSING DATE PALM SAP

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Drinking raw date palm sap is a seasonal delicacy in Bangladesh. During winter many rural Bangladeshis earn a living by collecting date palm sap. Fruit bats (*Pteropus giganteus*) drink raw date palm sap and can contaminate by shedding Nipah virus through saliva and urine. The aim of our study was to assess the feasibility of encouraging date palm sap collectors (*gachis*) to use bamboo net or lime (calcium carbonate), two interventions suggested by earlier research, to prevent bats accessing the sap.In February 2008, we conducted a pilot study in a village in Faridpur district, Bangladesh. We encouraged *gachis* to use either bamboo net or lime through interactive community meetings and individual discussion. We worked with *gachis* to prepare and apply these preventative methods, measured the time needed for preparation and application and calculated the cost. We documented their use of each method through short interviews. *Gachis* considered bamboo nets as an effective method and were interested to use to interrupt bat access, to ensure the quality and quantity of the sap. They were skeptical that lime would be effective as the lime was washed away by the sap flow. The application of each method took about one minute. *Gachis* prepared the bamboo nets, which took about 105 minutes for each. They proposed to prepare the bamboo nets during their leisure time or off season. They also suggested that once they make a net, they would use that for the next seasons. The cost of bamboo net is minimal because bamboo is widely available and they made net by the pieces of used bamboo. They recommended using bamboo nets on those trees which will be used for raw sap consumption or often visited by bats. In conclusion, the bamboo net method appeared practical and affordable to the local *gachis.* Further studies need to be done to asses if the use of bamboo nets produces a better quality and larger quantity of sap which would mean more income for *gachis* and so motivating them to use it regularly.

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PREDICTING HANTAVIRUS RISK IN CHILE

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Since the discovery of the Sin Nombre Virus, additional hantaviruses have been described in the Americas. Among these, Andes hantavirus has been shown to be responsible for several outbreaks in the southern cone. In Chile, 540 cases of Andes virus infection have been reported since 1995, with an average lethality of 37%. The main reservoir of Andes virus is the murid rodent *Oligoryzomys longicaudatus* (colilargo). This species possess ecological attributes (e.g. irruptive population dynamics, high vagility and abundance) that make the study of this reservoir species a great priority, given the risk it poses to human populations living within its distributional range. Here, we show two approaches aimed at assessing the spatial distribution of seropositive mice in Chile and we use this to forecast potential areas of high disease risk. The first approach uses Stepwise Generalized Linear Models (GLM) with binomial errors and the second is based on the application of Maximum Entropy techniques (MaxEnt). Models were based on the results of mice blood samples collected in 167 localities between latitudes 30° S and 46°S between 2000 and 2005 as the outcome variable. Predictive variables entered in models were obtained from Landsat Thematic Mapper imaginery. Selected GLM models for seropositive O. longicaudatus in the period 2000-2002 showed good performance in classifying cases with an area under de ROC curve or AUC= 0.81. The same model when used to predict the occurrence of seropositives individuals in the period 2003-2005 had an AUC= 0.63. The MaxEnt model, also provided highly significant fits with an AUC=0.78 for the training dataset (i.e. 2000-2002) and AUC=0.63 for the test data. Interestingly, when seropositive individuals of species other than *O. longicaudatus* where added to the models the performance of the models in predicting seropositive occurrences improved to AUC=0.81 and AUC=0.71 for the GLM and MaxEnt models respectively. We discuss our findings in relation to the occurrence of human cases and suggest further venues for research.

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DEVELOPMENT AND EVALUATION OF RECOMBINANT ARENAVIRUS PROTEINS FOR USE IN DIAGNOSTIC, PROPHYLACTIC AND THERAPEUTIC APPLICATIONS

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Lassa virus (LV), an arenavirus, is the causative agent of Lassa (LF), a febrile hemorrhagic syndrome associated with a non-specific clinical presentation and broad differential diagnoses. LV is acquired through contact with infected excreta of its rodent reservoir, Mastomys spp., or

by human-to-human transmission through contact with infected blood or bodily fluids. Although it is clear that the public health impact of LF in West Africa is immense, the true incidence of the disease is unknown. One major hindrance to diagnosing LF has been accessibility to diagnostic reagents, which must be produced in biosafety level (BSL)-4 laboratories. To address this shortcoming, we expressed LV (strain Josiah) glycoprotein (GP) 1, GP2, and nucleocapsid protein (NP) in E. coli for use in ELISAs. We incorporated these reagents into LV-specific antigen (Ag)-capture, IgM, and IgG ELISAs and evaluated them in eastern Sierra Leone, which has the highest known incidence of LF in the world. Kenema Government Hospital (KGH) in Kenema, Sierra Leone has for decades been the principle hospital managing patients with LF. KGH maintains a 25-bed LF Ward (LFW) and full-time staff completely dedicated to the care of patients with LF. Using archived patient serum collected in the LFW since 2004, we evaluated our recombinant Ag-capture, IgM, and IgG ELISAs and compared them with results from ELISAs using traditional BSL-4-generated antigens as well as a real-time RT-PCR assay targeting the LV (Josiah) nucleoprotein gene. The recombinant Ag-capture ELISA demonstrated 100% sensitivity when compared to real-time RT-PCR. By comparison, RT-PCR detected less than 50% of cases detected by Ag-capture ELISA. The recombinant IgM ELISA also demonstrated 100% sensitivity when compared to the assay performed with BSL4-generated reagents. We also analyzed the clinical chemistry profiles of a subset of surviving and fatal cases; The hepatic enzymes, ALT, AST, and ALP, levels were all extraordinarily high in both fatal and non-fatal cases. Interestingly, remarkably high creatinine levels were only observed in fatal cases, leading us to believe that this may be an important prognostic indicator. Although only preliminary, these findings offer a glimpse of the potential of this extraordinary research setting and opportunity to conduct in-depth study of a viral hemorrhagic fever and category A select agent while contributing to the control of the disease in the endemic area.

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HOME POULTRY RAISING PRACTICES IN BANGLADESH: THE SETTING FOR ANIMAL TO HUMAN INFLUENZA TRANSMISSION

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In Bangladesh, since March 2007, H5N1 has been identified in several commercial and backyard poultry farms in 47 districts and in 22 May 2008, government declared the first human case with H5N1. Backyard poultry represent approximately 50% of the total poultry population, 80% of rural households in Bangladesh raise poultry. The objective of this study was to explore everyday practices of poultry raising, which included human interactions with poultry and perceptions and practices regarding sick poultry within the household. In a village in Netrokona district, we conducted 20 indepth interviews, 10 observations (6-7 hours each) to document poultry raising and interaction of human and poultry. We did 20 household mapping and one village mapping to identify poultry raising households and poultry sheds. Out of 108 households, 86 were involved in backyard poultry raising and they described it as an important source of household income. Women were the primary caretakers of poultry. We observed poultry scavenge for food, sleep, and lay and hatch their eggs in homes, often under the bed. After slaughtering, entrails were thrown in the nearest bush and there was no effort to clean the slaughtering place. Respondents reported sick poultry were treated at home and kept in the same place as healthy poultry, even though they know about the contagiousness of diseases among birds. They expressed concern about unknown poultry disease. Villagers reported consuming sick poultry and throwing dead poultry in nearby water bodies used for bathing and washing. After physical contact with poultry we never observed anyone wash their hands with soap. In conclusion, the close interaction of humans and poultry in everyday life in rural Bangladesh puts people at risk for avian influenza infections. Simple health education messages that ignore the perspective of poultry producers including their limited resources, the importance of poultry for household income, and their habitual sharing of their house with the birds, are unlikely to be effective in reducing transmission risk.

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ENVELOPE REGION GENETIC CHARACTERIATION OF CHIKUNGUNYA VIRUS ISOLATES FROM INDONESIA

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Chikungunya virus (CHIKV) is an Alphavirus that normally causes a self-limiting febrile illness with symptoms similar to dengue. It is vectored to humans primarily through the *Aedes aegypti* and Aedes albopictus mosquitoes. Clinical manifestations include fever, rash and severe, sometimes prolonged, joint pain. CHIKV has been reported to cause human epidemics in many areas of Africa and Asia, and most recently in a limited area of Europe. CHIKV infection was first recognized in Indonesia in 1982 and has been reported in several provinces throughout the archipelago since then. To better understand the biology of CHIKV in Indonesia, we undertook a genetic characterization study. A phylogenetic approach was used to demonstrate virus evolution in isolates acquired throughout Indonesia. Partial sequencing of two envelope regions, comprising 260nt of the envelope-1 (E1) gene and 220nt of the envelope-2 (E2) gene was completed for each isolate. A phylogenetic tree of both genes was constructed and a multiple sequence alignment comparison of the Indonesian isolates with a candidate vaccine strain (TSI-GSD-218) as well as isolates from India, East/South Africa and West Africa was conducted. These data provide CHIKV sequences from Indonesian strains that are important for vaccine development and demonstrate the potential for microevolution of CHIKV in Indonesia.

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HOW TO IMPLEMENT A SUCCESSFUL TRAINING PROGRAM AT YOUR INSTITUTION?

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"Training", is the new buzz word! Training laboratory staff is nothing new but is somewhat problematic due to a shortage of people and funds. Institutions are responsible for training their staff and with the exponential growth of research facilities the realm of safety related to working in these facilities has drawn new attention from such areas as the government (congressional hearings), inspection agencies (NIH-OBA, Centers for Disease Control and Prevention, USDA, DHS, etc…) and the general public. The new attention has focused on the quality of training and documentation of the individuals working in these new facilities as well as existing facilities. Worldwide we have seen and heard about laboratory incidents that occur due to lack of improper training/understanding of the agent and equipment, staff complacency or just failing to follow or understand directions that have placed institutions in an onerous situation. This presentation will focus on what is expected from an institution in regards to training and how to develop a successful training program at your institution.

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WORLD RABIES DAY: A ONE HEALTH INITIATIVE TO...*MAKE RABIES HISTORY!*

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Although the word alone evokes a highly charged response, rabies remains a severely neglected disease. With the development of effective biologics more than 30 years ago, there is no reason why people exposed to rabies today should be at risk for developing and dying of rabies. And yet, it is estimated that more than 55,000 humans die of rabies every year - a large proportion of them are children from impoverished communities in Asia, Africa and Latin America. The continuing loss of lives to rabies infection is directly related to population-level risk factors. The World Rabies Day initiative is a rabies awareness tool intended as a catalyst for collaboration in animal and human health education, diagnostic capacity, and disease prevention - the foundation of which can then be applied to other major agricultural and human health problems. The initiative arose from a group of rabies prevention professionals who built partnerships with the World Organization for Animal Health (OIE), World Health Organization, the Pasteur Institute, World Veterinary Association, and many other organizations. The World Rabies Day initiative advocates for improving the health of the whole population, human and animal, - a "One Medicine Approach" - by raising awareness about the need to control rabies in the main global animal reservoir, the domestic dog, and prevent human rabies through education and appropriate medical prophylaxis - a need that arises primarily from economic and educational disparity. During the inaugural year, at least 74 countries participated through a wide variety of events including vaccination clinics, educational seminars, and media outreach. Summary data indicates that 400,000 people participated in a World Rabies Day event, over 600,000 animal vaccinations were administered and more than 54 million people were reached through media worldwide. Veterinary colleges around the world joined forces towards this effort, with activities at 24 in the US, 15 in India, 5 in Indonesia, and several in Mexico, Peru, the Philippines and in some African countries. The first World Rabies Day was a major achievement for the rabies prevention community. These efforts are envisioned to be part of comprehensive human and animal health delivery that would develop and augment public health and veterinary infrastructure in regions of greatest need. Now is the time for "Working Together to Make Rabies History!"

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AVIAN INFLUENZA IN WILD BIRDS FROM THE CENTRAL COAST OF PERU

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Migratory waterfowl are currently considered to be the primary reservoirs for avian influenza viruses. Although intense influenza virus surveillance in wild bird has been occurring in Europe, North America, Asia and Africa, few activities are present so far in South America. To our knowledge, only one serological study has been conducted and a single viral isolate recovered from Brazil and Bolivia, respectively. Environmental fecal samples were collected from 4 wetlands along the coast of central Peru from June 2006 to December 2007. Samples were pooled according to the collection date, avian species and location, and then processed for inoculation into specific-pathogen-free chicken eggs. Allantoic fluids from hemagglutination positive pools were sent to the US Naval Medical Research Center Detachment, Lima and CDC for viral and molecular characterization with immunofluorescence and genetic sequence analysis. A total of 2407 samples were collected from 26 avian species (12 families). We identified nine low pathogenic avian influenza (AI) viruses isolated from seven different species (five families) within 3 of the 4 wetlands sampled. Viral subtypes H3N8, H4N5, H10N9 and H13N2 were identified. Isolation was achieved during early spring and late summer, showing a possible pattern in temporal distribution of the viruses. Although majority of AI isolates were recovered from migratory waterfowl, we also found evidence of viral shedding in resident, non-migratory species. In conclusion, our findings provide additional evidence that AI viruses are present and circulate in wild birds in South America, and thus can be potential source of influenza viruses that may spread among a variety of species, as well as poultry, swine and humans, along their flyways between North and South America.

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DETECTION OF FEBRILE RESPONSES IN VENEZUELAN EQUINE ENCEPHALITIS VIRUS (VEEV) INFECTED MICE

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V3526, a live attenuated vaccine for VEEV subtype IA/B, induces mild fevers in nonhuman primates (NHP) and humans following vaccination. It is unknown if mice, the traditional model for encephalitic virus infections, develop a fever following vaccination with V3526. To date the murine response to V3526 and other VEEV vaccines has been limited to physical signs of disease (ruffled fur, hunched back, etc.) and generation of antibodies. The recent development of telemetric implants for small animals has made more sensitive assessment of murine responses to VEEV and vaccines a possibility. This study was designed to monitor body temperatures and activity in mice following administration of VEE IA/B Trinidad donkey strain (TrD) and V3526, and to evaluate the utility of telemetry as a diagnostic indicator of disease in nonclinical VEEV studies. Three groups of BALB/c mice were implanted with telemeters (Data Sciences International) and baseline data were collected for 7 days. Mice in each group were inoculated subcutaneously with either $1x10^4$ plaque forming units (pfu) of TrD, $1x10^4$ pfu of V3526 or control material. Temperature and activity data were collected on each mouse for 21 days post-inoculation or until death. Blood samples were collected prior to and at 21 days post-inoculation for monitoring VEEV neutralizing antibody responses. Mice in the TrD group presented the classical signs associated with infection. In the TrD inoculated mice, a loss of diurnal rhythm was observed at 48 hours post-inoculation. Temperatures in these mice began to increase at 96 hours post-inoculation and persisted for 48 hours at which time the temperatures decreased and remained subnormal until death. Temperatures in mice inoculated with V3526 or control material did not substantially differ from baseline values nor were physical signs of disease observed at any time during the study. From these studies we conclude: 1) telemetry is a sufficiently sensitive tool for detecting fevers in mice challenged with virulent VEEV and 2) V3526 does not induce a detectable febrile response in mice as seen in NHP and humans. Studies in progress will utilize telemetry to assess adverse effects in mice and NHP that receive various inactivated VEEV vaccine/adjuvant formulations.

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MAYARO FEVER VIRUS OUTBREAK IN SANTA BARBARA, PARÁ STATE, BRAZIL, 2008

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Mayaro fever virus (Togaviridae: Alphavirus) is associated with febrile disease in tropical South America, and transmitted by *Haemagogus janthinomys* mosquitoes. In February 2008 an outbreak of a dengue-like rash febrile disease was recognized in a settlement in the Santa Barbara municipality 38 km eastern Belém, Brazil. Febrile patients and their relatives were bled for attempts of virus isolation and IgM detection by ELISA, respectively. A total of 105 people (53 residents in the settlement and 52 students of a public university which were training in the area) were bled and blood samples taken in Santa Barbara municipality. All serum samples were submitted to an IgM ELISA against Mayaro fever virus antigens, while blood of acute febrile patients were inoculated into C6/36 cells and suckling mice. Two Mayaro virus strains from febrile patients were isolated in both systems and were confirmed by serologic and molecular techniques. By serology, 36 (34.3%) serum samples were positive to IgM detection; 43.4% (23/53) people resident in the settlement and 25% (13/52) students. Age of Mayaro confirmed cases ranged from 4 to 55 years old; 21 (58.3%) were male and 15 (41.7%) female; 47.8% were agriculture workers, 30.4% students, and 21.8% of other occupation. The clinical picture presented by infected people was characterized by a febrile illness with a sudden onset. The most important symptoms/signs were headache (63.8%), retroocular pain (44.4%), myalgia (75%), arthralgia (88.9%), articular edema (58.3%) and skin rash (63.8%). Other symptoms presents were dizziness (25%), vomiting (13.9%), itching (33.3%), anorexia (22.2%) and lymph nodes (16.7%). Differential diagnosis with other common maculopapular exanthema illnesses in Brazil including dengue fever resulted negative. After the rainy season, the transmission was interrupted in the area. This is the first Mayaro fever virus outbreak in Santa Barbara.

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ANTIGENIC DRIFT AND THE REASSORTMENT OF GENOMIC RNA SEGMENTS PROTAGONIST THE MICROEVOLUTION OF PUUMALA HANTAVIRUS IN A BANK VOLE (*MYODES GLAREOLUS*) POPULATION

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Hantaviruses are etiological agents of hemorrhagic fever with renal syndrome (HFRS) in Eurasia and hantavirus (HPS) in the Americas. Hantaviruses are enveloped negative-stranded RNA viruses. Here we analyze *Puumala hantavirus* (PUUV) genetics evolution. Genetic diversity of PUUV was studied in a local population of its natural host, the bank vole (*Myodes glareolus*). The trapping area (2.5 x 2.5 km) included 14 trapping sites, 500-1000 m apart; altogether 147 voles were captured during May and October 2005. Partial sequences of the S, M and L viral genome segments were recovered from 40 animals. For the S, M and L sequences were detected seven, twelve and seventeen variants, respectively. The genetic diversity of PUUV strains of the studied trapping area for the S segment was 0.2-4.9%, for the M segment was 0.2-4.8%, and 0.2-9.7% for the L segment. Most nucleotide substitutions were synonymous, most deduced amino acid substitutions in the N and L proteins were conservative, probably due to strong stabilizing selection operating at the protein level. Based on both, sequence markers and phylogenetic clustering, the S, M and L sequences could be assigned in two groups, "A" and "B". Notably, not all bank voles carried the S, M and L sequences that belong to the same group, i.e., $S_AM_AL_A$ or $S_BM_BL_B$. A substantial portion (8 of 40, or 20%) of the newly characterized PUUV strains possessed reassortant genomes such as $S_BM_AL_A$, $S_AM_BL_B$ or $S_BM_AL_B$. These results suggested that at least some of PUUV reassortants are viable and can survive in the presence of their parental strains.

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GENETIC CHARACTERIZATION OF THE RABIES VIRUS STRAIN QR 18867 (*RHABDOVIRIDAE*, *LYSSAVIRUS*) ISOLATED FROM THE *URODERMA BILOBATUM* BAT IN PORTEL MUNICIPALITY, PARÁ STATE, 2004

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Human rabies transmitted by hematophagous bats constitutes a serious concern for many Latin American Countries. Recently, between the years of 2004 and 2005, three rabies human outbreaks transmitted by bites of the vampire bat (*Desmodus rotundus*) were reported in Para state, wit a total of 22 deaths, revealing the alarming potential of rabies virus (RV) (*Rhabdoviridae*; *Lyssavirus*) transmission to humans associated with vampire bats attack in the Northern region of Brazil. During one of these outbreaks, a RV strain was isolated from the *Uroderma bilobatum* frugivorous bat (QR 18867). The aim of this study was to perform the genetic characterization of this strain QR 18867 isolated in Portel, Pará state, 2004. Samples of brain tissue were collected and used for viral isolation attempt in newborn mice by intracerebral (i.c) inoculation, as well as for antigenic characterization by using the Indirect Immunofluorescence Assay (IIFA) applying a monoclonal antibodies panel as recommended elsewhere (CDC/Atlanta). Virus RNA was extracted directed from the mice infected brain by the TRIZOL LS reagent technique and used for the full length N gene amplification by a standard two step RT-PCR protocol. Genetic and phylogenetic analyses were conducted of the studied strain for the determination of its genetic traits and evolutionary origin. The analysis using the IIFA, although positive, was not conclusive for the identification of the virus variant. The entire N gene was successfully sequenced revealing a total of 1353 nt (450 aa) in length. Silent and non-silent mutations were observed along the N gene of the strain QR 18867 in comparison with representative members of the vampire bat (AgV3), dog (AgV2), raccoon, and laboratorial strains. Phylogenetically, QR 18867 strain constituted a single clade more closely related to AgV3 strains. The genetic characterization and phylogenetic analysis suggests that the RV strain QR 18867 is a possible novel antigenic variant of RV found in the Brazilian Amazon which is more closely related to the hematophagous bat variant

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MATERNAL-FETAL TRANSMISSION OF CHIKUNGUNYA VIRUS IN MICE

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Chikungunya virus (CHIKV), an *Alphavirus* causing fever, severe myalgia and rash, is endemic in parts of Africa and sporadically epidemic in Asia and islands of the Indian Ocean. While CHIKV is not usually fatal in healthy adults, increased death rates have been observed in newborns and the elderly. In recent epidemics, maternal-fetal transmission of CHIKV also has been observed. This has been linked to aborted fetuses early in pregnancy and to newborn sickness in fetuses infected near term. While the exact mechanism of vertical transmission is unknown, aborted fetuses of acutely infected mothers were shown to be CHIKV positive by PCR. Likewise newborns of infected mothers developed a febrile illness with occasional hemorrhagic, cardiac and neurological manifestations resulting in at least one death. The development of an animal model for the maternal-fetal

transmission of CHIKV will be central to elucidating the mechanism of transmission. A model for vertical transmission of CHIKV is not known. Pregnant CD-1 mice were infected with CHIKV subcutaneously in the back approximately two days before delivery. Pups from these infected females exhibited lethargy, tremors and were unable to right themselves. CHIKV was isolated from the brains and leg muscle of newborn mice from these infected mothers, and HI antibody was detected in the pups three weeks after birth. Pups from theses infected females have little mortality and most recover from the infection. Preliminary results suggest that the neurological involvement observed in newborn mice is similar to that seen in newborn children. This mouse model should be helpful to understanding the mechanism(s) of maternal-fetal transmission of CHIKV and to develop interventions to reduce morbidity and mortality in newborn infants of CHIKV-infected mothers.

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INTERMITTENT PREVENTIVE TREATMENT (IPT) IN SCHOOLCHILDREN: A RANDOMIZED TRIAL TO COMPARE THE EFFICACY, SAFETY, AND TOLERABILITY OF ANTIMALARIAL REGIMENS IN UGANDA

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Intermittent preventive treatment (IPT) in pregnancy is an important component of malaria control, and may also benefit infants and children. A recent trial in Kenya found that provision of amodiaquine + sulfadoxine-pyrimethamine (AQ+SP) for IPT in schoolchildren reduced rates of malaria parasitemia and anemia. However, the optimal antimalarial regimen for IPT in schoolchildren remains unclear. We are currently conducting a randomized, blinded trial to compare the efficacy, safety, and tolerability of sulfadoxine-pyrimethamine, AQ+SP, dihydroartemisinin-piperaquine, and placebo among primary schoolchildren in Uganda. A blinded interim analysis including 324 participants (target sample size = 760) is presented here. Asymptomatic children aged 8 to 12 years (girls) and 8 to 14 years (boys) were randomized to receive one of the study regimens and were followed for 42 days. Participants were assessed for treatment outcomes over 42 days according to modified World Health Organization criteria. Rigorous assessment for adverse events was performed. Of 323 participants enrolled in the study, 321 (99%) completed follow-up and were assigned a treatment outcome; 3 participants were withdrawn or lost to follow-up. Almost half of the children (47%) were parasitemic at baseline. By day 42, clinical treatment failure occurred in 21 (7%) children. All clinical failures were due to development of fever/temperature in presence of parasitemia; no children developed severe malaria or danger signs. Parasitological failure occurred in 164 (51%) participants; approximately half of these occurred between days 14 and 28 (52%). At least one adverse event was reported in 216 (67%) participants, but no serious adverse events occurred. The most common adverse events included headache, cough, and abdominal pain, which were reported by 28%, 20%, and 19% of participants, respectively. Complete, unblinded results, including assessment of parasite isolates by genotyping, and full results of safety and tolerability, will be presented.

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A RANDOMISED TRIAL TO COMPARE THE SAFETY, TOLERABILITY AND EFFICACY OF THREE POTENTIAL DRUG COMBINATIONS FOR INTERMITTENT PREVENTIVE TREATMENT IN CHILDREN AGED ONE TO FIVE YEARS IN AN AREA OF SEASONAL MALARIA TRANSMISSION IN UPPER RIVER REGION, THE GAMBIA

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Intermittent preventive treatment (IPT) offers a potential way of preventing malaria infection without compromising the development of malaria immunity or encouraging drug resistance. The effect of IPT in infants and children in the prevention of malaria has been evaluated in a number of trials. Results from these trials have shown that IPT provided significant protection against clinical malaria. It seems likely that a long acting drug is needed for effective IPT and SP alone or in combination with other drugs have been used for IPT programmes. However, SP resistance is increasing in many parts of Africa. Thus it is important to investigate if other drug regimens might be equally effective in preventing malaria. Thus, during the 2007 malaria transmission season, 1009 children aged 1-5 years were individually randomized to receive amodiaquine plus SP, piperaquine plus SP or dihdroartemisinin plus piperaquine at monthly intervals on three occasions during the peak malaria transmission season (September, October, and November). To determine the prevalence of side effects following drug administration participants in each treatment group were visited at home three and seven days after each round of drug administration and a side effects questionnaire completed. To help establish whether these adverse events are drug related, the same questionnaire was administered after each treatment round, to 286 age-matched children who are not part of the trial. Morbidity was monitored throughout the malaria transmission season and malaria smears were prepared and haemoglobin concentration measured whenever a study subject presented to one of the health centres in the study area with symptoms compatible with malaria. Data on safety and efficacy of these antimalarial combinations will be presented.

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IMPACT OF ARTEMISININ-BASED COMBINATION THERAPY INTERMITTENT PREVENTIVE TREATMENT ON MALARIA MORBIDITY IN ELEMENTARY SCHOOL STUDENTS IN MALI

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Several studies have examined the impact of Intermittent Preventive Treatment (IPT) in pregnant women and infants, but few have focused on school-aged children. We assessed the impact of seasonal artemisinin-based combination therapy as intermittent preventive treatment in school children (ACT-IPTsc) on malaria incidence, school absenteeism, and school performance. We conducted a randomized, open, controlled trial of the impact of seasonal ACT-IPTsc among students aged 6-13 years in Kolle, Mali. The study began in September 2007 and completed follow-up in May 2008. Students were randomly assigned to one of three study arms: Sulfadoxine-pyrimethamine plus artesunate (SP/AS), amodiaquine plus artesunate (AQ/AS), or vitamin C (control arm). Two full treatment doses were given two months apart during the high transmission season in September and November. Student absenteeism and grades were noted for all study participants. Groups were then compared in terms of malaria incidence, hemoglobin levels, school absenteeism, and average school grades. 305 students were randomized from the first through sixth

grades with a total loss to follow-up of 3.6%. We observed a reduction in the incidence of malaria cases of 69.5%, 54.6% for SP/AS and AQ/AS respectively compared to vitamin C (p<0.001). The control group at 4-weeks and again at six months post-ACT-IPTsc doses had more than 5 times the rate of asymptomatic parasitemia than either SP/AS and AQ/AS (p<0.001). By month four of the study follow-up, SP/AS and AQ/AS had significantly higher hemoglobin averages (12.21mg/dL and 12.17mg/dL vs. 11.65mg/dL, p=0.010), and fewer cases of anemia. We observed a trend towards reduced school absenteeism in the SP/AS (0.375-1.00 days/child/season) and AQ/AS arms (0.60-1.5 days/child/season) as compared to the control arm (1.12-2.8 days/child/season). No effect was observed with regard to average school grades. This study showed that ACT-IPTsc reduces malaria morbidity in school age children and may have positive impact of school absenteeism.

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PUBLIC HEALTH IMPLICATIONS OF RECRUDESCENT VS. NEW INFECTIONS IN DRUG EFFICACY TRIALS

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Genotyping to distinguish recrudescent from new infections is standard in antimalarial drug efficacy trials, increasing their cost and complexity. When genotyping-corrected outcomes are reported, only recrudescent infections are considered treatment failures. However, the relative risks and benefits of recrudescent versus new infections are unknown, and we hypothesize that new infections are likelier to cause illness than are recrudescent parasites to which an immune response has already developed. If so, then in high transmission areas longer-acting drug combinations with imperfect initial efficacy might be preferable to highly efficacious but short-acting drug combinations. To determine if recrudescent and new infections carry different risks, we are comparing clinical, hematological and parasitological outcomes following recurrent infections experienced by children treated for uncomplicated malaria in drug efficacy studies in Blantyre, Malawi. Infections are classified as new or recrudescent by amplifying polymorphic regions of merozoite surface protein-2 (*msp-2*) and *msp-1* and using gel and capillary electrophoresis to assess the fragment lengths. The risk of fever and anemia will be compared by logistic regression and hemoglobin and recurrent parasite density will be compared by linear regression, after controlling for age, initial hemoglobin and initial parasitemia. Results from gel and capillary electrophoresis will be compared to determine if routine use of capillary electrophoresis to assess *msp-2* and *msp-1* genotyping is warranted or if agarose gel electrophoresis, a technology available in many malaria endemic countries, provides adequate information. The need to genotype episodes of recurrent parasitemia and the precision required for the method will be useful for malaria-endemic countries conducting drug efficacy studies to inform national treatment policies. If new infections are found to have higher risks of adverse clinical outcomes, this will argue for reporting rates of new infections as important endpoints in drug efficacy trials.

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ARTEMETHER-LUMEFANTRINE VERSUS DIHYDROARTEMISININ-PIPERAQUINE FOR THE TREATMENT OF UNCOMPLICATED MALARIA: A RANDOMIZED LONGITUDINAL TRIAL IN A COHORT OF UGANDAN INFANTS

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Artemisinin-based combination therapy (ACT) is widely advocated for the treatment of uncomplicated malaria, however limited data exists on efficacy in young children. This study compared two leading ACTs in a cohort of young children living in a high transmission area of Uganda. From August 2007 to January 2008 we recruited a cohort of 301 HIV-uninfected children aged 6weeks-9months. All children were provided with standard medical care, insecticide treated nets, and trimethoprim-sulfamethoxazole prophylaxis during breastfeeding if born to HIV-infected mothers. When children were diagnosed with their first episode of uncomplicated malaria, they were randomized to artemether-lumefantrine (AL) or dihydroartemisinin-piperaquine (DP). The same therapy was given for all subsequent episodes of uncomplicated malaria. Through March 2008, a total of 59 children were randomized to AL (121 treatments), and 71 children to DP (136 treatments). After 28 days of follow-up, AL was associated with a significantly higher risk of recurrent parasitemia (unadjusted by genotyping) compared to DP (35.0% vs. 11.2%, p<0.0001). Appearance of gametocytes after therapy was similar for AL and DP (0% vs. 3.2%, p=0.45). Mean hemoglobin recovery was similar for AL and DP (0.67 vs. 0.89 gm/dL, p=0.27). Both regimens were safe and well tolerated with the exception of one patient who developed severe anemia following repeated treatments with DP. Our preliminary results indicate that, in a high transmission region, the antimalarial efficacy of DP was superior to that of AL; Genotyping to distinguish recrudescence from new infections, is on going.

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PHARMACOKINETICS OF ARTEMISININ COMBINATION THERAPY IN CHILDREN IN KAMPALA, UGANDA

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The World Health Organization (WHO) recommends the use of artemisinin-based combination therapies (ACT) for the treatment of uncomplicated malaria. In response to increasing antimalarial drug resistance, the Uganda Ministry of Health advocates the use of ACTs, including artemether-lumefantrine (AL) and artesunate/amodiaquine (AQ/AS) as first-line antimalarial drugs. Despite wide-spread use, pharmacokinetic (PK) data informing optimum dosing of these drugs is limited, especially in children. Compared to adults, children may exhibit altered activity of cytochrome P450 and UDP-glucuronosyltransferase metabolic pathways, thereby affecting their ACT PK exposure and potentially altering their risk of treatment failure. We evaluated PK parameters in Ugandan children aged 5-13 years with uncomplicated malaria treated with AL or AQ/AS. Twenty children each were evaluated for the AL and AQ/AS treatment arms. Blood samples were drawn just prior to treatment and at 0, 2, 4, 8, 24 and 120 hours following

the last dose of the 3 day regimen of AL (twice daily) or AQ/AS (once daily). Sample collection has been completed, and plasma is currently being analyzed for concentrations of artemether, the active metabolite dihydroartemisinin (DHA), lumefantrine, and the metabolite desbutyl-lumefantrine for the AL regimen and artesunate, DHA, amodiaquine, and the active metabolite desethylamodiaquine for the AQ/AS regimen. These data will allow the calculation of PK parameters, including the area under the plasma concentration versus time curve (AUC), apparent clearance (CL/F) and half-life, which will be presented and compared to historical data from adult subjects. This will be, to our knowledge, the first intensive PK study carried out in children in subSaharan Africa. Results will allow optimization of antimalarial treatment guidelines for African children.

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REGIONAL AGE-BASED DOSE REGIMENS FOR A NEW FIXED-DOSE COMBINATION OF ARTESUNATE-MEFLOQUINE FOR THE TREATMENT OF UNCOMPLICATED FALCIPARUM MALARIA IN LATIN AMERICA AND ASIA

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The recently launched fixed-dose combination of artesunate-mefloquine (ASMQ, Far-Manguinhos, Brazil) was developed as part of an international collaboration, coordinated by DNDi. The target dose is 4mg/kg of artesunate (AS) and 8mg/kg of mefloquine (MQ) daily for 3 days. ASMQ is formulated in paediatric (p=25/55 mg AS/MQ hydrochloride (HCl), corresponding to 50 mg MQ base) and adult strength tablets (a=100/220 mg AS/MQ HCl, corresponding to 200 mg MQ base). With weight-based dosing, the co-formulation was efficacious and well tolerated in field trials in Asia. In practice, however, antimalarial treatments are often dosed by age resulting in a proportion of patients receiving doses outside of the therapeutic range. We previously defined the optimal Brazilian dose regimen using nationally representative weight-for-age database of >180,000 individuals from the malaria-endemic Amazon region (IBGE, POF2002-2003). The therapeutic range was defined as 5-11 mg/kg/day for MQ and 2-10 mg/kg/day for AS. Modelling predicted that the optimal 4-category regimen for intended programmatic use in Brazil that maximised efficacy and minimised toxicity, was: 6-11mo (1p), 1-5yr (2p), 6-11yr (1a) and 12+yr (2a). We will now extend these observations to determine the optimal age-based regimen for ASMQ for Latin America and Asia.Using data from population-based health surveys, we compiled a weight-for-age reference database that is representative of the populations at risk of malaria in both regions. Smoothed regional reference growth curves were generated using a new, extended version of the LMS method with the R GAMLSS package (R2.7.0). Using weight-for-age centiles, we compared the proportions of patients predicted to receive doses within the above therapeutic ranges for different age-dose categories. Inter- and intra-regional differences in modelled growth distributions and corresponding impact on optimal age-dose categories were assessed to guide decisions whether regimens can be harmonized at global level or should be region or sub-region specific. Results will be presented.

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DENGUE VIRUS-INFECTED *AEDES AEGYPTI* IN THE HOME ENVIRONMENT

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We conducted a study in Merida, Yucatan, Mexico, aiming to determine abundance of the yellow fever mosquito *Aedes aegypti* and presence of dengue virus (DENV) in female mosquitoes collected from premises of laboratory confirmed dengue patients over a 12-month period from March 2007 - February 2008. Dengue cases occurred in all months (range per month, 20-451) but there was a distinct peak in cases from late August to late November. Seasonal patterns of abundance of *Ae. aegypti* and number of dengue cases were positively correlated with rainfall. Backpack aspiration yielded *Ae. aegypti* from 583 (46%) of 1,255 examined dengue patient premises; totals of 1,836 females and 1,292 males were collected from the indoor environment and an additional 102 females and 108 males from patios and backyards. *Ae. aegypti* females were collected most commonly from bedrooms (60.3% of all females collected indoors) followed by living/dining rooms (18.4%), kitchens (7.5%), bathrooms (6.6%), and storage rooms (3.9%). A similar intradomicile use pattern was seen for males. This finding indicates that indoor interventions such as fogging with insecticide should place special emphasis on bedrooms and living/dining rooms. Blood feeding status (Sella's stages) did not influence use by *Ae. aegypti* females of different room types as they were found most commonly in bedrooms regardless of blood feeding status. Testing of pooled females from 336 premises produced 34 pools infected with DENV (10.1% of pools infected; overall minimum DENV infection rate of 1.8% and monthly range of 0-4%). DENV-infected *Ae. aegypti* females were commonly collected from the indoor home environment of dengue patients 7-21 d after the onset of symptoms which demonstrates the usefulness of indoor insecticide application in the homes of suspected dengue patients to prevent their homes from becoming sources for dispersal of DENV by persons visiting and being bitten by infected mosquitoes.

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IMPACT ON SEROLOGICAL, ENTOMOLOGICAL, AND BEHAVIORAL INDICES OF AN EVIDENCE-BASED COMMUNITY-DERIVED COMMUNICATION PROGRAM FOR THE CONTROL OF *AEDES AEGYPTI* AND DENGUE IN MANAGUA, NICARAGUA

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For the past four years, we have implemented a step-wise strategy, Socializing Evidence for Participatory Action (SePA), that emphasizes community-derived ways of communicating evidence to encourage informed decisions and actions aimed at reducing dengue risk. CIETmethods provided the epidemiological backbone for this study, based on a modified stratified cluster sampling with 30 sentinel sites in Managua, Nicaragua, that include 10 intervention and 20 reference barrios (132 houses each, ~3,960 households and ~23,300 individuals). Three levels of evidence (serological, entomological and

behavioral), gathered by community members in each barrio during 2 yearly measurement cycles, were used to promote dialogue and inform interventions. Serological analysis of paired saliva samples showed a decrease in children infected with dengue virus, from equivalent numbers in intervention vs reference barrios in 2004 (OR 0.99 (95%CI 0.72-1.35)) to an OR of 0.43 (0.26-0.71) in 2007. Improvements in entomological indicators in intervention households were maintained from 2005 through 2007. In October 2007, a household in an intervention barrio had ~25% less probability of being positive for *Aedes aegypti* larvae and/or pupae than a home in a reference barrio (OR 0.7 (0.6-0.9), contrasting with the 2004 baseline (OR 1.3 (1.0-1.6)). The number of productive containers also decreased, with 55% reduction in positive water storage barrels in intervention barrios in 2007 vs 2004, while the reduction was only 21% in reference barrios; OR 0.6 (0.4-0.8) of intervention vs reference barrios in 2007 vs OR 1.1 (0.9-1.5) in 2004. The positivity of discarded containers was also reduced; OR 0.7 (0.6-0.8) in 2007 vs OR 1.3 (1.1-1.6) in 2004. Relevant behavioral gains in intervention vs reference barrios that were maintained through to the most recent measurement in 2007 included improved ability to "search on one's own for larvae every 8 days" (OR 1.4 (1.3-1.7)), reduced dependence on pesticides (OR 2.6, (CI 2.1-3.2)), and the ability to identify community leadership (OR 4.6 (3.9-5.4)). A positive impact of the program on reference barrios and "spillover" homes in intervention barrios was also documented. In 2008, 7 new barrios were incorporated into the SePA intervention by the Ministry of Health together with community governance organizations following the methodology established for this project, thus paving the way for its future expansion.

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A RESIDUAL DEMOGRAPHY METHOD FOR ESTIMATING AGE STRUCTURE OF WILD MOSQUITO VECTOR POPULATIONS

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Age structure of mosquito populations can profoundly affect the number of vectors that are infected with a pathogen, duration of their infective life, and thus the force of pathogen transmission. Currently available methods for estimating age structure, however, are based on difficult to validate assumptions, subject to considerable bias, unable to estimate age of older most epidemiology important mosquitoes, and too expensive and technically challenging for large scale field studies. To fill this gap, we used a deconvolution model to estimate age patterns of wild *Aedes aegypti* in a dengue endemic area of Thailand. The model required information derived from two different age- and sex-specific life tables, including (1) reference life tables constructed by monitoring daily lab survival throughout the lives of newly-eclosed mosquitoes that were collected as wild pupae and (2) wild life tables constructed by monitoring wild adults of unknown age that were collected by aspiration from inside houses, brought to the lab, and monitored through their death. To test this approach we constructed both types of life tables using between 200 to 350 mosquitoes of each sex that were collected in 2007 during either March-April or Sept-Oct. Life expectancies for reference (newly-eclosed) females and males collected in March-April were 23.6 and 24.3 days, respectively, and for Sept-Oct were 18.9 and 29.7 days, respectively. In contrast, the average times to death of captured free-ranging males and females collected in March-April were 12.2 and 14.5 days, respectively, and for Sept-Oct were 15.5 and 19.3 days, respectively. Shorter times to death in wild-caught (free-ranging) relative to reference mosquitoes reflect higher mortality rates experienced by older mosquitoes that were brought into the lab. Estimates of population age structure revealed that the average male and female mosquito in the wild population was between 11 and 17 days old with from 13 to 18% over 3 weeks old and from 5 to 7% over 4 weeks old. Seasonal differences support the hypothesis that vector population age structures vary temporally in concert with patterns of virus transmission. The residual demography method is a powerful, simple, field amenable technique for generating previously unavailable detail on mosquito vector age structures. Assumptions, caveats, and possible extensions of this method will be discussed.

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SCREENING HOMES TO PREVENT MALARIA: A RANDOMISED CONTROLLED TRIAL

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Historically malaria was controlled in many parts of the tropics by mosquito-proofing houses with screens. Today in Africa most people receive infective bites at night indoors, so screening houses should protect against malaria by preventing mosquito house entry. A three-arm randomized-controlled trial was conducted in Farafenni town and surrounding villages in The Gambia to assess whether installing full screening (screened doors, windows, and closing the eaves) or screened ceilings in local houses can a) reduce house-entry by malaria-transmitting mosquitoes, b) increase hemoglobin concentration, reduce anemia prevalence and reduce parasitemia prevalence in children sleeping in these houses, and c) be comfortable, durable and acceptable to local communities, when compared to control houses (no screening). Over two years 200 homes received full screening, 200 received screened ceilings and 100 had no protection. Mosquitoes were collected biweekly from each house during the rainy season using light traps hung near a household member sleeping under an untreated net (in all arms of the trial). The children sleeping in these homes were fingerpricked at the end of the rainy season to see whether they had malaria and/or anemia. Focus group discussions and questionnaires were used to sample the views of house occupants about the screening, and indoor temperatures were recorded to find out whether the screening makes it hotter than normal indoors. There were 6-month and 12-month assessments of the durability of the screening. The primary outcomes of the trial and their implications will be presented.

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THE ROLE OF SUGAR IN THE MATING BEHAVIOR OF *ANOPHELES GAMBIAE S.S*

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The value of sugar feeding for female *Anopheles gambiae s.s.* remains controversial, as does its pervasiveness in nature. Here we report on a study on the frequency of sugar feeding during early adult life of *An. gambiae* under cage and semi-field conditions, and its influence on the mating behavior of this species. Cohorts of two-hundred females were released into mesocosms where nectariferous plants were present or absent. Two-hundred five day old sugar-fed males were released on the first day and every second day after that, to ensure the presence of competent males throughout the experiment. Females were allowed to blood feed on a human volunteer each night. Every morning a sample of twenty females was taken for five consecutive days. The same experiment was also done in cages, using wicks with either water or a honey solution instead of plants, and half the number of mosquitoes. The Sella's stage, wing length, insemination status, and parity of each sampled female were recorded and nutritional reserves (lipid, glycogen and fructose content) were quantified. The experiment was replicated four times. Blood feeding was more prevalent when sugar was absent, with 49.8% of females over five days having taken a blood meal, against 18.6% when sugar was present. Sugar feeding was consistently high, with an average of 50.2% of females being fructose positive in mesocosms where nectariferous plants were present. After correcting for differences in numbers of males present, females in mesocosms were more likely to be inseminated when sugar was present. This difference was not significant in cages, which

stresses the importance of using appropriately scaled enclosures for behavioral studies on this species. Mating status of females was found to be influenced by their fructose content, with mated females having lower amounts of fructose; indicating a tendency to nectar feed before mating. Blood feeding was opportunistic, i.e. as likely to occur before as after mating. Implications and relevance of the study for control of this important vector are discussed.

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HUMAN IGG RESPONSE TO *ANOPHELES GAMBIAE* SALIVARY PROTEINS AS AN IMMUNO-EPIDEMIOLOGICAL MARKER OF EXPOSURE TO MALARIA VECTOR BITES

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The evaluation of the human antibody response to arthropod salivary proteins could be an epidemiological indicator of exposure to vector bites, and therefore to risk of pathogen transmission. In the case of malaria, which is transmitted only by anopheline mosquitoes, our group has indeed demonstrated that, in Senegal, IgG response to whole saliva extracts of *Anopheles gambiae* represents a marker of exposure to *An. gambiae* bites. In the objective to increase specificity of malaria exposure, we identified the SG6 salivary protein as *Anopheles* genus specific and antigenic in children living in a malaria endemic area. The objective of the present study was to determine whether the IgG response to the *An. gambiae* gSG6 protein, from its recombinant form to derived synthetic peptides, could be an immunological marker of exposure specific to *An. gambiae* bites. Specific IgG antibodies to recombinant gSG6 protein were observed in children living in a Senegalese area exposed to malaria. With the objective of optimizing *Anopheles* specificity and reproducibility, we designed five gSG6-based peptide sequences using a bioinformatic approach, taking into consideration i) their potential antigenic properties and ii) the absence of cross-reactivity with protein sequences of other arthropods/organisms. The five gSG6 peptides showed differing antigenic properties, with gSG6-P1 and gSG6-P2 exhibiting the highest antigenicity. However, a significant increase in the specific IgG response during the rainy season and a positive association between the IgG level and the level of exposure to *An. gambiae* bites was significant only for gSG6-P1. This step-by-step approach suggests that gSG6-P1 could be an optimal candidate marker for evaluating exposure to An. gambiae bites. Furthermore, a complementary study seems to indicate that gSG6-P1 could be a marker in low exposure area. This marker could be employed as a geographic indicator, like remote sensing techniques, for mapping the risk of malaria and especially in low Anopheles density conditions, where entomological studies are not sensitive enough (dry season, malaria according to altitude, urban exposure) or adequate (travelers, military corps). It could also represent a direct criterion of efficacy in evaluation of vector control strategies.

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CHARACTERIZATION OF HOST-SEEKING ACTIVITY OF *ANOPHELES MELAS* IN RESPONSE TO INDOOR-BASED ANTI-VECTOR INTERVENTIONS ON BIOKO ISLAND, EQUATORIAL GUINEA

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An intensive anti-malaria intervention focused on indoor application of residual insecticides and distribution of insecticide-treated bednets was initiated in 2003 to reduce the burden of malaria on the island of Bioko. Despite documented reductions in prevalence of *Plasmodium falciparum* infections throughout the island and sporozoite infection rates among the dominant vectors *Anopheles gambiae* s.s., *An. melas* and *An. funestus*, prevalence of malaria infection in humans remains high in some areas. Surveillance measures indicate *An. melas* is the most abundant anopheline vector in some of these infection foci. It has been reported from other areas in West- Africa that *An. melas* may have exophilic tendencies. This could explain the relatively high prevalence of malaria infections in these specific localities, despite the widespread application of indoor control measures. We are evaluating this possibility by characterizing the host-seeking behavior of *An. melas.* Indoor and outdoor landing counts and time-segregated trapping were conducted and will provide information on these operationally-relevant behaviors. If a preference of *An. melas* for outdoor blood feeding is demonstrated, this would support the need for supplementary interventions in areas where indoor control measures are not appropriate due to this vector's behavioral characteristics.

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INTEGRATION OF REPORTER TRANSGENES INTO *SCHISTOSOMA MANSONI* CHROMOSOMES MEDIATED BY PSEUDOTYPED MURINE LEUKEMIA VIRUS

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The recent release of draft genome sequences of *Schistosoma mansoni* has underscored the pressing need to develop functional genomics approaches for this significant human pathogen. The sequence information makes feasible genome-scale investigation of transgene integration into schistosome chromosomes. Retrovirus mediated transduction offers a means to establish transgenic lines of schistosomes, to elucidate schistosome gene function and expression, and to advance functional genomics approaches for these parasites. We investigated the utility of the Moloney murine leukemia retrovirus (MLV) pseudotyped with vesicular stomatitis virus glycoprotein (VSVG) for the transduction of *S. mansoni*. Schistosomes were exposed to VSVG-MLV virions after which genomic DNA and protein were extracted from transduced parasites. Southern hybridization analysis indicated the presence of proviral MLV retrovirus in the transduced schistosomes. Fragments of the MLV transgene and flanking schistosome sequences recovered using an anchored PCR-based approach demonstrated definitively that somatic transgenesis of schistosomes had occurred and revealed widespread retrovirus integration into the chromosomes. MLV transgenes inserted in the vicinity of genes encoding immunophilin, zinc finger protein and others, and also near endogenous retrotransposons such as the *fugitive* and *SR1*. Proviral integration appeared to exhibit primary sequence site specificity, targeting a gGATcc-like motif. Reporter luciferase transgene activity driven by the schistosome actin gene promoter was expressed in transduced schistosomules and adults. Luciferase activity appeared to be developmentally regulated in schistosomules with increased activity

after 1-2 weeks in culture. These findings indicate the utility of VSVG-MLV for transgenesis of *S. mansoni*, herald a tractable pathway forward towards germline transgenesis and functional genomics of parasitic helminths, and provide the basis for comparative molecular pathogenesis studies of retroviral integration into human compared with schistosome chromosomes.

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METABOLOMIC APPROACH TO ONCHOCERCIASIS DIAGNOSTICS

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Onchocerciasis, caused by the parasitic nematode *Onchocerca volvulus,* is one of several persistent neglected tropical diseases, afflicting nearly 37 million people worldwide with symptoms of blindness and acute dermatitis. With many eradication programs nearing two decades of continuous ivermectin treatment, novel approaches toward accurate, sensitive diagnostics are urgently needed. We hypothesized that given the complexity of chemical signals present within *O. volvulus* infected individuals, including worm excretory-secretory products, host immune response and angiogenic factors, onchocerciasis-specific small molecule metabolites can be isolated and used as chemical beacons for identification and characterization of infection. In this study, metabolite profiling involving liquid chromatography electrospray ionization time of flight (LC/ESI-TOF) mass spectrometry analysis was used to screen blood sera from *O. volvulus* infected individuals and uninfected controls for the identification of onchocerciasis disease biomarkers. Serum samples from greater than 300 individuals encompassing both the Eastern and Western hemispheres (e.g., Cameroon, Ghana, Liberia, Guatemala) were analyzed in multiple ionization modes, revealing a set of candidate biomarkers with a high degree of statistical significance ($p < 0.0001$). Visual analysis of this large data set by multivariate statistical methods has demonstrated the utility of the technology, and uncovered subsets of biomarkers that can be used as a "fingerprint" of onchocerciasis. Screening of sera with high-resolution TOF mass analysis has provided the mass accuracy (<5 ppm) necessary for empirical formula assignment of candidate compounds and initiated an exploration of the *in vivo* biological roles of these molecules. This research demonstrates the power of metabolomics toward the study of infectious disease, particularly in the identification of onchocerciasis specific biomarkers and drug targets and in providing a broader biochemical understanding of the disease.

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DEORPHANIZATION OF TWO NOVEL *SCHISTOSOMA MANSONI* G-PROTEIN COUPLED RECEPTORS (GPCRS), USING A YEAST EXPRESSION SYSTEM

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A bioinformatics analysis of the *Schistosoma mansoni* genome database has revealed many new G protein-coupled receptors (GPCRs) that share some sequence similarity with the biogenic amine receptor family but have no identifiable mammalian orthologues. These GPCRs have been designated as "orphan" receptors and may be schistosome-specific. We have cloned three of these receptors, one of which was previously shown to be activated by the biogenic amine, histamine (HA). Here we describe the functional analysis of the other two GPCRs (Smp_043290 and Smp_043340). To test for receptor activity, we have used a recently described yeast system, which is uniquely designed for functional expression of GPCRs, as reported prevuously, and can be easily adapted to high-throughput ligand screens. A first assessment of this system revealed that it was suitable for expression of *S. mansoni* GPCRs, even those receptors that could not be expressed in other heterologous systems, such as mammalian cells. Smp_043290 and Smp_043340 were both expressed in yeast and then tested with all known biogenic amines at various concentrations. Smp_043290 was activated by dopamine (DA) and, to a lesser extent, the structurally related catecholamines, adrenaline (A) and noradrenaline (NA), but not by any of the other biogenic amines tested. Further analyses with various classical agonists/antagonists showed the receptor was selective for dopaminergic ligands and their $IC5_0$ values were determined. The second GPCR, Smp_043340, was found to be preferentially stimulated by histamine (HA) and 1-methyl histamine, a common agonist of histamine receptors. Several histamine antagonists were tested and the results confirmed the specificity of this response. These results describe a powerful new system for de-orphanization of schistosome GPCRs and for large-scale screens of receptor blockers.

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DIFFERENTIAL PATTERNS OF PROTEIN EXPRESSION IN HEPATOSPLENIC SCHISTOSOMIASIS

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Schistosomiasis is a parasitic disease caused by infection with *Schistosoma spp.* helminths and is endemic in 74 countries, primarily in the equatorial region. The eggs laid by adult *S. mansoni* females in the microvasculature of the liver induce granulomatous lesions with a strong Th2-type immune response. In 90-95% of infected individuals, the inflammatory response to eggs is well-regulated, resulting in low to moderate pathology schistosomiasis. However, in 5-10% of patients, the inflammatory response can lead to hepatic and periportal fibrosis, portal hypertension, and portal shunting, resulting in hepatosplenic schistosomiasis. We wished to identify differentially expressed marker proteins associated with the different pathologies of chronic schistosomiasis by using the CBA/J mouse model. In this model 20% of infected mice spontaneously develop severe hypersplenomegaly syndrome (HSS) by 20 weeks of infection while the remaining 80% develop moderate splenomegaly syndrome (MSS). Using Two Dimensional Differential In-Gel Electrophoresis (2D-DIGE), we identified 406 distinct changes induced by schistosome infection, with 172 of these distinct changes specifically associated with HSS. Hepatic levels of collagen isoforms, annexin5, carbonic anhydrase III, major urinary protein, sarcosine dehydrogenase, glutathione S-transferase mu1 and transferrin were specifically altered during HSS. The findings indicate that the expression patterns of liver proteins during MSS and HSS are distinct. As a result, they have potential for use as diagnostic markers of hepatosplenic pathology during schistosome infection. Early detection of hepatosplenic disease would help identify patients in need of closer monitoring for infection and treatment to prevent life-threatening disease.

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HIGH THROUGHPUT QUANTITATIVE ANALYSIS OF ICAM-1 BINDING TO 3D7 DUFFY-BINDING LIKE (DBL) DOMAINS

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ICAM-1 supports adhesion of *Plasmodium falciparum*-infected erythrocytes (IEs), and has been implicated as an endothelial receptor for IE sequestration during cerebral malaria. ICAM-1 binds *in vitro* to DBLβ-C2 domains of the PfEMP1 proteins (a variant antigen family), including 6 out of 21 recombinant DBLβ-C2 domains from the IT4 genome that were recently surveyed; all DBLβ-C2 domains that bound ICAM-1 in that study belong to group B and C PfEMP1 proteins. We cloned and expressed a complete collection of 16 DBLβ-C2 domains from the 3D7 parasite line, and analyzed these for ICAM-1 binding in a high throughput quantitative assay using the BioPlex platform. Only one domain showed strong and specific binding to ICAM-1, and this was encoded by the group A PfEMP1 protein PF11_0521. Two other DBL2β-C2 domains (from groups A and

A/B) bound to ICAM-1 weakly. Most of the residues identified previously as important for ICAM-1 binding are preserved in DBL2β-$C2_{PF11\_0521}$, but less so in the other 15 domains. The binding of ICAM-1 to DBL2β-$C2_{PF11\_0521}$ was almost completely inhibited by pre-incubation of the domain with pooled serum samples obtained from adults living in endemic areas of east Africa, but not by pre-incubation with non-immune serum from the US. The reversal of ICAM-1 binding was much less efficient than blocking, indicating the strong association between DBL domain and ICAM-1 molecule. These data contribute further into understanding of PfEMP1-ICAM-1 interactions, and our high throughput approach will significantly accelerate studies of binding and binding-inhibition between PfEMP1 domains and various cellular ligands. We are presently using this platform to measure anti-adhesion antibody levels in Tanzanian children, and to relate these functional antibodies to malaria outcomes.

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CHARACTERIZATION OF A DOMESTIC TRANSMISSION FOCUS OF AMERICAN CUTANEOUS LEISHMANIASIS IN RURAL COLOMBIA

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Between 2003 and 2004, a large outbreak of American cutaneous leishmaniasis occurred in Chaparral County, Tolima, located in a rural mountainous area in Central Colombia. Although no cases were reported here in the past, the initial outbreak documented 2,400 cases. Prevalence among the 69 (157) townships with reported cases in Chaparral varied from 1 to 95%. Thirty percent of the cases were women and children, indicating domestic transmission. The potential risk factors associated with domestic transmission were evaluated in a township with high prevalence (Agua Bonita,74%) and low prevalence (Irco dos Aguas, 1%). Sandfly and wild mammal trapping was undertaken in a concentrated transect design within a 100 m radius from selected houses. For sand fly capture, CDC light traps were placed indoors (n=1) and outdoors (n=16) during three consecutive nights, and for animal capture, Sherman (n=72) and National (n=16) traps were oriented around the house for 4 nights per trapping period. Epidemiological and land use data were recorded at each house to capture environmental parameters associated with leishmania transmission. A great difference between the two townships was seen in sand fly composition and abundance; 1,446 sandflies were capture in Agua Bonita (12 houses) compared with 80 in Irco dos Aguas (10 houses). The most abundant species was *Lutzomyia Longiflocosa*,70% in Aqua Bonita and 49% in Irco dos Aguas. Ten and 7 sand fly species were identified from the two sites, respectively. Eighteen mammals were captured in Agua Bonita and 14 in Irco dos Aguas. Two animals (*Didelphis marsupialis* and *Marmosops impavidus*) tested positive for Leishmania by kDNA PCR at the high endemicity site. No lesions were observed in wild and domestic dogs. Prevalence of infection in the inhabitants of the houses sampled was 45% in Agua Bonitas and 0% in Irco dos Aguas. Although peridomestic land use was similar in the two sites (coffee and scrub brush), adjacent forests occurred only in Agua Bonita. In conclusion, the high risk of domestic leishmania transmission was associated with high densities of a relative uncommon vector species, *L. longiflocosa*, and the proximity of tropical forest.

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MOLECULAR SYSTEMATICS OF THE BARBIROSTRIS SUBGROUP AND HYRCANUS GROUP OF THE GENUS *ANOPHELES* IN SOUTHEAST ASIA

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The *Anopheles barbirostris* subgroup includes six mosquito species that are almost identical in adult morphology: *Anopheles barbirostris*, *An. campestris, An. donaldi, An. hodgkini, An. pollicaris* and *An. franciscoi*. Some of these species are implicated in the transmission of malaria and filariasis in Southeast Asia. Specimens of the Barbirostris Subgroup are also confused in the field with those from the Hyrcanus Group. Such mistakes in identification are an obstacle to the implementation of effective vector control. A phylogenetic analysis of 756 bp of Cytochrome Oxidase I (COI) in the mitochondrial genome revealed five clades within the Barbirostris Subgroup. The same clades were shown using Neighbour Joining and Maximum Parsimony trees, although internal branch points were different. A parsimony-based nested clade analysis also showed five separate networks, congruent with the phylogenetic clades. Analysis of the nuclear rDNA ITS2 region revealed five clades, congruent with those from the COI analysis. In all specimens of the Barbirostris Subgroup, ITS2 was >1.5kb, the largest so far recorded in any insect. The extreme length of the ITS2 was a most interesting finding and resulted from the presence of four or five internal repeats of c.220bp within the ITS2, each repeat comprised of two c.110 bp sub-repeats of variable homology. Within the Barbirostris Subgroup, clade I, II and III were morphologically compatible with *Anopheles barbirostris Van der Wulp,* suggesting that *Anopheles barbirostris Van der Wulp* is a species complex comprising at least 3 species (clades I, II and III). There is limited information on host preferences for clades I and II, but clade III appears to be zoophilic. Clade V was identified as the anthropophilic species *Anopheles campestris.* Clade IV is a zoophilic species with morphological characters intermediate between those of *An. campestris* and *An. barbirostris*, with which it is found in sympatry in Sa Kaeo (Thailand). Clade IV appears to be a new species.

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CHROMOSOMAL INVERSIONS, NATURAL SELECTION AND ADAPTATION IN THE MALARIA VECTOR *ANOPHELES FUNESTUS*

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Chromosomal polymorphisms, such as inversions, are presumably involved into the rapid adaptation of populations to local environmental conditions. Reduced recombination between alternative arrangements in heterokaryotypes might protect sets of co-adapted genes promoting ecological divergence and assortative mating, eventually leading to reproductive isolation and speciation. The adaptive significance of polymorphic paracentric chromosomal inversions has been evidenced in a number of Diptera such as flies and mosquitoes. Through a comparative analysis of chromosomal inversions and microsatellite markers polymorphisms, we hereby present biological evidence that strengthens this view in the mosquito, *Anopheles funestus* s.s, one of the most powerful and widespread malaria vector in Africa. Specimens were collected across a wide range of geographical, ecological and climatic conditions in Cameroon. We observed a sharp contrast between population structure measured at presumably neutral microsatellite markers and chromosomal inversions. Microsatellite data detected only a weak signal for population structuring among geographical populations (Fst<0.013). By contrast, strong differentiation among ecological zones was revealed by chromosomal inversions (Fst>0.190). Using standardized estimates of Fst, we show that inversions behave at odds with neutral expectations, strongly suggesting a role of environmental selection in shaping their distribution. Using Canonical Correspondance analysis, we demonstrate that heterogeneity in eco-geographical variables (EGVs)

measured at specimens sampling sites explain 89% of chromosomal variance in *An. funestus*. These results are in agreement with a role of chromosomal inversions in ecotypic adaptation in this species. We argue that this widespread mosquito represents an interesting model system for the study of chromosomal speciation mechanisms and should provide ample opportunity for comparative studies on the evolution of reproduction isolation and speciation in major human malaria vectors.

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DOES HEMOLYMPH FLOW DRIVE MALARIA SPOROZOITE MIGRATION THROUGH THE MOSQUITO HEMOCOEL?

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Mosquitoes are obligate vectors of malaria parasites. For successful transmission to the vertebrate host, malaria sporozoites must migrate from the mosquito midgut to the salivary glands. It has long been considered that this migration is parasite-driven by means of active motility. However, our initial experiments showed that after release from oocysts, sporozoites are swept toward the posterior of the insect, enter the dorsal vessel, and traverse the length of the mosquito at speeds much faster than can be accounted for by sporozoite motility alone. Based on these data, we hypothesize that sporozoites passively migrate through the hemocoel to the vicinity of the salivary glands using the mosquito's circulatory system, and once in the anterior ventral thorax, active sporozoite motility allows them to locate and invade the salivary glands. Given the above data, and in prelude to continuing studies on sporozoite migration, we have been conducting a comprehensive assessment of the mosquito circulatory system by tracking the movement of inoculated fluorescent particulates throughout the hemocoel of the malaria vector *Anopheles gambiae*. Here, we will present data that corroborate the general anatomy of the mosquito dorsal vessel, characterize the mechanics of dorsal vessel contraction, unveil novel hemolymph channels, and describe in detail hemolymph flow in mosquitoes. Data on absolute rates of flow speed, acceleration, and direction in different regions of the insect will also be presented, and the general implications of hemolymph flow on *Plasmodium* sporozoite migration will be discussed.

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IDENTIFICATION OF THE BARRIERS PREVENTING SUCCESSFUL DEVELOPMENT OF *PLASMODIUM FALCIPARUM* IN *CULEX* MOSQUITOES

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Culex mosquitoes are extremely prevalent across the malarious world serving as important vectors for a number of infectious diseases and are primary vectors of avian Plasmodia, yet they are known to be totally refractory to human Plasmodia species. This is especially fascinating because many species, such as *Culex quinquefasciatus*, are highly anthropophilic and consequently, have repeatedly been exposed to human Plasmodia. However, despite extensive and prolonged exposure, human malaria parasites have never adapted to exploit *Culex* mosquitoes. Thus, there is something unique about the *Culex* physiology that prevents the successful development of human Plasmodia, but to date the exact processes remain unknown. Here, we compare the developmental success of *Plasmodium falciparum* in two African mosquitoes (*Cx. quinquefasciatus* and *An. gambiae*) in key events: fertilization, midgut invasion, oocyst maturation, sporozoite invasion and accumulation in salivary glands. We identify successful fertilization events and subsequent ookinete invasion in *Culex* mosquitoes. However, transformation of ookinetes to oocysts does not occur, suggesting that a developmental barrier exists at the midgut stage preventing successful parasite development. We investigate whether bypassing the midgut allows successful development or whether multiple barriers are responsible for *P. falciparum* refractoriness in *Cx. quinquefasciatus.*

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ENVIRONMENTAL FACTORS INFLUENCE *CULEX PIPIENS QUINQUEFASCIATUS* (DIPTERA: CULICIDAE) SUSCEPTIBILITY TO WEST NILE AND ST. LOUIS ENCEPHALITIS VIRUSES

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Three age cohorts from two *Culex pipiens quinquefasciatus* colonies were fed blood meals containing a low or high dose of St. Louis encephalitis virus (SLEV) or West Nile virus (WNV), and each group was held at two different extrinsic incubation temperatures (EITs) for 13 days. The vector competence effects of age, EIT, and dose were complex with interactions between them for WNV and SLEV. The effect of the environment showed differences depending on the virus and colony. Susceptibility of *Cx. p. quinquefasciatus* to both viruses increased with both increasing virus dose and EIT, except for WNV in one of the colonies where infection rates were inversely related to dose. Mosquito age had an effect on vector competence for both viruses, but the effect differed depending on virus, colony, EIT and dose. The relationship between infection and dissemination rates for both viruses changed between colonies, and was dependant on age, EIT and dose where the proportion of disseminated infections relative to infected mosquitoes varied. We observed that the effects of the environment change depending on the virus and the vector strain, here two different laboratory colonies. The complex effects of the environment must be considered in laboratory studies of vector competence that are used to generalize to nature where the extent of the genetic and environmental variation controlling vector competence is largely unknown.

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BLOOD FEEDING IN MOSQUITOES PROMPTS EXPRESSION OF TWO HEAT SHOCK PROTEINS

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Blood feeding in mosquitoes poses two significant stresses: huge changes in size that alters internal pressure, and increases in internal temperature, up to 10°C within 1 minute. In this study, we evaluated expression of two heat shock protein genes (Hsp70 and Hsp90) in three mosquito species (*Culex pipiens*, *Anopheles gambiae*, and *Aedes aegypti*) to see if feeding elicited up-regulation of these genes. High amino acid homology between species was evident for the portions of the genes used for northern blot hybridizations. When exposed to heat shock at temperatures equivalent to the changes that occur during blood feeding (37°C for 5 min), the genes for Hsp70 and Hsp90 where highly up-regulated in all three mosquito species, suggesting that feeding may evoke a stress response. Expression of Hsp70 in response to blood feeding increased within one hour in all three species, and expression persisted above normal background for at least 6h. Blood feeding caused a higher expression of Hsp90 in *Cx. pipiens* and *Ae. aegypti*, but failed to change expression in *An. gambiae*. Our preliminary results suggest that engorging the mosquitoes by injecting blood held at room temperature does not induce Hsp expression, indicating that the high temperature of blood is the critical feature evoking expression. The responses of Hsp70 and Hsp90 to blood feeding are not identical among the three species: responses of *Cx. pipiens* and *Ae. aegypti* are more similar to each other, while the response of *An. gambiae* is more distinct (only Hsp70), suggesting a possible Culicine and Anopheline difference. Our current goal is to understand how heat shock proteins are regulated during blood feeding.

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STUDIES OF *SCHISTOSOMA MANSONI* POPULATION STRUCTURE BY MICROSATELLITE ANALYSIS OF AGGREGATED SAMPLES

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Many parasite populations are difficult to sample since they are distributed non-uniformly in several host species and are often not easily collected from living individuals. One approach is to passage parasite eggs through laboratory hosts in order to amplify them, but small sample size and selection in these hosts may distort the representation of the population. For the parasite *Schistosoma mansoni*, the simplest and most representative sample may be found aggregated in the stool eggs of infected individuals. Previously, we have described 7 microsatellite loci from *S. mansoni* and demonstrated that microsatellite allele frequencies can be accurately estimated from DNA pools of *S. mansoni* clones. Here, we show that 9 reproductively isolated populations from laboratory strains of *S. mansoni* can be identified by characteristic patterns of allele frequencies. F_{ST} values, a measure of genetic differentiation, were calculated from relative allele frequencies by the Arlequin software package. The weighted average F_{ST} across these lab populations was 0.651. The microsatellite loci were also used to genotype *S. mansoni* eggs isolated from stools of infected individuals from one location in Kenya and two locations in Brazil. The results produced were consistent with the geographic distribution of the samples. The Kenyan population had a weighted average F_{ST} across all loci tested of 0.029, while that of the Brazilian populations was 0.057. However, division of the Brazilian population into its two component locales yielded F_{ST} values of 0.031 in Itaquara (a small municipality) and 0.066 in Salvador (a larger city). In addition, aggregated samples obtained directly from stool can be used to calculate the important population indices N_e (effective population size) and m (migration rate). Microsatellite analysis of these kinds of samples will enable us to effectively and efficiently probe the genetic structure of *S. mansoni* populations in response to repeated large-scale chemotherapy as well as to examine the effects of migration and microgeographic influences.

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THE EFFECT OF PRAZIQUANTEL TREATMENT ON THE GENETIC DIVERSITY OF *SCHISTOSOMA MANSONI* INFECTIONS IN PRIMARY SCHOOL CHILDREN WITHIN MAYUGE DISTRICT, UGANDA

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The Ugandan Government, with assistance from the Schistosomiasis Control Initiative, aims to reduce *Schistosoma mansoni* infection intensity, prevalence and associated morbidity through mass treatment with praziquantel (PZQ). Knowledge on how the genetic structure of *S. mansoni* populations may change in response to such PZQ pressure may have important implications for the success of this and other control programmes. Data will be presented on miracidial samples of *S. mansoni* collected from children at three primary schools in Mayuge district, Uganda. Samples were collected over three years, from the same individual children where possible, providing miracidia from children pre-treatment (PZQ-naïve infections) and from those that have received up to six PZQ treatments. Population genetic analyses using seven microsatellite loci were carried out to investigate the effect of PZQ treatment on heterozygosity, allele number and population structure both within and between individuals and schools. Population structure at the individual human host level was not clearly apparent, but structuring was observed between all samples collected pre-treatment in comparison to those collected from subsequent time points after PZQ. Allele numbers were reduced by PZQ exposure in the treated population as a whole, including the newly recruited PZQ-naïve populations, as PZQ coverage of the community continued over three years. This suggests both direct and indirect effects of PZQ selective pressure on the local parasite population. Such results could have contrasting implications for the future establishment of PZQ resistance. Firstly, altered parasite genetic diversity, induced through PZQ exposure could be a precursor to the evolution of resistance with the risk that alleles associated with resistance could become fixed within this reduced population size. However, such reduced diversity may become so low, that the *S. mansoni* population could be less capable of responding to coevolution, including possible costs associated with resistance.

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INTEGRATION OF LASER MICRODISSECTION AND MICROARRAY ANALYSIS FOR TISSUE SPECIFIC GENE EXPRESSION PROFILES OF *SCHISTOSOMA JAPONICUM*

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We have integrated precise dissection and isolation of specific tissues of the *Schistosoma japonicum* by Laser Microdissection Microscopy with the broad gene expression analysis tools of a microarray. This has allowed us, for the first time with any parasite, to identify tissue-specific gene expression profiles. We examined the following tissues from the adult female parasite: gastrodermis, ovary and vitelline. These tissues were selected due to their well delineated morphology and the critical biological functions of nutritional uptake and fecundity they provide. From these "enriched" tissue preparations, isolated total RNA was hybridised to a custom microarray containing 19,222 probes to schistosome contiguous sequences. Genes representing the specific biological functions of each tissue, such as red blood cell digestion, egg shell formation and oocyte development were identified as relatively over expressed. Our method also allowed us to identify a multitude of unannotated genes that are highly expressed in each tissue type. These genes may represent key but as of yet uncharacterised components of important biological functions of this human parasite.

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THE IDENTIFICATION OF PUTATIVE MOLECULAR PATHWAYS REGULATING *SCHISTOSOMA MANSONI* MIRACIDIAL TRANSFORMATION BY THE USE OF A HIGH-THROUGHPUT SMALL-MOLECULE SCREEN

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The transition from the free-living miracidia stage to the parasitic sporocyst stage is associated with a number of physiological, morphological and biochemical changes. We have utilized a small molecule screen to identify putative pathways involved the transformation of *Schistosoma mansoni* miracidia. The Sigma-Aldrich Library of Pharmacologically Active Compounds (LOPAC) contains 1280 chemical compounds. The library contains all major drug target classes including well-characterized enzyme inhibitors, antibiotics, cell-cycle regulators, apoptosis inducers and GPCR ligands. A preliminary screen of 80 compounds was used to optimize drug and carrier (DMSO) concentrations and establish normal baseline controls for larval development. Freshly hatched miracidia were concentrated on ice and resuspended in complete Biomphalaria glabrata embryonic cell-line media (cBGE). 120 miracidia were then placed into each well of a 96-well plate containing a single compound per well and 16 wells per plate containing DMSO carrier only (control wells). Due to a limit on parasite numbers, the experiment was run on three separate occasions in which 480, 480 and 320 compounds were screened on freshly hatched miracidia. In control wells, miracidia commenced shedding

of ciliary plates within 6 hours and were typically fully transformed within 12 hours, while drug-associated phenotypes included no transformation, late transformation (between 12-24 hours), very late transformation (between 24-48 hours), low transformation (25-50% transformed within 48 hours) and very low transformation (5-25% transformed within 48 hours). Of the 1280 compounds tested, 55 exhibited an observable phenotype. The majority transformation-inhibiting phenotypes were caused by D2 dopamine antagonists, calcium channel blockers, serotonin reuptake inhibitors and calmodulin inhibitors. Other chemical compounds also exerting adverse effects on miracidia transformation included a phosphatase inhibitor, a PKC signaling inhibitor and a cyclin dependent kinase (CDK) inhibitor.

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NEW SCHISTOSOMIASIS DRUGS

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Schistosomiasis is a neglected tropical disease with more than 200 million people infected and a yearly death toll of >20.000 people. Current treatment of schistosomiasis is solely dependent on the drug Praziquantel (PZQ). PZQ is very advantageous in terms of safety, efficacy and cost-of-goods (COG). However, the dependence on one drug for such a high incidence disease is very concerning regarding the possible scenario of occurrence of resistance. There have been several reports already on PZQ resistant schistosomes in the field as well as in the laboratory. The discovery and development of alternative back-up drugs is therefore of uttermost importance. However future schistosomiasis drugs have to be measured against PZQ. Recently, we have discovered the shortest and most convergent access to PZQ using a chemical technology called multicomponent reaction (MCR). This approach reduces the number of production steps of PZQ considerably thus has the potential to reduce the price for the drug. It is well known that the COG is a critical factor for disease treatment in third world countries. In addition this new MCR allows for the rapid generation of novel PZQ derivatives. More molecular sites of PZQ than ever can now be changed at the same time and a theoretical chemical space of several million compounds can be investigated. The most successful and economic strategies to overcome drug resistance is the derivatisation of the prototypical parent compound. Classical and highly successful examples are the β-lactame and macrolide antibiotics against bacterial infections. They are still the mainstay of infectious disease control even after more than half a century of use. In addition we have access to PZQ resistant field isolated schistosomes for testing and preclinical Hamster disease models for efficacy and safety studies. We will present the new synthesis strategy, first Praziquantel derivatives and biological screening results.

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RANDOMIZED DOUBLE BLIND CLINICAL TRIAL, COMPARING THE EFFECTIVENESS OF ARTESUNATE+SULFAMETHOXYPYRAZINE/PYRIMETHAMINE VERSUS PRAZIQUANTEL IN THE TREATMENT OF *SCHISTOSOMA HAEMATOBIUM* IN MALIAN CHILDREN

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This study aimed to determine the relative effectiveness and safety of Artesunate+Sulfamethoxypyrazine-Pyrimethamine (AS+SMP), a novel ACT in fixed dose combination, administered over 24 h identical to the current malaria treatment, in comparison to Praziquantel to treat *Schistosoma haematobium* in children from 6 to 15 years old living in a peri-urban area of Bamako. The study had a double blind set up. Parasitological assessments were done on days -1, 0, 28 and 29, detection of haematuria, haematology and biochemistry on days 0 and 28, and clinical examinations on days 0, 1, 2, and 28. Eight hundred children (400 for each treatment arm) were included. The cure rate was 97.7% in Praziquantel group compared to 81.4% in As+SMP group, (chi-square=55.26, p=0.000). The geometric mean of eggs counted under Praziquantel treatment (1.801) was less than the geometric mean under As+SMP treatment (2.969), (p=0.000). Eggs reduction rate was 95.6% in the Praziquantel arm compared to 92.8% in the As+SMP treatment arm. Mild abdominal pain and vomiting were the common adverse events related to medication for both treatment arms: 0.5% (2/400) in As+SMP treatment group compared to 2.3% (9/399) in Praziquantel group (p=0.033). The study demonstrates that As+SMP, administered in its dosage for curing malaria, is effective for treating *S. haematobium*. It is easily used and safe, with less adverse events in the As+SMP treatment arm in comparison to the praziquantel group.

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MOLECULAR AND BIOCHEMICAL CHARACTERIZATION OF *SCHISTOSOMA MANSONI* CAMP-DEPENDENT PROTEIN KINASE (PKA): A POTENTIAL NEW DRUG TARGET

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Protein kinases represent novel drug targets for the treatment of diseases caused by eukaryotic pathogens such as helminth parasites. We therefore explored the anti-parasite potential of targeting cAMP-dependent protein kinase (PKA) enzymes in *Schistosoma mansoni*. Examination of the *S. mansoni* genomic sequence database (SchistoDB) identified sequences of four putative PKA genes in the *S. mansoni* genome. Using reverse transcriptase-PCR and RACE, transcripts from two distinct PKA genes were identified in adult *S. mansoni* cDNA, one of which is expressed as two distinct splice variants that utilize different exons at the 5′ end. Western blot analysis of adult *S. mansoni* proteins, using a polyclonal antibody directed against conserved sequences of the PKA α catalytic subunit, identified several protein species with expected molecular weights of PKAs. PKA activity was detectable in adult *S. mansoni* lysates at various nanogram concentrations, confirming that *S. mansoni* worms express active PKAs. Further, schistosome PKA activity was readily inhibited by commercially available PKA inhibitors. Finally, three PKA inhibitors were shown to have schistosomocidal effects on adult worms *in vitro* at various micromolar concentrations within four hours to six days. These data suggest that inhibitors of PKA and perhaps other protein kinases have potential as novel chemotherapeutics for the treatment of schistosomiasis and other helminth infections.

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ANTI-MALARIAL ACTIVITY OF MIRINCAMYCIN AND ITS ANALOGS *IN VITRO* AND IN AN *IN VIVO* PRESUMPTIVE CAUSAL PROPHYLACTIC MOUSE MODEL

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Historical data suggests that mirincamycin, a clindamycin analog, may be superior to clindamycin against malaria liver stages in animal models and similarly active against blood stages of malaria. The possibility of exoerythrocytic activity of mirincamycin encourages exploration into this class as radical cure agents. A similarity search of the Walter Reed Army Institute of Research chemical inventory system identified over 50 mirincamycin analogs. Compounds with sufficient quantity available were screened for *in vitro* activity against several strains of *Plasmodium falciparum*. Clindamycin, mirincamycin racemate (65% trans-, 35 % cis-), and mirincamycin cis- and trans- isomers, were screened for activity in the Walter Reed Army Institute of Research "*Plasmodium berghei - Anopheles*

dirus Sporozoite -Mice Malaria Model for Screening Exoerythrocytic Antimalarial Drugs" using 3 day subcutaneous dosing. Efficacy was determined by number of mice with delayed onset of parasitemia and mice that were protected and remained malaria free by blood smears follow-up for 31 days and no malaria gross lesions at necropsy on day 31. Lincomycin and 6 other analogs are currently undergoing similar screening. 34 analogs were screened in a 72 hour *in vitro* assay against blood stage *P. falciparum.* Twelve showed IC_{50}'s similar to or better than clindamycin, with one as low as 30 ng/ml. Cis-mirincamycin protected 5/5 mice at all dose levels from 1.1 to 40 mg/kg/d x 3 days. Racemic and trans- mirincamycin prevented malaria in 3/5 mice and delayed patency in 2/5 mice at the lowest dose tested of 1.1 mg/kg/day x 3 days, and protected all mice at dose regimens of 3.3 to 40 mg/kg/day x 3 days. There were no toxicities found at any dose levels tested. Clindamycin showed a delay in patency in 5/5 mice at doses of 10 mg/kg/d x 3 days, and 3/5 cures and 2/5 delayed patency at 40 mg/kg/d x 3 days with no toxicity seen. Cis-mirincamycin shows 100% causal prophylactic activity in a mouse model at the lowest dose tested, 1.1 mg/kg/d x 3 days. Racemic and trans-mirincamycin show 60% causal prophylactic activity at the lowest dose tested, 1.1 mg/kg/d x 3 days, while clindamycin is only active at higher doses. Assessment of lower and single doses of mirincamycin is ongoing. Reassessment in a Rhesus radical cure model and assessment of promising analogs is planned.

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MALARIA-INFECTED MICE ARE CURED BY NEW TRIOXANE DIMERS

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Malaria is a leading cause of death and disease within the developing world affecting people who live in tropical climates the most. Artemisinin is a naturally occuring product from a Chinese plant that has been known for centuries to have antimalarial activity and has shown little to no resistance in modern usage. Short half-life coupled with poor bioavailability have prompted the search for a better artemisinin-based drug. We have rationally designed and easily synthesized (4 or 5 high-yielding steps from artemisinin) a series of new artemisinin-derived trioxane dimers. Several of these new C-10 carbon dimers completely cure malaria-infected mice at low oral doses without any observed toxicity or behavioral change. An update will be given on SAR generalization and on selection of a trioxane dimer antimalarial drug development candidate.

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OPTIMIZATION OF DUAL-FUNCTION ACRIDONE ANTIMALARIALS: IMPROVED EFFICACY AND SYNERGY WITH PIPERAQUINE

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Preventing and delaying the emergence of drug-resistance is an essential goal of antimalarial drug development. Monotherapy and highly mutable drug targets have each facilitated resistance, and both are undesirable in effective long-term strategies against multi-drug resistant (MDR) malaria. We have previously reported the discovery of dual-functional antimalarial acridones as a new strategy to maximize drug potential and sustainability. These acridone derivatives with an innovative design that merges intrinsic potency and resistance-counteracting features into a single molecule possess unique synergy with quinine (QN) in both QN-sensitive and QN-resistant parasites, in addition to verapamil-like chemosensitization with chloroquine (CQ) and amodiaquine in MDR parasites. The latest lead candidate, T16.5, demonstrates improved efficacy both *in vitro* and *in vivo*, with an ED_{50} value of 17mg/kg/day after oral administration in a 3-day regimen against patent infection with *Plasmodium yoelii* in mice. The newly optimized derivative also demonstrates synergy in combination with piperaquine against quinoline-sensitive and MDR *P. falciparum*. Details of the design, chemistry, structure-activity relationships (SAR), safety, metabolic studies, and assessment with PfCRT mutant lines will be presented.

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RANDOMIZED CROSSOVER TRIAL TO EXAMINE THE SAFETY AND PHARMACOKINETICS OF 2100 MG DOSE OF AQ-13 AND THE FOOD EFFECT ON ITS BIOAVAILABILITY

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AQ-13 is a candidate antimalarial active against chloroquine- (CQ) resistant *Plasmodium falciparum.* AQ-13 doses up to 1750 mg have been found safe and to have pharmacokinetics similar to CQ in human volunteers. We are currently performing a randomized crossover trial to examine the safety of a 2100 mg dose of AQ-13 and the effect of a standard fatty meal on its bioavailability. This crossover trial design increases the efficiency of the study by allowing each volunteer to serve as their own control. Twelve healthy volunteers are being randomized to receive the drug on an empty stomach or after a standard fatty meal while being monitored as inpatients. After an outpatient washout period of 8 weeks (4X the terminal elimination rate, $t_{1/2}$, of AQ-13), volunteers are re-admitted to receive a second 2100 mg dose of AQ-13 after a fatty meal or on an empty stomach in reverse order. Preliminary analysis of adverse events (AE) data from 11 volunteers after the first 2100 mg AQ-13 dose indicates that headache was the most frequent AE (8/11), followed by dizziness (5/11) and gastrointestinal AEs (nausea 5/11; vomiting 4/11; loss of appetite 3/11 and diarrhea 3/11). Other AEs included weakness (2/11), difficulty focusing (2/11) and rash (1/11). These proportions are unstable due to the small sample size, however, these AEs were similar to those observed at lower doses and resolved rapidly. Based on Holter monitoring data from 8 volunteers (beginning before the first dose and concluding 24 hours after the last dose on day 3), the 2100 mg dose of AQ-13 prolonged the cardiac QT interval by 23 ms on average (range: 0-52 ms). At the 2 week follow-up, the QT interval had returned to baseline in all volunteers except one, in whom it was still 20 msec greater than baseline. As with lower doses of AQ-13, no volunteers experienced any cardiac AEs. The data on the fatty meal effect will be presented at the time of the meeting after breaking the randomization code.

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ASSESSMENT OF THE CAUSAL PROPHYLACTIC ACTIVITY OF DB289 IN HEALTHY VOLUNTEERS CHALLENGED WITH *PLASMODIUM FALCIPARUM*

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The causal prophylactic antimalarial activity of DB289 (pafuramidine), an experimental prodrug of the active metabolite DB75, was evaluated in a randomized, double-blind, placebo-controlled trial. Non-immune healthy volunteers were randomly assigned to one of three treatment arms: seven received a single dose of 100 mg pafuramidine 8 days before challenge (Day -8); seven received the same dose one day before challenge (Day -1) and five received placebo. This dosing regimen was designed to exclude the suppressive activity of DB75 at the initiation of the erythrocytic stage in the parasites' lifecycle, and to maintain adequate hepatic concentrations of DB75 during the hepatic stage development. Sixteen of 19 volunteers were then challenged by the bites of *Plasmodium falciparum*-infected

Anopheles gambiae. Efficacy, safety and pharmacokinetics of DB289 were evaluated in an outpatient setting. Patent malaria was identified by the detection of parasitemia by one or more of four diagnostic methods: light microscopy, acridine orange microscopy (QBC), PCR and culture. Fifteen of the 16 challenged volunteers developed patent parasitemia and one in the Day -8 arm did not. There were no significant differences in the frequency and severity of adverse effects between the placebo and the DB289-treated group. Plasma concentrations of DB75 achieved in this study were lower than previous observations. We conclude that a single dose of 100 milligram pafuramidine does not protect non-immune individuals against *P. falciparum* and shows no evidence of causal prophylactic activity. No conclusions about suppressive prophylactic activity of DB289 can be drawn from this study.

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A PHASE II, RANDOMIZED, OPEN-LABEL, DOSE-RANGING STUDY OF GMP INTRAVENOUS ARTESUNATE FOR OPTIMIZING PARASITE CLEARANCE IN UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA

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Parenteral antimalarial drugs are indicated for the treatment severe malaria and when oral therapy cannot be given. The goals of treatment are prevention of death and reduction of morbidity. Rapid reduction in parasite clearance appears to be a good surrogate for these endpoints. While artemisinins is known to clear malaria more rapidly than other antimalarials, the available data on optimal dosing for pharmacodynamic effect is difficult to interpret and does not include the higher or multiple daily doses that are now in common clinical usage. As part of the US Army's goal to secure FDA approval of a GMP formulation of intravenous artesunate, a multi-center, phase II, open-label, dose-ranging study in Thai adults and African children and adults was conducted from May to December 2007. One hundred study subjects from Thailand (n=33) and Kenya (n=67) with uncomplicated *Plasmodium falciparum* malaria and parasite densities >5000 asexual parasites/µL were randomized into one of four intravenous artesunate treatment arms: 1.2 mg/kg once daily, 2.4 mg/kg once daily, 2.4 mg/kg initially and at 12 hours and then daily, or 4.8 mg/kg once daily. All regimens were given for 3 days. Patients remained in the hospital for the initial 4 days with frequent parasite monitoring using standardized quantitative microscopy and were then followed up as outpatients weekly until day 28. Follow-on oral therapy with mefloquine 25 mg/kg in 2 split doses (Thailand) or atovaquone proguanil HCL (Kenya) was given to all patients following the completion of artesunate treatment to ensure cure. Safety, tolerability and efficacy data were collected and pharmacodynamic endpoints including parasite reduction ratios and parasite and fever clearance were determined, and compared between treatment arms. These data will be presented and discussed. The results of this study will help define optimal dosing regimens for future testing in severe malaria.

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CHLORPROGUANIL-DAPSONE-ARTESUNATE VS. ARTEMETHER-LUMEFANTRINE: A RANDOMISED, DOUBLE-BLIND PHASE III TRIAL FOR THE TREATMENT OF ACUTE, UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA IN AFRICAN CHILDREN AND ADOLESCENTS

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The objective of this study was to compare chlorproguanil-dapsone-artesunate (CDA) and artemether-lumefantrine (AL) efficacy and safety in acute uncomplicated *Plasmodium falciparum* malaria. Haematological safety in glucose-6-phosphate dehydrogenase (G6PD)-deficient patients was studied carefully. Non-inferiority of CDA to AL for efficacy was tested in a randomised parallel-group, double-blind, double-dummy study conducted at 11 sites in 5 African countries. Patients (>=1 to 14 years) were randomised (2:1) to CDA 2.0/2.5/4.0 mg/kg od x 3 days or six-dose AL over 3 days. G6PD genotype and phenotype were determined. A haematological safety composite endpoint was defined as haemoglobin (Hb) drop of >=40 g/L or >=40% vs. baseline or Hb <50 g/L or blood transfusion. 1372 subjects were randomised; 914 to CDA, 458 to AL. Baseline demographic/clinical characteristics were similar between treatment groups: mean age 4.1 (1-14) years, weight 16.1 (8-62) kg, 51% male. Parasitological cure rate (polymerase chain reaction-corrected) at Day 28 for the per-protocol population (primary efficacy comparison) was 94.1% (703/747) for CDA and 97.4% (369/379) for AL (treatment difference -3.3% 95%CI -5.6 to -0.9%). CDA met the non-inferiority criterion of a lower 95% CI of >=-7%; however, the upper 95% CI limit of <0 also indicated statistical superiority of AL. 282/1201 (23%) patients were G6PD A-; 94 males, 22 homozygous females and 166 heterozygous females. For CDA, but not AL, occurrences of the composite haematological safety endpoint and blood transfusions were significantly more frequent in G6PD-deficient vs. normal patients. There were no other remarkable safety observations. There were three deaths, unrelated to study medication (2 with CDA [severe malaria/herbal intoxication, sickle-cell anaemia crisis/severe sepsis], 1 with AL [severe pyrexia]). In conclusion, CDA was efficacious for the treatment of uncomplicated *P. falciparum* malaria, despite rapid elimination. However, the haematological safety profile of CDA in G6PD-deficient patients precludes its use in African countries.

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INSENSITIVE ACETYLCHOLINESTERASE (*ACE-1^R*) OF *ANOPHELES GAMBIAE* S.S.: EVENTS OF INTROGRESSION AND DUPLICATION BETWEEN THE M AND S MOLECULAR FORMS

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Studies on insecticide resistance genes provide data on the evolutionary processes involved in the adaptation of insects to environmental changes. Understanding the dynamics and the evolution of genes associated with insecticide resistance between closely related taxa represents a great interest, in terms of understanding resistance evolution in the field. This is a key component in establishing effective long-term resistance management strategies to eventually adapt vector control. In an upstream study, the mutation G119S (generating *ace-1^R* allele) was found in both molecular forms of *An. gambiae* s.s from Benin, Burkina Faso and Côte d'Ivoire. To establish whether the G119S mutation has arisen independently in each form or by genetic introgression, we analysed coding and non-coding sequences of *ace-1* alleles in M and S mosquitoes from representative field populations from West Africa. Our data revealed many polymorphic sites shared by S and M forms, but no diversity was associated with the G119S mutation. This indicates that the G119S mutation was a unique event and that genetic introgression explains its distribution within the two forms. Unexpectedly, sequence analysis of some resistant individuals revealed a duplication of the *ace-1* gene in both *An. gambiae* s.s. M and S forms. Again, the distribution of this duplication in the two forms most likely occurred through introgression. These results impacts on the question of actual levels of gene flow between the two molecular forms in tropical savannah areas. We can conclude that the G119S mutation could spread rapidly in the field and then compromises the use of organophosphate and carbamate compounds in public health as an alternative for indoor residual spraying in areas where malaria vectors are resistant to DDT and pyrethroids. This study underlines the necessity to monitor the G119S mutation in natural populations before planning and implementing malaria control programs based on the use of organophosphate and carbamate.

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ENTOMOLOGICAL EVALUATION OF PERMETHRIN IMPREGNATED BEDNETS AGAINST *ANOPHELES DARLINGI* IN THE PERUVIAN AMAZON

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Millions of Long Lasting Insecticide Treated Nets (LLINs) are to be distributed throughout the Amazon basin between 2007 and 2010. There is however, little understanding of how such interventions might impact upon the number of bites (and hence infections) that the people of that region receive. Bednets are particularly suitable for insects that bite and rest indoors and at night but *Anopheles darlingi*, the principal and anthropophilic vector of malaria in the Amazon, typically bites early in the evening, and is relatively exophilic and exophagic. In this study we present a simple prefabricated design for an experimental hut and a basic window trap. We show that these collect sufficient An. darlingi for the evaluation of interdomiciliary vector-control interventions. Using these tools, we report on the effect of LLINs on entrance and exit rates, and mortality of *An. darlingi*. Huts containing bednets collected ca. 50% fewer mosquitoes in window traps than untreated huts. Treated huts also contained fewer mosquitoes that had entered by other routes. Of those, >90% were collected dead or dying; presumably as a result of contact with bednets. This was in dramatic contrast to untreated huts in which there was less than 5% mortality among mosquitoes that had entered by means other than the window traps. We also note some unusual patterns in parous / nulliparous rates inside and outside the huts that suggest that *An. darlingi* becomes more endophilic and endophagic with experience and age.

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SPATIO-TEMPORAL ORDERING OF A CHAGAS DISEASE VECTOR ELIMINATION CAMPAIGN

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The rational design of interventions is critical to the control of communicable diseases. The Chagas disease vector *Triatoma infestans* has been eliminated from large areas of South America through application of insecticide, but vector control campaigns often fail when the insect returns to treated areas after insecticide wanes. Here we utilize a genetic algorithm, originally developed for the "traveling salesman" problem, in order to identify spatio-temporal designs of insecticide application that maximize the probability of vector elimination. Calibrated to empirical data on *T. infestans* migration, we find that the success of a control strategy is sensitive to the duration of insecticide efficacy, and shows chaotic fluctuations in response to unforeseen delays in the control campaign. Successful strategies feature both coarse and fine-grained geographic correlations. Nevertheless, successful designs generally deviate from a simple, sequential treatment of geographically proximate communities, instead "jumping ahead" to treat distal communities prior to completing treatment of a single area. We study the conditions under which this "jumping-ahead" strategy improves outcomes in a simplified system. Our analysis provides a detailed method to optimize elimination campaigns for an important disease vector as well as elucidates general guidelines to improve other control measures in time and space.

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EFFECTS OF FOREST FRAGMENTATION ON RELATIVE ABUNDANCE, BLOOD MEAL SPECIES COMPOSITION, AND TRYPANOSOME INFECTION OF THE CHAGAS DISEASE VECTOR *RHODNIUS PALLESCENS* IN A PANAMANIAN LANDSCAPE

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Deforestation and forest fragmentation are associated with infectious disease emergence in humans and animals. The objective of this study is to investigate relationships between forest fragmentation, vector populations, and transmission of the zoonotic vector-borne parasite *Trypanosoma cruzi* in a rural landscape of Panama. *Rhodnius pallescens,* the principal reduviid bug vector of Chagas disease in Panama, was collected from its primary habitat, the palm *Attalea butyracea*, from habitat types reflecting a range of anthropogenic disturbance (continuous undisturbed forest, forest fragments, cattle pasture, and peridomicile). *R. pallescens* abundance was estimated for each habitat type, bugs (N=641) were tested for infection with *T. cruzi* and *T. rangeli* by a duplex PCR assay, and

blood meal species composition was evaluated by amplifying vertebrate ribosomal DNA in vector samples. Across all habitat types, 79.43% (139/175) of palms were infested with *R. pallescens.* The number of palms infested with *R. pallescens* was significantly higher in forest fragments than in continuous forests ($X^2 = 11.6984$, df = 1, p= 0.0006255). Mean *R. pallescens* abundance was also significantly higher in forest fragments as compared to continuous forests (Wilcoxon rank sum test, p= 0.0005527). The proportion of *T. cruzi* infected vectors was significantly higher in forest fragments (76.9%) as compared to continuous forests (58.4%) ($X^2 = 11.8405$, df = 1, p = 0.0005796). Conversely, the proportion of vectors infected with *T. rangeli* was significantly higher in continuous forests (56.4%) as compared to forest fragments (31.3%) ($X^2 = 19.17$, df=1 p=0.000001). We discuss potential mechanisms for habitat-related differences of vector trypanosome infection in relation to changes in host blood meal species composition. Results suggest that fragmented forests are sources for *R. pallescens* populations and *T. cruzi* transmission in anthropogenically altered landscapes.

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THE EFFECT OF IVERMECTIN (MECTIZAN®) TREATMENT OF HUMANS ON FIELD-CAUGHT BLOODFED *ANOPHELES* SPP. SURVIVAL RATES IN SENEGAL

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In order to control *Onchocerca volvulus* transmission in Southeastern Senegal, mass-drug administration (MDA) of Mectizan® (active ingredient, ivermectin) is performed twice/year at a dose of 150µg/kg. This same area is hyperendemic for malaria transmission and ivermectin is distributed at the height of the rainy season when malaria transmission is most prevalent. We have demonstrated significantly reduced daily survival rates of colony-raised *Anopheles gambiae* s.s. mosquitoes in the laboratory when bloodfed on ivermectin concentrations equivalent to that which circulates in human plasma for up to four days post-Mectizan® treatment. To determine if this effect translates to the field, we performed daily hut aspirations in treated and untreated villages to determine adult *Anopheles* spp. survival rates. Preliminary data indicates that MDA affects *Anopheles* spp. survival rates. We are examining models and performing further collections and testing to determine whether MDA can transiently reduce malaria transmission via its affect on *Anopheles* spp. survival. CDC light traps hung beside humans sleeping under bed nets were used to collect host-seeking *Anopheles* spp. mosquitoes to determine the abundance and species composition of host-seeking mosquitoes. We then determined their sporozoite infection status and parity. Changes in the daily survival rates of various *Anopheles* spp. resulting from ivermectin treatment could dramatically affect the entomological inoculation rate, and the basic reproductive rate of malaria.

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DEVELOPMENT OF A MOSQUITOCIDAL VACCINE AGAINST *AEDES AEGYPTI* USING THE MOSQUITO LYSOSOMAL ASPARTIC PROTEASE (MLAP) AS AN IMMUNIZATION ANTIGEN

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Aedes aegypti remains the most important vector of dengue viruses worldwide. Mosquitocidal vaccines (vaccines which elicit mosquito-killing activity) are a promising strategy that may be effective in reducing transmission of dengue. We describe the use of the *Ae. aegypti* mosquito lysosomal aspartic protease (AeMLAP) as an antigen to generate mosquitocidal activity against *Ae. aegypti* and provide data characterizing the mechanism of mosquitocidal activity. Two vaccination regimens, DNA immunization and immunization with recombinant protein, were tested for mosquitocidal efficacy. For DNA immunizations, AeMLAP was cloned into the immunization vector pCDNA3.1. Balb/c mice were immunized concurrently with pCDNA3.1/AeMLAP and a mouse IL-12(mIL-12) adjuvant plasmid. Recombinant AeMLAP was produced by cloning the AeMLAP gene and expressing it in a bacterial expression system. Recombinant protein was excised from an SDS-PAGE gel, and gel strips containing recombinant AeMLAP were used to immunize Balb/c mice. Mosquitoes fed on mice immunized with pCDNA3.1/AeMLAP plus mIL-12 adjuvant or recombinant AeMLAP protein exhibited significantly reduced survival over mosquitoes fed on control immunized mice, with the majority of death occurring at 3-7 days post blood meal ingestion. We have detected specific anti-MLAP antibody in immunized mice. Using TUNEL analysis, we found an increased number of apoptotic cells localized in ovarian follicles and the terminal abdominal segment of mosquitoes fed on DNA immunized mice compared to age-matched blood fed controls. Apoptotic cells were detected at 3 and 5 days post-bloodmeal, coinciding with the days when the majority of death occurs in mosquitocidal blood fed mosquitoes. Additionally, preliminary data shows that RNAi-mediated silencing of MLAP reduces mosquito survival following a bloodmeal. Using MLAP as an antigen for immunization, we show that a mosquitocidal response can be consistently produced in *Ae. aegypti*. The research described lays the groundwork for stream-lined discovery of novel mosquitocidal targets.

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DEVELOPMENT OF CONTROLLED VOCABULARIES AND ONTOLOGIES FOR SURVEILLANCE AND CONTROL OF VECTORS OF HUMAN DISEASE AGENTS

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An ontology is composed of a controlled vocabulary of terms, including the definitions of these terms, and a description of how the terms are inter-related. Standardization and unambiguous definitions of controlled vocabulary terms support consistent annotation and interpretation of data as well as data interoperability. The establishment of an ontology is a process that requires participation of and consensus among the expert community. To achieve such mutual ownership, subject matter experts should be invited to contribute at an early stage of development. We are developing a web interface (http://www.rams-aid.org/ontologies/) to provide access to a visual framework and structure of terms suggested for inclusion in controlled vocabularies and ontologies for surveillance and control of arthropod vectors of human disease agents. Participants will be able to: 1) propose new terms, 2) propose modifications to existing terms and/or their definitions, and 3) comment on structures and undefined relationships. No restrictions will apply to participation and all contributions will be validated. The focus of this initiative is to develop controlled vocabularies and ontologies for surveillance and control of arthropod vectors of human disease agents. The development of a Dengue Decision Support System at Colorado State University and a Malaria Decision Support System at the Medical Research Council of South Africa currently support the use of these ontologies and controlled vocabularies. Global data warehouses are then able to annotate and incorporate data from all sources ensuring standardization and supporting data interpretation and analysis.

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PLASMODIUM FALCIPARUM MOLECULAR BARCODE ASSESSMENT OF PARASITES SEQUESTERED IN TISSUES AT AUTOPSY

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One aspect of the pathogenesis of *Plasmodium falciparum* malaria is the prolonged period of the life cycle which the parasites spend sequestered or adherent to the endothelium of the deep tissues. In cerebral malaria, this sequestration into cerebral vessels is thought to be of crucial importance in pathogenesis. Previously, 20 patients who had autopsies performed as part of the Blantyre Malaria Project/Malawi-Liverpool-Wellcome Trust Research Programme's autopsy study based in Blantyre, Malawi were studied including 5 with cerebral malaria (CM), 4 with CM and severe malaria anemia (SMA), 1 with SMA, 3 with pneumonia, and 7 with other non-malaria causes of death. These results showed a variable number of genotypes in different body tissues by routine MSP 1 and 2 genotyping with cerebral malaria infections appearing to be less genetically complex than controls. The *P. falciparum* molecular barcode, a set of 24 single nucleotide polymorphisms with high minor allele frequencies, allows for unique identification of a parasite strain in culture and from single clone infections in patient samples. We applied the molecular barcode to these 20 DNA samples directly and found that patients with a very high total body parasitemia and known tissue sequestration by light microscopy could be barcoded directly (CM, CM+SMA, and SMA). The barcodes revealed a dominant strain in 4 of 5 patients with clinically defined CM. The barcodes in the organs examined (three brain sites, lung, heart, and colon) had identical barcodes within each CM patient. Patients with CM + SMA and SMA alone had mixed barcodes, but the same mixed pattern was found in all tissues for these cases. Patients who died of non-malarial causes did not have sufficient parasite material for analysis directly. We performed whole genome amplification on all 20 patients' samples and repeated the barcode analysis. The CM and SMA patients had identical barcodes to pre-amplification. The tissue samples from non-malaria diagnosis yielded a mixture of non-amplifying signals and/or random alleles across the tissues suggesting the assay was picking up partial signals from previous malaria infections. In summary, the *P. falciparum* molecular barcode appears to not only confirm our previous conclusion that cerebral malaria is less genetically complex than controls but also suggests a dominant parasite or group of parasites may be an important aspect of severe malaria.

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GENOME-WIDE SURVEY OF GENE COPY NUMBER VARIATION IN THE MALARIA PARASITE *PLASMODIUM FALCIPARUM*

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Gene copy number variation (CNV) is responsible for several major phenotypes of the malaria parasite *Plasmodium falciparum*, including drug resistance, loss of cytoadherence, and alteration of erythrocyte invasion pathways. Despite the importance of CNV in *P. falciparum* biology little is known about its extent throughout the genome. Here, we report a whole-genome survey of CNV genes in *P. falciparum* using comparative genome hybridisation (CGH) to a custom designed high density Affymetrix GeneChip. Results indicate that the generation of CNV in malaria parasites involves highly non-random mutational and selective processes. There is a strong association of CNV with gene length, genomic location, low orthology to genes in other *Plasmodium* species and elevated nucleotide diversity. Sub-telomeric regions of all chromosomes are strongly associated with CNV genes independent from members of the previously described *var*, *rifin* and *stevor* multigene families. Among the previously undescribed CNV genes are several that are of potential phenotypic relevance. These results have led to a study into genetic changes associated with the adaptation of clinical isolates from The Gambia into long-term *in vitro* culture. Preliminary findings and the potential of newly emerging tools to assay these genetic changes will be discussed.

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ANALYSIS OF DRUG RESISTANCE USING *PLASMODIUM FALCIPARUM* GENETIC CROSSES

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Resistance to a broad diversity of antimalarials is one of the major problems in malaria control. Amodiaquine (AQ) has been extensively used and is currently recommended by the World Health Organization to treat chloroquine (CQ) resistant malaria. However, uncertainty about cross-resistance between AQ and CQ results in a constraint on this alternative. Quinine (QN) and mefloquine (MF) have been effectively used for decades against *P. falciparum*, but reports of decreased sensitivity in several endemic areas also indicate the limited use of these drugs. Nevertheless, the extensive use of the available antimalarials, including piperaquine (PPQ) and artemisinin (ART), may eventually result in appearance of resistance. The search for genetic markers that might affect resistance to these antimalarials is of high importance to monitor effectiveness of malaria treatment. Previous analysis of a genetic cross between a CQ-resistant *P. falciparum* clone, Dd2, and a CQ-sensitive clone, Hb3, led to identification of genes linked to CQ and QN resistance. Another genetic cross between two CQ-resistant clones with different levels of response, 7G8 and GB4, was recently completed in our lab. We employed quantitative trait loci (QTL) analysis of these two genetic crosses to search for genes linked to responses to the antimalarials described above. A total of 88 *P. falciparum* clones were evaluated for *in vitro* response to CQ, AQ, QN, MF, PPQ and ART. We also evaluated the effect of the main metabolites of CQ and AQ, monodesethylchloroquine and monodesethylchloroquine, respectively, considering the extent of metabolism of these drugs and the fact that AQ is a pro-drug. An overview of our results will be presented with an emphasis on AQ resistance findings and its impact on the current malaria treatment policy.

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INSIGHTS INTO GENE EXPRESSION THROUGH ANALYSIS OF TRANSCRIPTIONAL ACTIVITY DURING THE INTRAERYTHROCYTIC DEVELOPMENTAL CYCLE OF *PLASMODIUM FALCIPARUM*

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Plasmodium falciparum transitions through several distinct morphological stages during its intraerythrocytic developmental cycle (IDC). Microarray studies report stage-specific changes in the steady-state mRNA levels of most genes, suggesting that differential gene expression is related to development, though the mechanisms driving it remain poorly understood. Here, we examine the role of transcriptional activity in the

expression of specific genes during the IDC by first characterizing a bulk trend in the activity of the transcriptome, then integrating this data into our analysis of individual genes. Nuclear run-on assays on whole-transcriptome samples from timepoints throughout the IDC revealed a strong peak in RNA Polymerase II-dependent transcriptional activity. We mathematically attributed increased levels of transcriptional activity to multinucleate stages (late trophozoites and schizonts). This increase correlated with the increasing genome-copy number per parasite during S-phase and schizogony, indicating that during these stages RNAP II utilizes all DNA templates available. This newfound differential activity of the bulk transcriptome was then used for normalization of the activities of a set of specific genes from the same timepoints to render their absolute transcriptional activity levels. The majority of these genes experienced peak sense-strand transcriptional activity at the same timepoint as the bulk trend. Furthermore, antisense strands of several genes were substantially transcriptionally active, corroborating previous studies which found unusually abundant antisense RNA in *P. falciparum*. Comparison of the transcriptional activity of the sense strand of each gene to its steady-state RNA level across the timepoints revealed cases in which transcriptional activity correlated with steady-state expression, as well as cases where anticorrelation suggested that regulated RNA stability was modulating steady-state expression. This work brings together data on a stage-specific, bulk transcriptional activity trend with the transcriptional activity of individual genes, thereby providing unique insight into the mechanisms of RNA regulation relevant to those genes throughout the IDC.

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IDENTIFICATION OF BIOLOGICAL PATHWAYS CRITICAL FOR MALARIA PARASITE DEVELOPMENT, THROUGH TRANSPOSON-MEDIATED MUTAGENESIS

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Persistent problems with drug resistance and the critical need to identify novel targets for therapeutic intervention creates a continuing need to improve our understanding of what is important for growth and development of malaria parasites. A strategic hurdle for development of new anti-malarial therapeutics remains the lack of functional information about most *Plasmodium falciparum* genes and target discovery relies on the identification of molecules and pathways vital for parasite growth. Here, we used piggyBac as a molecular genetic tool for functional characterization of the *P. falciparum* genome and identified several genes and pathways crucial for intra-erythrocytic development of the parasite. Some of the biological pathways that significantly affected parasite growth include: cell cycle control, mRNA metabolism, intracellular signalling and redox metabolism. Our results provide the experimental basis for further evaluation of the components of these pathways as antimalarial drug targets and clearly validate piggyBac as an indispensable tool for forward functional genomics in malaria parasites.

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INVESTIGATION OF AN OUTBREAK OF A FATAL FEBRILE ILLNESS IN GUATEMALA, 2007

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Although rickettsioses are endemic in the Western Hemisphere, the presence of rickettsiae and rickettsioses has not been previously documented in Guatemala. Fifteen patients were identified in the spring of 2007 in the farming community of Moyuta, Jutiapa, presenting with fever, chills, headache, myalgias and abdominal pain. Two of these were fatal cases. The purpose of this study was to evaluate if these cases had a rickettsial etiology. Acute and convalescent sera, plasma and acute whole blood samples were collected from each patient and tested by indirect immunofluorescence assay (IFA), western blotting (WB) and PCR, respectively. *Amblyomma* (12) and *Boophilus* (85) ticks were also collected from the property and several cows belonging to one of the patients and tested for the presence of typhus (TG) and spotted fever group (SFG) rickettsiae using nested and quantitative PCR assays. One plasma sample, from 1 of the 2 fatal cases, out of a total of 14 DNA samples prepared either from acute serum, plasma or whole blood was PCR positive for SFG rickettsia DNA. Sera of 9 patients with one or more positive titers contained low level of IgM or IgG antibodies to *Rickettsia typhi* (IFA geometric mean titer, GMT IgM/IgG 128/68, respectively), *R. akari* (91/91 GMT), or *R. rickettsii* (29/18 GMT). IgM antibodies against high molecular weight protein antigens of *R. typhi*, *R. rickettsii* and *R. akari* were detected by WB in sera of 4 patients. PCR screening of ticks detected the presence of SFG DNA in 2 (16%) *Amblyomma*. Sequences of the OmpA gene fragments of these ticks are most similar but not identical to *omp*A from *R. sibirica*. SFG rickettsiae, *R. prowazekii* and *R. typhi* were not detected in any of the 85 *Boophilus* tested. In conclusion, SFG rickettsiae other than *R. rickettsii* are present and may cause febrile illness in Guatemala. Further clinical surveillance and environmental assessment are needed to establish the true prevalence of rickettsioses and to identify their etiologic agents and vectors in Guatemala.

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TBRF IN EAST AFRICA: EPIDEMIOLOGY AND CLINICAL DIAGNOSIS IN CENTRAL TANZANIA

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Tick-borne relapsing fever (TBRF), primarily caused by the spirochete *Borrelia duttonii*, occurs throughout East Africa and is common in parts of Tanzania. Although a serious public health problem, the extent and true burden of TBRF in Tanzania, and indeed elsewhere in Africa, remain unknown. This situation has arisen in part because TBRF infections typically present with signs and symptoms that are indistinguishable from malaria, obscuring the incidence of TBRF and falsely elevating malaria data. Using PCR to identify the presence of *Plasmodium* and/or *Borrelia* infection in 69 fever cases presenting at hospital clinic within a highly endemic TBRF locality in Dodoma region, central Tanzania, we compared over forty clinical signs and symptoms. Statistical analysis did not reveal

significant differences that could be useful in distinguishing between these two infections in resource-poor locations. Following up the same individual cases, we investigated risk factors associated with *Borrelia* infection. Results of these final analyses will be presented and discussed in the light of our recently published findings indicating that a domestic animal reservoir of infection may exist in this region. How these findings inform the simple measures known to prevent and control TBRF in Africa, particularly insecticide-treated bednets and methods used to control other vector-borne diseases, will also be discussed.

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QUANTUM OF TULAREMIA INFECTION WITHIN QUESTING DOG TICKS

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The island of Martha's Vineyard, MA has been the site of an outbreak of tularemia for the last 7 years. Dog ticks are prevalent on the island and tick bites are common. We previously reported that as many as 5% of the ticks there contain DNA of *Francisella tularensis tularensis* (Ftt). Despite this great potential entomological inoculation rate, only 14% of 68 reported cases there have been classified as the ulceroglandular form, most likely due to dog tick bites. Little work has been done to characterize the transmission dynamics of the agent of tularemia and in particular the vector-pathogen interface. It may be that solely ticks with a threshold bacterial load are capable of efficiently transmitting infection. As the first step in testing this hypothesis, we measured the quantum of infection in ticks that were previously identified to contain Ftt DNA by a nested PCR targeting the fopA gene. The amount of Ftt DNA contained by each tick was determined by quantitative real-time PCR targeting the tul4 gene and using known amounts of Live Vaccine Strain as a standard. The amount of tick DNA per sample was standardized using a *Dermacentor variabilis* 28S rRNA gene target. Of 61 ticks tested, the amount of Ftt DNA ranged from <1 genome equivalent per pg of 28S (ge/pg) to 123,000 ge/pg. The quantum of infection was not normally distributed but skewed to the left with a long right-hand tail. The mode of the distribution is approximately 10,000 ge/pg. Such a distribution is similar to that reported previously for *Borrelia burgdorferi* in host-seeking *Ixodes dammini* ticks. We conclude that if the quantum of infection influences the probability of transmission, individual dog ticks naturally vary in their capacity to efficiently infect by their bites, thereby helping to explain why there are relatively few tick-associated cases of tularemia on Martha's Vineyard.

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IDENTIFICATION OF BACTERIAL PATHOGENS AND HOSTS OF BLOOD MEALS IN QUESTING IXODID TICKS IN THE NORTH CAROLINA PIEDMONT

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Questing ticks were collected by flagging vegetation in residential areas of Chatham County, North Carolina. *Ambloyomma americanum* was the predominant species collected. Dermacentor variablis and Ixodes scapularus were occasionally collected. Genomic DNA was extracted from individual ticks and species-specific primers were used in quantitative PCR assays to detect *Rickettsia rickettsia, R. amblyommii, Borrelia burgdorferi, B. lonestari, Anaplasma phagocytophilum, Ehrlichia chaffeensis*, and *E. ewingii*. Pathogen prevalence will be presented. A two-step nested PCR assay, with primer sets taken from conserved areas of the 12s and 16s vertebrate mitochondrial genome, was used to amplify vertebrate DNA from the genomic DNA of individual ticks and other blood fed arthropods. Amplicons were separated by denaturing gradient gel electrophoresis (DGGE) and individual DNA fragments were identified to host species by comparison to a ladder of known vertebrate samples. The use of PCR amplification of DNA combined with DGGE was demonstrated to be an effective method for identifying the vertebrate hosts of blood feeding arthropods.

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ISOLATION OF *FRANCISELLA TULARENSIS TULARENSIS* SUBPOPULATION A.I. FROM MISSOURI LONE STAR TICKS

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Over half of the tularemia cases reported annually in the United States occur in the southcentral states of Missouri, Arkansas, Kansas and Oklahoma; tick bites account for about 60% of the tularemia cases in this endemic area. Lone Star ticks (*Amblyomma americanum*; LST) and dog ticks (*Dermacentor variabilis*) are thought to be the main vectors, but their relative contributions remain to be described. Although *Francisella tularensis* was reported to have been isolated from LSTs (Calhoun 1954), the role of these ticks in tularemia epidemiology remains as poorly described as it is for Rocky Mountain spotted fever. To our knowledge, there are no existing isolates or definitive subspecies identifications of *F. tularensis* from LSTs. To determine whether LSTs are frequently infected in endemic areas, we sampled one site in Camden County, Missouri and two sites in Linn County, Kansas, based upon convenience and confirmed reports of tularemia cases from these areas. Host seeking ticks were sampled by dragging vegetation during May and July 2006 and were tested by PCR targeting the *fop*A gene for evidence of *F. tularensis* infection. LSTs accounted for the majority of the total tick collections: *F. tularensis* was detected by PCR from one adult male LST from the Missouri field site (0.16% of 607 LSTs from all sites; 1 % of LST adults from MO). None of 120 dog ticks contained *F. tularensis* DNA. *F. tularensis* was isolated from the one LST by plating the tick homogenate onto cysteine heart agar containing 8% sheep blood; colonies that were morphologically consistent with *F. tularensis* were visible after 24 hours. The isolate was characterized as *F. tularensis* subspecies *tularensis* (Type A) by sequencing of a portion of the PPI helicase gene. Interestingly, the isolate groups with subpopulation A.I. based on molecular typing of the Ft-M20 VNTR locus, confirming the suggestion that the subpopulation A.I. is not necessarily associated only with *D. variabilis*, as speculated previously. We conclude that LSTs from endemic sites are naturally infected by *F. tularensis tularensis* and that because these aggressive human biting ticks are more common than are dog ticks, are likely to be the main vectors of tularemia.

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EARLY INNATE IMMUNE EVENTS IN THE SKIN AFTER TRANSMISSION OF *YERSINIA PESTIS* BY FLEAS

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Transmission of pathogens by arthropod vectors is known to affect early immune events in host animals in ways that cannot be mimicked by needle injection. This phenomenon has not been well studied in the transmission of *Yersinia pestis* by fleas. In this study we compare early innate immune events in the ear tissue of mice after infection with *Y. pestis* delivered by both intradermal needle inoculation and transmission by blocked *Xenopsylla cheopis*. Mice were injected intradermally with purified *E. coli* lipopolysaccharide (LPS) as a positive inflammatory control; saline diluent alone served as a negative control. Paired ear and draining lymph node samples were taken at 3, 6, 12, 24, and 48 hours post infection. Skin and lymph node cells were analyzed by flow cytometry to measure the nature and dynamics of the inflammatory response. Additional whole tissue samples were fixed, sectioned, and examined for histological changes. Infection in the ear by needle with virulent *Y. pestis* elicited moderate

infiltration by neutrophils and monocytes early in infection, comparable to or less than that seen with the LPS control. Intensity of inflammation was not correlated with bacterial load. In contrast to LPS controls, virtually no recruitment of macrophages was detected at any time point. In the lymph node, there was an almost complete absence of inflammation until the end stages of disease, even when 5-6 logs of bacteria were present. These results will be compared with trials using infection by flea bite.

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RICKETTSIA FELIS INFECTION IN A MURINE MODEL

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Rickettsia felis is a globally distributed flea-borne rickettsial pathogen. The vertical transmission of *R. felis* by the cat flea, *Ctenocephalides felis* has been examined; however, no studies have described the horizontal transmission of viable *R. felis* to a mammalian host. Outside of the symptoms reported for human cases including: fever, rash and myalgia, little is known about *R. felis*-infection biology within a mammalian host. Towards understanding *R. felis* pathogenesis, we developed an animal model of *R. felis* infection. C3H/HeN mice were injected intravenously with either high ($1x10^6$) or low ($1x10^3$) dose of *R. felis*, sacrificed at 1-19 days post-inoculation, and assessed for *R. felis* infection using quantitative real-time PCR. To date *R. felis* was detected in samples of heart, lung, liver, spleen, kidney, skin, reproductive tissue, and brain from infected mice. The development of a murine model of *R. felis* infection will allow for in-depth examination of *R. felis* pathogenesis in mammalian hosts and elucidation of the transmission dynamics to arthropod vectors.

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A COHORT STUDY EVALUATING IMMUNOLOGICAL AND CLINICAL CONSEQUENCES OF THE CO-INFECTION HTLV-1 AND *SCHISTOSOMA MANSONI*

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The frequency of schistosomiasis is increased in patients infected with HTLV-1 but no evidence of deleterious effect of HTLV-1 infection in schistosomiasis was observed. In this ongoing cohort study, clinical manifestations related to schistosomiasis and to HTLV-1 were evaluated in HTLV-1 infected subjects with and without schistosomiasis and immunological responses were determined at different time points including before and after treatment for shistosomiasis. Participants included 32 HTLV-1 carriers co-infected with *Schistosoma mansoni* and 64 HTLV-1 carriers without helminthic infection. Cytokine measurements were performed by ELISA in supernates of cell cultures and the frequency of T cells expressing cytokines were determined by FACs analysis. IFN-γ and TNF-α levels were lower ($P<0.01$) in patients co-infected with HTLV-1 and *S. mansoni* than in individuals only infected with HTLV-1. Neutralization of IL-10 did not enhance IFN-γ or TNF-α levels. IFN-γ, TNF-α and IL-10 levels were similar before and up to two years after treatment and cure of *S. mansoni* infection. The decreasing in the frequency of T cells expressing TNF-α and IFN-γ was observed in both CD4 and CD8 population. There was no documentation of liver fibrosis in patients co-infected with HTLV-1 and *Schistosoma mansoni*. An interim analysis of this ongoing study regarding the influence of schistosomiasis on clinical manifestations of HTLV-1 have indicated a trend to a higher frequency of neurological manifestations on patients with HTLV-1 without helminthic infection than in the group with HTLV-1 and schistosomiasis. Down modulation of the immune response mediated by *S. mansoni* in HTLV-1 infected individuals is independent of IL-10 and remain even after therapy and cure of schistosomiasis. As HTLV-1 associated diseases are related to the exaggerated inflammatory response, *S. mansoni* infection may attenuate clinical manifestations of HTLV-1.

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

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Pregnant women are often chronically infected with helminthic parasites, whose soluble products can cross the placenta and prime the fetus. This *in utero* exposure may induce an immunomodulatory phenotype that persists into infancy and thus affect efficacy to childhood vaccines. To test this hypothesis we examined the impact of schistosomiasis, lymphatic filariasis (LF), and/or hookworm infection during gestation on immunoglobulin G (IgG) responses to the protective epitope PRP (poly-ribitol phosphate) in infants, who had previously been vaccinated with Hib vaccine (*H. influenza* type b conjugate with tetanus toxoid, TT). A total of 241 Kenyan maternal-infant pairs were assayed at 6, 10, 14 and 26 weeks of age for PRP-IgG following Hib immunization at 6, 10 and 14 weeks. Overall 57% of the pregnant women were infected with one or more helminths. 210 infants (87%) received 2 or 3 Hib immunizations, attaining a geometric mean±SEM PRP-IgG of 6.7±3.6 µg/ml at 26wks, with >99% of children having protective levels of antibodies (>0.15 µg/ml). Offspring of helminth-negative women had 1.5-, 2.0- and 2.2-fold higher geometric mean PRP-IgG levels at 10, 14 and 26 weeks of age compared to offspring of women with ≥2 helminthic infections ($P<0.01$-0.001). Children of women with one helminth infection had intermediate PRP-IgG levels. Maternal infection with LF and/or schistosomiasis had a stronger effect on reduced PRP-IgG levels, when compared to hookworm infections. Children of women infected with LF and/or schistosomiasis had impaired lymphocyte recall responses to TT compared to children of uninfected women, suggesting a mechanism by which the conjugate vaccine was less effective. Thus, chronic helminthic infections during pregnancy may impair childhood vaccine efficacy, highlighting the importance of national programs to eradicate these infections in pregnant women.

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INHIBITION OF TYPE I DIABETES IN FILARIA INFECTED NOD MICE IS ASSOCIATED WITH A TH2 SHIFT AND INDUCTION OF REGULATORY T CELLS

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We investigated whether *Litomosoides sigmodontis*, a tissue-invasive filarial nematode, prevents the onset of type I diabetes in nonobese diabetic (NOD) mice. As a broad range of helminth parasites have been found to have beneficial effects on autoimmune diseases, we hypothesized that filarial infection would be protective against Type I diabetes. Further, we hypothesized that helminth-mediated protection against autoimmunity would be associated with an autoantigen-specific Th2 shift and with increases in regulatory T-cell numbers. While regulatory T-cells play an important role in chronic filariasis, to date their presence has not been evaluated in models of helminth-mediated protection against autoimmunity. 6-week old NOD mice were sham-infected or infected with L3 larvae, adult male worms, or adult female *L. sigmodontis* worms. Whereas 82% of uninfected NOD mice developed diabetes by 25 weeks of age, no *L. sigmodontis*-infected mice developed disease. Although all mice had evidence of ongoing islet cell inflammation by histology, *L. sigmodontis*-infected mice had greater numbers of total islets and non-infiltrated islets than control mice. Protection against diabetes was associated with a Th2 shift, as IL-4 and IL-5 release from α-CD3/α-CD28-

stimulated splenocytes was greater in *L. sigmodontis*-infected mice than in uninfected mice. Increased circulating levels of insulin-specific IgG1, but not insulin-specific IgG2a, showed that this Th2 shift occurs in response to one of the main autoantigens in diabetes. Multicolor flow cytometry studies demonstrated that protection of diabetes in *L. sigmodontis*-infected NOD mice was associated with significantly increased numbers of splenic $CD4^+CD25^+FoxP3^+$ regulatory T cells. Interestingly, injection of crude worm antigen into NOD mice also resulted in protection against type I diabetes, though to a lesser degree than live *L. sigmodontis* worms. These studies demonstrate that filarial worms protect against the onset of Type 1 diabetes in NOD mice. This protection is associated with a Th2 shift, as demonstrated by cytokine and antibody production, and with an increase in $CD4^+CD25^+FoxP3^+$ regulatory T cells. Current studies to determine whether these immunologic changes are responsible for helminth-mediated protection against autoimmunity are ongoing.

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INFLUENCE OF PRE-EXISTING FILARIAL INFECTION ON THE INCIDENCE AND SEVERITY OF CLINICAL MALARIA IN CHILDREN AND YOUNG ADULTS IN A COENDEMIC REGION OF MALI

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In Mali, infection with *Wuchereria bancrofti* and/or *Mansonella perstans* exists in several regions highly endemic for malaria, and co-infection is common. Pre-existing filarial infection and the resultant bias toward a modulated Type 1 immune response could alter the response to incoming malarial parasites, clearance of which is thought to depend on robust IFN and TNF responses. In turn, this could affect the clinical manifestations and outcome of malaria infection. To determine the effect of filarial infection on the prevalence and severity of malaria infection, 41 filaria-positive (FP) and 42 filaria-negative (FN) children and young adults (1-20 years of age) matched for age, gender and HbS/HbC status were followed weekly through the malaria transmission season (July-December 2007). Anthelmintic treatment was administered to diminish the potential confounding effect of geohelminth infection. Clinical malaria was defined as signs and symptoms consistent with malaria infection in the presence of parasitemia. Baseline parameters, including Hgb level, eosinophil count, G6PD status and malaria parasitemia, were comparable between the groups. Overall, 28/41 (68%) subjects in the FP group and 33/42 (79%) subjects in the FN group had at least one episode of clinical malaria. Fever ($T>37^oC$) was present at the time of first malaria diagnosis in 19/28 (68%) of the FP and 21/33 (64%) of the FN subjects. The geometric mean (GM) time to first episode was also comparable (58 days in the FP subjects and 69 days in the FN subjects (p=0.07)). In contrast, GM parasitemia at the time of the first malaria episode was significantly higher in the FN group (926 parasites/mm^3 vs. 353 parasites/ mm^3 in the FP group, p=0.04). These findings suggest that filarial infection does not influence susceptibility to malaria infection but may lower the threshold for developing symptomatic infection. Analysis of symptom scores and cytokines during clinical malaria episodes is currently underway and should help elucidate the role of immune effectors in this process.

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T LYMPHOCYTE SUBSETS IN CHILDREN WITH SCHISTOSOMIASIS MANSONI COMPARED TO CHILDREN WITH *SCHISTOSOMA MANSONI* AND *PLASMODIUM FALCIPARUM* CO-INFECTIONS IN WESTERN KENYA

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We are pursuing longitudinal studies of immune profiles of 153 children attending schools located within 3 km of Lake Victoria in western Kenya. Herein we present data from the baseline time point comparing T cells subsets between children with schistosomiasis mansoni and those with dual infections of both *Schistosoma mansoni* and *Plasmodium falciparum*. We also examined the impact of soil-transmitted helminths on these subsets of T lymphocytes, but co-infections with these intestinal parasites were found not to correlate with any differences relative to having *S. mansoni* infection. Four-color flow cytometry of T lymphocytes was done using combinations of directly labeled monoclonal antibodies. The intensity of schistosome infection had no influence on either the percentage of T regulatory cells (Treg; $CD3^+/CD4^+/CD25^{hi}$) ($p = 0.763$) or their expression of PD-1 ($p = 0.236$), or activated T cell (Tact; $CD3^+/CD4^+/CD25^{med}$) that co-express HLA-DR ($p = 0.579$). There was also no significant difference in the mean percentage of $CD3^+$ T cells (73.7%) or CD3+/CD4+ cells (55.4%) in *S. mansoni* single infection and these populations in *S. mansoni* and malaria co-infection (76.1% and 55.4%, respectively). Likewise single or dual infections did not differ in overall circulating Treg percentages, 1.6 % and 1.7 %, respectively. However, children with *S. mansoni* and *P. falciparum* malaria had significantly lower mean percentages of HLA-DR+ Tact cells (5.2%; $p = 0.017$) than those with schistosomiasis only (16.1%) ($p = 0.017$). Similarly, the mean percentage of Treg that expressed CD45RO ($CD3^+/CD4^+/CD25^{hi}$/CD45RO) (considered to be memory Treg) was significantly lower in children with *S. mansoni* and *P. falciparum* double infections (60.3%) compared to those with *S. mansoni* alone (71.2%; p=0.003). These differences in activated and regulatory T cell subsets may contribute to the differential findings reported in morbidity and resistance due to co-infections with *S. mansoni* and *P. falciparum* compared to schistosomiasis alone.

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WUCHERERIA BANCROFTI AND *MANSONELLA PERSTANS* INFECTIONS MAY PROTECT AGAINST *PLASMODIUM FALCIPARUM* INDUCED ANEMIA IN FILARIA/MALARIA CO-INFECTED POPULATIONS

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In contrast to co-infections with intestinal helminths and *Plasmodium falciparum* (Pf), blood-borne filarial parasites that share the circulatory compartment with Pf might have a greater potential to influence the clinical and parasitological outcomes of Pf infection. We conducted a longitudinal study among filarial-infected and uninfected residents of a village in Mali with seasonal malaria transmission, to compare demographic and parasitological characteristics of malarial and filarial infections in the two groups. Two cohorts of 30 villagers each (aged

18-65 yrs), filaria-infected (Fil+), defined either by a positive ELISA for circulating Wb antigen (Ag) and/or Mp microfilaremia (mf), and filaria-uninfected (Fil-), defined by a negative Wb Ag and absence of Mp mf, were evaluated at the start (V1), middle (V2) and two months after the end (V3) of one transmission season of Pf malaria (Jun 2006-Jan 2007). Among Fil+, 43% had both Wb and Mp, 50% had Mp alone, and 7% were amicrofilaremic but positive for Wb Ag. There were no significant differences in the prevalence of asymptomatic Pf parasitemia in the Fil+ and Fil- cohorts during any of the three visits (7 in Fil+ vs. 6 in Fil-; p=NS). Moreover, there were no episodes of clinical malaria at the time of the study visits in the Fil+ and only 1 in the Fil- cohort. There was a trend towards a lower level of Pf parasitemia in the Fil+ cohort (GM 85 trophozoites/10,000 RBC [25-175]) compared to Fil- (GM 338 [50-3850]; p=0.11). White cell and eosinophil counts were similar in the two groups. Interestingly, hemoglobin (Hb) levels decreased significantly between V1 and V3 in the Fil- (mean reduction 1.4 ± 0.5 g/dL; p<0.005) but not in the Fil+ cohort,(0.9 ± 0.6 g/dL; p=0.18). These data suggest that pre-existing filarial infection does not significantly affect the prevalence or level of Pf parasitemia, but may ameliorate malaria-related anemia through a transmission season. Further analysis of data from a smaller cohort of infants in the same village may help to elucidate the effects of filarial infections in the age group most susceptible to clinically apparent malaria.

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STEEP INCREASE IN CHILD SURVIVAL AFTER FOUR YEARS OF INTEGRATED MALARIA CONTROL IN BIOKO ISLAND, EQUATORIAL GUINEA

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The effect of insecticide treated nets on child survival is well documented. Less is known about the combined effect of indoor residual spraying and other malaria control measures on child mortality in settings of high malaria transmission intensity. The Bioko Island Malaria Control Project, in collaboration with the government of Equatorial Guinea introduced an integrated malaria control programme consisting of IRS of all houses, case management based on ACTs and definitive diagnosis, IPT and information and education campaigns in 2004. All cause under 5 mortality was monitored as one of the project impact indicators. In a pre-intervention household survey a random sample of women of reproductive age gave information on all previous live births, recording date of birth of the child, whether still alive, and age at death if deceased using the standard reproductive history module of the Demographic and Health Surveys questionnaire. A similar survey was repeated four years after the start of the project in Bioko on a random sample of women. Using life tables, under 5 mortality rates were calculated for pre- and post-intervention periods respectively. Proportional hazards analysis was used to compare pre-and post intervention mortality, adjusted for potential confounders. All cause under five mortality, estimated from survey data reporting on 2418 birth histories before the introduction of malaria control measures, was reduced by approximately 60% after the introduction of malaria control, estimated from survey data reporting on 2390 births four years after the launch of the program. In mainland Equatorial Guinea, where similar control measures had not been introduced by the time of the second mortality survey, a similar survey showed that child mortality remained high. In conclusion, comprehensive malaria control based on IRS, effective treatment and IPT can substantially reduce child mortality in areas of high malaria transmission intensity.

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THE IMPACT OF HOME BASED MANAGEMENT OF MALARIA (HMM) ON UNDER FIVE MALARIA MORTALITY: THE RWANDAN EXPERIENCE

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Falciparum malaria is the most important cause of death of Under Fives in Africa. The backbone of any control program is prompt and efficacious case management. Despite limited evidence, the World Health Organization advocates Home-based Management of Malaria (HMM) as one of the major strategies. Rwanda adopted this strategy in 2004. From 2004 until 2006, the malaria morbidity and mortality data in 48 Health Centres (HC) with 1,096,922 inhabitants and 4,374 Community Health Workers (CHW) implementing the HMM strategy were compared to 37 Control HC with 833,852 people. Data were analysed in a repeated measures Poisson regression model with HCs as clusters and assessed for possible confounders. In 2004, control and HMM districts were comparable for all monitored parameters. The malaria mortality rate decreased 64% more in the HMM districts than in the control districts (Ratio of IRR:0·36; p<0.001) The case fatality ratio decreased 46% more in the HMM districts (Ratio of IRR:0·54;p=0.003) and the severe malaria incidence was 33% lower in HMM districts compared to the control districts (Ratio of IRR:0·67;p=0.005). Between 2004 and 2006, the HMM induced an almost 3-fold increase in Under Five contacts for fever (Ratio of IRR:2·75;p<0.001). Control and HMM districts did not differ for possible confounders including adherence to the health insurance, overall consultations, bed net coverage and hospital case fatality ratio. Iconclusion, the reported direct malaria mortality rate dropped by two thirds in the intervention districts due to a lower case fatality ratio at peripheral level. The HMM program tripled accessibility to care and improved promptness of care.

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COMPARISON OF THE EFFECTIVENESS OF ITNS, IRS, AND CHEMOTHERAPEUTIC INTERVENTIONS, IN REDUCING MALARIA TRANSMISSION, USED INDIVIDUALLY AND IN COMBINATION, THROUGH A MATHEMATICAL MODEL

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ITNs and IRS form the prevention cornerstone of most current malaria control strategies. While some programs begin to combine the two interventions, little is known about the effectiveness and cost-effectiveness of such combinations. We use mathematical models to compare the effectiveness of adding IRS to populations with high levels of ITN coverage, and vice versa, and of adding a chemotherapeutic intervention that reduces human infectiousness to mosquitoes. We have developed a deterministic dynamical systems model for mosquito infection and survival, where the host exposure to infectious bites, and mosquito survival probabilities, vary with the host. We link this model to an individual-based stochastic simulation model for malaria in humans that incorporates naturally acquired immunity, effects of co-infection, and variations in human infectiousness. This full model allows us to compare the effects on transmission of vector control and/or chemotherapeutic interventions (achieving high clearance of gametocytes). For given transmission levels, the model then allows calculation of morbidity and mortality rates. The model can reproduce malaria patterns in endemic areas, including non-monotonic relationships between human infectivity to mosquitoes

and EIR, and quantitative relationships between increasing ITN and IRS coverage and a range of entomological outcomes. When applied in a population with *An. gambiae* as the primary vector, our model indicates that while both IRS (with DDT) and ITNs provide some personal protection, humans with only ITNs are better protected than those with only IRS. When adding a second vector control intervention, it is more effective to cover the unprotected population first. Very high coverage with at least two transmission reduction interventions can potentially lead to interruption of transmission. In conclusion, we can simultaneously capture in a mathematical model the dynamics of mosquito ecology, malaria epidemiology, interventions, and human demography. Combining the human and entomological models leads to plausible quantitative predictions and comparisons of effects of a comprehensive set of different interventions and their combinations. Although our model does not fully represent transmission heterogeneity, these findings should be useful for prioritizing and designing controlled field trials of combined interventions.

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IMPACT OF LARVICIDING ON MALARIA IN THE GAMBIA

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Anti-larval measures may be a useful component of integrated vector management for malaria control in sub-Saharan Africa. As part of a large scale intervention of microbial larviciding in a rural area of The Gambia with large-scale seasonal flooding, we examined the impact on malaria infection, incidence and prevalence in children and adults. In four zones of approximately 100km^2 each located around the middle reaches of the Gambia River, microbial larvicide (Bti) was applied weekly to breeding sites during the main period of transmission in each of two years (2006 and 2007) using a cross-over study design (see abstract S. Majambere for further details and entomology results). Fifty sentinel villages were selected across the zones as representative of the population and all residents were enumerated (tot. pop.: 14,112; 4656 (33%) < 10 years old) and data on known malaria risk factors collected. The impact on clinical disease was measured during the main transmission season primarily by passive detection of malaria attacks in cohorts of approximately 500 children/zone, using two study nurses posted to each zone who worked in close collaboration with village health workers (VHW). The impact on transmission was assessed by time to infection in four cohorts of approximately 140 aparasitemic adults/zone who agreed to provide a capillary blood sample every two weeks. In addition, prevalence of *Plasmodium* infection and anaemia was measured at the start of each malaria season in adults and children and the child cohort was also surveyed at the end of the season. The intervention was successful at the entomological level although the impact on adult female *Anopheles gambiae* s.l. was less marked than that on larvae and varied across the zones. Analysis of the epidemiological impact is ongoing. Compliance was good, over 80%, in all the cohorts and this was assisted by the excellent study nurse/VHW collaboration. Preliminary results indicate a minimal effect of the intervention but show considerable variation of both malaria attacks and infection rates among the four zones and between years. The zones varied considerably for known malaria risk factors, including distance of study villages from the floodplain, the use of bed nets, house construction (open or closed eaves) and ethnicity. The results will be presented taking into account the entomological results and the possible effects of known malaria risk factors.

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A CROSS-NATIONAL COMPARISON OF INSECTICIDE-TREATED NET HOUSEHOLD POSSESSION AND USE AMONG CHILDREN UNDER FIVE YEARS OLD AND PREGNANT WOMEN

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Nationally representative data between 2003-2006 from 13 Demographic and Health Surveys (DHS), 2 Multiple Indicator Cluster Surveys (MICS), and 1 Malaria Indicator Survey (MIS) were used to investigate relationships between household insecticide-treated net (ITN) possession and use among children under 5 years old and pregnant women. The proportion of households with a child under 5 years old reporting ownership of at least one ITN ranged from 3.0% to 42.0%, while the proportion of children who slept under an ITN ranged from 1.5% to 20.0%. The proportion of pregnant women who slept under an ITN ranged from 1.1% to 19.7%. An inverse relationship exists between the proportion of children under 5 using an ITN and the mean intra-household ratio of residents per ITN ($F_{1,12} = 7.83$, $P = 0.016$, $R^2 = 0.395$), after controlling for season and survey year. Within-country analyses showed that intra-household access to ITNs (ratio of resident per ITN), was the strongest and most consistent household factor associated with ITN use among children. Within households that have at least one ITN, many children and pregnant women are still not sleeping under them. We argue that gaps between ITN use and possession will likely persist in the absence of achieving an intra-household ratio of no more than 2 people per ITN.

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POTENTIAL CONTRIBUTION OF SERO-EPIDEMIOLOGICAL ANALYSIS FOR MALARIA ELIMINATION: HISTORICAL AND CURRENT PERSPECTIVES

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As malaria transmission falls, whether due to successful control programmes or changing environmental or socio-economic conditions, accurately measuring transmission will require alternative tools. The classical gold standard measures such as entomological inoculation rate (EIR) and parasite rate are subject to seasonal variation and lack precision at low endemicity. Anti-malarial antibody sero-conversion rates derived from age sero-prevalence profiles reflect cumulative malaria exposure and are an alternate measure of transmission refracted through the human population. Sero-conversion rates have been shown to correlate with EIR and could be used to monitor changes in transmission, as reported previously. This approach is not new and was used in the 1970's to evaluate malaria eradication attempts. Bruce-Chwatt and colleagues examined more than 6000 sera from Mauritius and found a virtual absence of sero-positivity in children under 5 years of age, confirming the absence of transmission in the past 5 years. A similar phenomenon was observed in Greece with a waning of immunity in older individuals indicating a drop in exposure. Serological evaluation during the landmark Garki study showed these measures to be sensitive to the initial reduction in transmission and its subsequent return to pre-intervention levels, as reported previously. However, these historical studies were limited by a lack of standardised antigens and of standardisable high throughput assays. Current techniques using recombinant antigens with a range of immunogenicities, high throughput ELISA assays and statistical analysis which incorporates uses both the prevalence and intensity of antibody response to better understand the factors affecting malaria transmission. Here we present a reexamination of the historical data with current analytical tools and compare it with newly collected data from Tanzania

and elsewhere. Special emphasis will be made on the serological contribution to monitoring malaria elimination.

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MULTIDRUG RESISTANT VIVAX MALARIA: A MAJOR CAUSE OF MORBIDITY IN EARLY LIFE

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In malaria endemic areas, infants are relatively protected from symptomatic and severe *Plasmodium falciparum* malaria, but less is known on the effect of *P. vivax* infections. To define the epidemiology of malaria in the first year of life in an area with highly prevalent multidrug resistant *P. falciparum* and *P. vivax,* data were gathered on all infants attending a referral hospital in Timika, southern Papua, Indonesia. Between April 2004 to December 2007 14% (3,825/26,890) of all infants reviewed in outpatients were treated for malaria. During the same period 3,619 infants were admitted to hospital of whom 1,396 (39%) had malaria, with infection equally attributable to *P. falciparum* and *P. vivax* (43% each). The mean haemoglobin concentration in infants admitted with malaria was 6.8g/dl [95%CI: 6.7-6.9], compared to 8.0g/dl [95%CI 7.8-8.1] recorded in 686 infants admitted without malaria during the same period ($p<0.001$). Severe anaemia was present in 31% (175/572) of infants with *P. vivax* compared to (24% 137/560) with *P. falciparum*; OR=1.36 [95%CI 1.04-1.78], p=0.025. Overall the case fatality rate was 2.2% (13/595) in those with *P. falciparum* compared to 1.0% (6/592) with *P. vivax* (p=0.272). Further details were available in 70% (161/229) of babies less than 3 months of age, with *P. falciparum* infection present in 45 (28%), *P. vivax* in 97 (60%), mixed infections in 17 (11%) and *P. malariae* in two (1.2%) cases. 30% (46/155)of babies were severely anaemic (46/155). Fever, spleen enlargements and respiratory insufficiency occurred in 81%, 47% and 57% and were equally present in infants with different species of infections.In conclusion, our results demonstrate a significant health risk to infants, including a neglected burden in the first 3 months of life, particularly due to *P. vivax* infections. The findings highlight the need for early diagnosis and prompt treatment. The spectrum of disease secondary to multidrug resistant *P. falciparum* and *P. vivax* in this age group will be discussed.

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PNEUMOCOCCAL DISEASE IN MALI AND THE INTRODUCTION OF 7-VALENT VACCINE INTO THE EPI

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Streptococcus pneumoniae is a major public help problem worldwide. Based on assessment of disease burden and predicted impact, policymakers in industrialized countries are increasingly incorporating conjugate pneumococcal vaccine into their routine infant immunization programs. In developing countries the paucity of similar analysis and the lack of data on serotype distribution have impeded decisions about the need for routine immunization and the specifications of a suitable vaccine to prevent this infection. Mali, a Sub-Saharan country, in West Africa has been able to demonstrate the *Haemophilus influenzae* type b (Hib) and pneumococcal disease burden and to introduce Hib vaccine into the routine immunization program since 2005. More recently, Mali has completed an application for pneumococcal 7-valent vaccine. Since February 2002, the Center for Vaccine Development of Mali has established hospital based surveillance in the main pediatric hospital in Bamako. Children age 0-15 years admitted to that principal pediatric hospital with a fever $\geq 39^{o}$ C or a syndrome compatible with an invasive bacterial infection were invited to participate in a study in which blood and relevant body fluid (e.g. cerebrospinal fluid (CSF)) were cultured to identify bacteria using standard microbiologic techniques. Data were generated and used to convince decision makers to introduce Hib and pneumococcal 7-vaccine into the routine immunization program in Mali. From July 2002 to June 2007, the incidence of pneumococcal disease has been 127.6, 20.5 and 4.8 per 100,000 among 0- to 11-month old, 1- to 4-year olds and 5- to 15-year olds. The case fatality rate has been 18.5%. Among the isolates that have been serotyped to date, 22% of them are included in the 7-valent vaccine. The Malian Ministry of Health has decided to introduce pneumococcal 7-valent vaccine into the routine immunization program of Mali in 2008-2009. In conclusion, these preliminary results demonstrate that pneumococcal infections are an important cause of morbidity and mortality among Malian children and suggest that multivalent conjugate pneumococcal vaccines have the potential to substantially reduce this disease burden.

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NEW DIAGNOSTIC APPROACHES FOR PEDIATRIC TB AMONG PERUVIAN CHILDREN

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The diagnosis of pulmonary tuberculosis (PTB) presents challenges in children, as symptoms are non-specific, sputa are absent, and *Mycobacterium tuberculosis* (MTB) cultures and smears are often negative. Children with HIV co-infection present special challenges, as they present with atypical symptoms of PTB. To investigate new diagnostic approaches for TB in children for resource-poor countries, we conducted a clinical trial in Lima, Peru among children without evidence of HIV infection (218 cases with clinical evidence of PTB and 241 controls) and children with confirmed HIV infection (85 cases with clinical evidence of PTB and 46 controls). Cases of PTB were defined by Stegen-Toledo clinical score. Cases were investigated with two specimens of each type (gastric aspirate [GA], nasopharyngeal aspirate [NPA], and stool specimens). Specimens collected from controls included one NPA and two stools. Specimens were examined by 1) auramine stain, 2) hemi-nested IS6110 PCR assay, and 3) cultured by the Microscopic Observation Drug Susceptibility (MODS) technique and standard Lowenstein Jensen (LJ) technique. 22 HIV-negative subjects (10%) had at least one positive MTB culture (from stool in 4 cases, NPA 12 cases, and GA 22 cases). 60 culture-positive specimens were obtained from this group, and MODS provided significantly more positive cultures than LJ (33/38 specimens culture-positive by MODS vs. 21/38 by LJ, $p<0.001$). Isolation was faster by MODS than LJ (mean 10 days vs. 25 days, $p<0.001$). A single HIV-infected case was culture positive (GA only), and recovery of MTB from HIV negative cases was significantly more frequent than from HIV+ cases (p=0.008). MTB was not cultured from any controls. HIV-negative cases who were culture-positive were more likely to have at least one PCR-positive specimen than culture-negative cases (61.9% vs. 20.3%, p=0.0001), but concordance between culture and PCR was poor. In conclusion, recovery of MTB from children with clinical evidence of PTB was profoundly lower among HIV + children. Isolation of MTB by MODS demonstrated greater yield and faster recovery than by LJ. Recovery of MTB from NPA and stool specimens was significantly lower than from GA specimens. Children with culture-confirmed TB were three

times more likely to have at least one PCR-positive specimen than culture-negative counterparts; concordance between PCR and culture was poor.

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A REPORT OF THE FIRST TWO AND A HALF YEARS OF A COMPREHENSIVE INFLUENZA SENTINEL SURVEILLANCE SYSTEM IN KENYA AND ITS IMPLICATIONS FOR VACCINE STRAIN SELECTION IN THE EAST AFRICA REGION

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Sentinel influenza surveillance is critical for the detection of antigenic drift and shift and vaccine selection. No consistent influenza surveillance has been conducted in East Africa despite evidence of ILI. The Southern Hemisphere influenza vaccine is used in the region. The United States Army Medical Research Unit-Kenya (USAMRU-K) and the Kenya Medical Research Institute developed an influenza surveillance network at 8 hospitals dispersed throughout Kenya in 2006. Nasopharyngeal specimens are screened by real-time RT-PCR and then identified by HAI after culture. Isolates are characterized by sequencing. 878 of 4615 specimens (19%) tested positive for influenza A and 230 (5%) for influenza B. 94 isolates were characterized and submitted to GenBank and WHO. Influenza activity increased during the cool months-May through October. This seasonal trend disappears on Kenya's tropical coast. Influenza B (Victoria lineage) predominated in 2006. Midway through the 2007 season, influenza A (H1N1) replaced influenza A (H3N2) as the dominant strain. From 2006 through early 2007, all detected strains matched the S. Hemisphere vaccine strains, but the emerging H1N1 did not. Phylogenetic analysis of HA1 sequences revealed that the H1N1 isolates formed distinct branches from A/New Caledonia/20/99(H1N1) and A/Solomon Islands/3/2006 (H1N1), the 2007 and 2008 S. Hemisphere vaccine strains. The Kenya strains-first detected in August 2007-clustered around A/Brisbane/59/2007(H1N1), the 2007-08 N. Hemisphere vaccine strain. 60% of our H1N1 isolates demonstrated at least four-fold lower HAI titers compared to the S. Hemisphere vaccine reference antigen. Our surveillance establishes influenza as a significant cause of respiratory disease in Kenya displaying clear seasonal trends. Detection of the circulating Kenya H1N1 virus challenges current influenza vaccine policy though future trends must be studied. Because the emergence of the Kenya H1N1 strain precedes its usage in vaccine formulations, Kenya may be an epidemiologically interesting site and warrants continued monitoring.

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THE EPIDEMIOLOGY OF HUMAN PARAINFLUENZA VIRUS-ASSOCIATED PNEUMONIA IN THAILAND

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In the US, human parainfluenza virus (HPIV) is an important cause of croup, bronchiolitis, and pneumonia in young children, with peaks of HPIV1 biennially in the fall and HPIV3 circulating annually in spring/summer months. Little is known about the epidemiology of human parainfluenza viruses (HPIV) in the tropics. We conducted active surveillance in all 8 hospitals in Sa Kaeo Province, Thailand, to identify patients admitted between Sep 2003 - Aug 2006 with clinical pneumonia (signs or symptoms of acute respiratory infection) and chest radiographs performed. An independent panel of radiologists determined radiographic evidence of pneumonia. We tested nasopharyngeal swabs for HPIV types 1, 2, and 3 by reverse transcriptase polymerase chain reaction (RT-PCR) and enzyme immunoassay on paired sera (first 2 years only). We considered negative RT-PCR with positive serology for both HPIV1 and HPIV3 as positive for HPIV of indeterminate type. Of 3248 pneumonia cases, 198 (6%) had HPIV infection (types 1=83, 2=10, 3=96, indeterminate=9). Of, 54 HPIV patient radiographs evaluated to date, 53 (98%) had radiographic evidence of pneumonia. Average annual incidence of HPIV pneumonia per 100,000 population was 114 for children <5 years, 3 for 5-64 years, and 29 for ≥ 65 years. Five patients (ages 39-76 years) died: two with HPIV1 and three with HPIV3. Viral co-infections occurred in 19 (14%) cases; 9 (7%) with influenza, 6 (4%) with adenovirus and 4 (2%) with respiratory syncytial virus (RSV). There were peaks in HPIV1 incidence in Feb - Apr in 2004 and 2006. HPIV3 peaked in Feb - Mar in 2005 and 2006, with smaller numbers in 2004. In Thailand, the incidence of patients hospitalized with HPIV pneumonia was high in children <5 years and the elderly, and was similar to RSV, a common cause of severe respiratory infection among children. HPIV seasonality was different compared to temperate climates and the proportion of HPIV1 and 3 infections appeared to be interdependent. These findings may have important implications for development and evaluation of candidate HPIV vaccines.

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RESPIRATORY DISEASE SURVEILLANCE IN 6 ROYAL THAI ARMY HOSPITALS ALONG THAI BORDERS

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The Royal Thai Army (RTA) has 34 hospitals that serve the military and surrounding civilian population with 10 located in rural areas with high border traffic near Myanmar, Malaysia, Laos, and Cambodia. In order to determine the seasonal and regional etiology of respiratory disease, enhance outbreak response capability and detection of avian influenza, and to enable the safe collection of clinical samples, the RTA established a network of respiratory disease surveillance sites at 6 border area hospitals. Adults who present with a history of fever and cough or sore throat or rhinorrhea are consented and enrolled. Respiratory samples are tested with a rapid test for influenza A and B on-site. Aliquots are sent to AFRIMS in Bangkok for influenza genotyping using realtime RT-PCR and to identify other respiratory pathogens by MassTag PCR. MassTag PCR amplifies genetic material utilizing domain-specific primers tagged with a unique mass to allow spectrometric analysis and detection of up to 30 respiratory pathogens. Our panel identifies influenza A viruses, influenza B, both genotypes of respiratory syncytial virus (RSV) and human metapnuemovirus (hMPV), enterovirus, adenovirus, 2 human corinavirus, 3 human coronaviruses including the causative agent of SARS, 4 subtypes of human parainfluenza virus (PIV), *Chlamydia pneumoniae, Mycoplasma pneumoniae, Streptococcus pneumoniae, Legionella pneumophila*, *Haemophilus influenzae* and *Neisseria meningitidis*. We collected 630 samples from March 2007 to January 2008. Using rapid testing, 53 (8%) were positive for influenza A and 17 (3%) for influenza B. To date,

409 samples have been tested by realtime RT-PCR, 89 (21.8%) were positive for influenza A (54 H1 and 35 H3 subtypes) and 17 (4.2%) were influenza B. MassTag PCR revealed 143 of 409 samples were infected by virus (Influenza A, 20-B, RSV-A, -B, hMPV, enterovirus, adenovirus and PIV 4 subtypes) and bacteria (*M. pneumoniae*, *H. influenzae* and *S. pneumoniae*). In addition MassTag PCR identified co-infected of pathogens such as RSV-B with *S. pneumoniae*, adenovirus + corinavirus-OC43+ *H. influenzae,* and *H. influenzae* with *S. pneumoniae,* influenza A, or PIV-1. 51% of samples remained unknown. Testing for many respiratory pathogens provides a comprehensive picture of disease burden in this population and greatly improves our ability to detect and respond to emerging diseases including avian influenza.

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EPIDEMIOLOGY AND GENETIC CHARACTERIZATION OF INFLUENZA VIRUSES ISOLATED FROM PATIENTS ENROLLED IN A HOSPITAL-BASED FEBRILE SURVEILLANCE STUDY IN CAMBODIA

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Globally, influenza remains a significant cause of morbidity and mortality. In Indochina, few reports have described the epidemiology of influenza. In December 2006, we established a hospital-based surveillance network in two Cambodian provinces to determine the etiologies contributing to acute febrile illness of unknown origin. From December 2006 through January 2008, seven health care centers collected samples from patients with undifferentiated fever. Samples from 951 subjects were screened by real-time RT-PCR for human influenza. A total of 279 (29.3%) patient samples were positive for influenza viruses. Febrile cases and influenza infection rates climbed between June 2008 and January 2008, coinciding with the peak during the rainy season. Influenza A (H1N1 and H3N2) viruses were identified in 169 (60.6%) of the influenza virus positive population, while influenza B was detected in 110 (39.4%). While influenza transmission was evident year round, infection from AH3N2 and B viruses emerged later in the year. There was a statistically significant difference between the age of patients positive for influenza compared to those who were negative, 10 years (5 - 16) and 12 (6 - 27), respectively ($p < 0.0001$). The symptoms that were statistically more prevalent among the influenza positive participants were cough (70.1% vs 52.8%; $p < 0.0001$) and sore throat (52.8% vs 43.5%; $p = 0.01$). Malaise (34.8% vs 44.0%; $p = 0.009$), muscle aches (24.4% vs 34.8%; $p = 0.002$) and shortness of breath (28.4% vs 36.9%; $p = 0.01$) were more prevalent among the influenza negative participants as compared to influenza positive participants. Influenza viruses were isolated by culturing swab samples in MDCK cells or in 9- to 11-day-old embryonated hen's eggs. The predominant subtype identified was influenza A (H1N1); the majority of H1N1 viruses tested were antigenically related to A/Solomon Islands/03/2006 virus. All the influenza B viruses tested belonged to B/Victoria lineage. The majority of H3N2 viruses tested were closely related to reference strain A/Brisbane/10/2007. Phylogenetic analysis of the H1 HA1 gene was conducted to determine the relationship between the Cambodian isolates to other viruses worldwide. These data provide evidence that during the study period viral influenza infections in Cambodia contributed to nearly one-third of all surveyed febrile illness cases and point to the importance of ILI surveillance in tropical regions.

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EVALUATION OF SYMPTOM RECALL DURING A TWO-WEEK INTERVAL IN HOME-BASED MORBIDITY SURVEILLANCE, KISUMU AND NAIROBI, KENYA

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In African settings with poor access to health care, more disease may be identified in home-based than facility-based surveillance. However, the optimal recall period to capture symptoms is unknown. We evaluated the impact of recall time on the calculation of disease rates. From July 2006 - June 2007, fortnightly home visits were made in rural western Kenya (n =25,000 people) and a Nairobi informal settlement (n=30,000). Symptoms in the last 2 weeks were recorded, including specific dates for cough, fever, and diarrhea. In Memba village in the western Kenya site (n=1,396) in November 2006, twice per week home visits were conducted in addition to the fortnightly visits. Severe illnesses in children were defined as those in which a key symptom was accompanied by a danger sign from WHO's Integrated Management of Childhood Illness. Prevalence rates were modeled using Poisson regression. More recent symptoms were more frequently reported in both sites. Among children < 5 years in the rural site, the prevalence rate ratios of fever, diarrhea and cough comparing 0-6 days with 7-13 days before the home visit were 2.6 (95% CI 2.5-2.7), 2.5 (95% CI 2.3-2.6), and 2.6 (2.5-2.7), respectively. Among adults, the rate ratios for fever, diarrhea and cough were 2.2 (95% CI, 2.2-2.3), 2.1 (95% CI 1.9-2.2) and 2.3 (95% CI, 2.2-2.3), respectively. Rate ratios were similar in rural and urban sites. Rates peaked 1 day before the visit date and began to decrease significantly by 3 days before the visit for children and 4 days for adults. Severe illnesses among children also showed decay in recall, although to a lesser degree (rate ratios ranged from 1.8 to 2.3). In Memba village, rates were 1.5 - 3 times higher for all symptoms, except adult diarrhea, with twice per week visits than with fortnightly visits. In conclusion, using 2-week recall periods underestimates true disease prevalence. The finding was consistent across syndrome, age group, and study site. More accurate disease rates would be achieved by using a 3-5 day recall period.

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THE STOICHIOMETRY OF ANTIBODY-MEDIATED NEUTRALIZATION OF WEST NILE VIRUS INFECTION: FACTORS THAT GOVERN ANTIBODY POTENCY

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Flaviviruses are a group of positive-stranded RNA viruses that have a global impact on public health due to their widespread distribution and their ability to cause morbidity and mortality in humans. West Nile virus (WNV) is a mosquito-borne encephalitic member of this genus that has emerged as a significant pathogen in North America since its introduction in 1999. WNV incorporate 180 envelope (E) proteins that orchestrate the process of virus entry and are the primary target of neutralizing antibodies. We have previously established that neutralization of WNV is a "multiple hit" phenomenon that requires engagement by approximately 30 antibodies. Antibody affinity and epitope accessibility are two important factors that govern whether a particular antibody can dock on the virion with a stoichiometry that exceeds the threshold required for neutralization. Serum complement has been shown previously to enhance the protective activity of antibodies *in vitro* through undefined mechanisms. In this study,

we employed a series of quantitative functional approaches to define precisely how complement augments antibody-mediated neutralization of West Nile virus. Remarkably, we found that the complement opsonin C1q, by itself, reduces the stoichiometric threshold required for neutralization, and improves the efficiency of neutralization *in vitro* and *in vivo* in an isotype-dependent fashion. Importantly, for particular IgG isotypes, this reduced stoichiometric threshold falls below the minimum number of antibodies required for the antibody-dependent enhancement phenomena associated with more severe manifestations of secondary dengue virus infection, and explains mechanistically our previous findings that purified C1q can limit ADE *in vitro* and *in vivo*.

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MOLECULAR BASIS FOR THE RESISTANCE OF WEST NILE VIRUS TO ANTIVIRAL ACTIVITY OF OAS1B

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West Nile virus (WNV) is an emerging mosquito-borne flavivirus of global significance that can infect the central nervous system and cause severe neurological disease. Host genetic factors that influence the antiviral effects of type-I interferons have previously been defined in a mouse model of WNV-induced encephalitis. Mice derived from wild species are resistant to WNV-induced disease while WNV infection of inbred laboratory strains is uniformly fatal. A WNV resistant locus, termed Wnv, was mapped to a cluster of 2'-5'-Oligoadenylate synthetase (Oas) genes, which encodes a group of interferon-induced enzymes that regulate innate immunity. The Oas system activates RNase L and targets RNA virus destruction by degrading single-stranded RNA. Analysis of the Wnv locus in resistant and sensitive mice revealed a point mutation in the Oas1b gene in all sensitive mice that is absent from resistant mice. This mutation results in a premature stop codon, leading to a defective enzyme. We previously demonstrated that ectopic expression of Oas1b in murine cells inhibits WNV infection at the early stages of the virus life cycle. Serial passage of a neurovirulent WNV strain in murine fibroblasts overexpressing Oas1b gave rise to an escape variant that resists the antiviral activity of Oas1b. We observe that Oas1b-overexpressing cells infected with the variant virus produce more viral RNA and proteins than cells infected with the parental virus. This might result from enhanced virus replication by the variant virus. The escape variant contains four amino acid substitutions in the genes encoding the prM, E, NS3, and the 2K fragment at the junction between NS4A and NS4B. In vivo experiments show that these substitutions attenuate neuroinvasiveness in mice. We plan to investigate the impact of each of the observed amino acid substitutions on WNV pathogenesis and Oas1b activity in comparison to the parental WNV strain. These experiments may help to define molecular mechanism by which WNV resists Oas1b and, more generally, how arboviruses circumvent innate immunity.

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WEST NILE VIRUS-VECTOR INTERACTIONS ARE AFFECTED BY GLYCOSYLATION OF THE VIRAL ENVELOPE PROTEIN

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Many, but not all, strains of *West Nile virus* (WNV) contain a single N-linked glycosylation site on their envelope (E) proteins. Previous studies have shown that E-glycosylated strains are more neuroinvasive in mice than non-glycosylated strains. E protein glycosylation also appears to play a role in attachment and entry of WNV into host cells *in vitro*; however, studies examining how E protein glycosylation affects the interactions of WNV with its mosquito vectors *in vivo* have not yet been performed. We mutated the E protein glycosylation site in a previously described full-length clone of the NY99 genotype of WNV (WT) from NYS to IYS. Digestion of the WT E protein with PNGase-F resulted in a mobility shift by SDS-PAGE, consistent with deglycosylation, whereas both the digested and undigested N154I E protein migrated at the same position as the digested WT E protein, confirming that the N154I mutation resulted in a non-glycosylated protein. The N154I virus replicated with similar efficiency to WT *in vitro* in both mosquito and avian cells, but its *in vivo* replication in *Culex pipiens* and *Cx. tarsalis* mosquitoes was decreased relative to that of the WT virus. To determine whether glycosylation of the WNV E protein affected viral infectivity and transmission *in vivo*, we fed *Cx. pipiens* and *Cx. tarsalis* mosquitoes a bloodmeal containing either the WT virus or the N154I virus and examined infection, dissemination, and transmission rates at 5, 7, 9, and 14 days post-feeding (dpf). At early times post-feeding, infection rates were significantly greater in *Cx. pipiens* that fed on the WT virus; however, in *Cx. tarsalis* infection rates were significantly greater in mosquitoes that fed on the N154I virus. By 9dpf, dissemination rates in both species were significantly greater in mosquitoes that fed on WT than N154I. In addition, transmission of WNV by mosquitoes that fed on the N154I virus was associated with viral reversion to a WT glycosylation phenotype. These results suggest that loss of E protein glycosylation can severely inhibit WNV spread in the mosquito vector.

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REPLIVAX WN, A SINGLE-CYCLE FLAVIVIRUS VACCINE, IS SAFE AND EFFICACIOUS IN A RHESUS MACAQUE MODEL OF WEST NILE DISEASE

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Safer vaccines are needed to prevent flavivirus diseases. To develop these products we produced a single-cycle West Nile virus (RepliVAX WN) that blends the replicative capacity of live-attenuated viral vaccines with the safety of inactivated formulations. RepliVAX WN contains a truncated capsid protein (C) that allows for propagation in cells where C is supplied in trans, but does not produce infectious progeny in normal cells. Instead, RepliVAX WN-infected cells produce subviral particles containing prM, M and E that are likely the basis for RepliVAX WN-induced protective immunity. We have previously demonstrated that RepliVAX WN can be safely propagated in C-expressing cell lines, and in the process developed a second-generation RepliVAX WN with enhanced growth characteristics and potency capable of protecting mice and hamsters from West Nile encephalitis, as reported previously. Here we describe the initial evaluation of potency and efficacy of RepliVAX WN in a non-human primate model of WN disease. A single subcutaneous vaccination of rhesus macaques with 10^6 IU of RepliVAX WN was safe and well-tolerated, and elicited neutralizing antibody (neut) titers ranging from 1:32 to 1:64 and readily detectible anti-WNV IgM and IgG responses. An additional dose of 10^6 IU administered to two of the animals resulted in neut titers of 1:32 and 1:128. After challenge with 10^5 pfu of WNV, three of four vaccinated animals were completely protected from viremia, compared to an unvaccinated animal which developed a sustained viremia of greater than 2 logs for 4 days. Taken together, these results demonstrate the utility of RepliVAX WN as a safe, economical, and efficacious vaccine candidate to prevent West Nile encephalitis.

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ECOLOGY OF WEST NILE VIRUS IN GUATEMALA

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West Nile virus (WNV), a mosquito-borne *Flavivirus (*Flaviviridae), spread to Guatemala in 2003 but is not currenty recognized as a significant public health pathogen in tropical America, due to lack of confirmed neurologic disease cases in humans. In 2005, we established a network of sentinel chickens in four Departments of Guatemala in order to detect a WNV transmission focus, and detected multiple seroconversions in the Atlantic Coast Department of Izabal in 2006. An ecology study was established in the municipality of Puerto Barrios, Izabal, beginning in 2006. We monitored transmission rates in 10 sentinel chickens flocks over a 3-year period in Puerto Barrios. In July 2007, transmission spiked, and isolations were made from several species of Culex mosquitoes. High seroprevalence rates were detected in free-ranging bird species, particularly in the orders Passeriformes and Columbiformes. Seroprevalence combined with relative abundance data implicates two passerine species, Great-tailed Grackle (*Quiscalus mexicanus*) and Clay-colored Robin (*Turdus grayi*), as important vertebrate reservoirs. Implications for public health are discussed.

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DETECTION OF RNA FROM A NOVEL WEST NILE-LIKE VIRUS AND HIGH PREVALENCE OF AN INSECT-SPECIFIC FLAVIVIRUS IN MOSQUITOES IN THE YUCATAN PENINSULA OF MEXICO

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As part of a comprehensive surveillance program for arboviruses in the Yucatan Peninsula of Mexico, 96,687 mosquitoes from 26 species were collected in the states of Yucatan and Quintana Roo from January to December 2007. Three mosquito pools caused cytopathic effect in Vero cells. Two isolates were orthobunyaviruses (Cache Valley virus and Kairi virus) whereas the identity of the third virus was not determined. A subset of mosquitoes was also tested directly by RT-PCR using West Nile virus (WNV), flavivirus, alphavirus and orthobunyavirus-specific primers. A total of 7009 *Culex quinquefasciatus* in 210 pools were analyzed. Flavivirus RNA was detected in 146 (70%) pools, and the flavivirus minimum infection rate was 20.8. Sixty-seven PCR products were sequenced. The nucleotide sequence of one PCR product was most closely related (71-73% identical) to the homologous regions of St. Louis encephalitis (SLEV), Ilheus (ILHV), Japanese encephalitis (JEV), Usutu, Rocio, Murray Valley encephalitis (MVEV) and West Nile viruses. The deduced amino acid sequence was most closely related (77-79% identical, 90-92% similar) to the homologous regions of WNV, ILHV, SLEV, JEV and MVEV. These data suggest that a novel flavivirus that is genetically equidistant from a number of other flaviviruses, including WNV, is circulating in Mexico. Although we successfully amplified viral RNA, we were not able to obtain an isolate by virus isolation in Vero cells or suckling mouse brain inoculation. The other 66 PCR products correspond to *Culex* flavivirus (CxFV), an insect-specific flavivirus first isolated in Japan in 2003. CxFV was isolated from approximately one in four homogenates positive by RT-PCR after 3 blind passages in C6/36 cells. CxFV genomic sequencing experiments are in progress.

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TEMPORAL AND SPATIAL RELATIONSHIP BETWEEN FLANDERS VIRUS AND WEST NILE VIRUS IN THE SOUTHEASTERN UNITED STATES

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Flanders virus is a Rhabdovirus that has been identified in various parts of the U.S. as being associated with *Culex* mosquitoes. Mosquito samples from Georgia and Tennessee were tested for West Nile virus (WNV) and Flanders virus (FLDV). Over 24,000 *Culex* mosquito pools from Georgia from 2004-2007 and 9,738 pools from Tennessee from 2006-2007 were tested via virus isolation and Realtime RT-PCR. The rate of infected pools was as high as 30% for FLDV and 60% for WNV in certain locations. The appearance of FLDV in mosquito pools consistently preceded isolation of WNV at the zip code level. The average time between the first FLDV and the first WNV was four weeks (range 1-13 weeks). Ten percent of mosquito pools were coinfected with both FLDV and WNV. We found that FLDV typically appears in May and peaks in July, whereas WNV appears in June and peaks in August in the southeastern U.S. FLDV appears to share the same ecological niche with WNV, being identified from the same locations and mosquito species as WNV. We discuss the potential use of FLDV as an early warning for the surveillance of WNV and the implications of large populations of *Culex* mosquitoes that are infected with FLDV.

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HDP- A NOVEL HEME DETOXIFICATION PROTEIN IN THE MALARIA PARASITE

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During the intraerythrocytic stages, the malaria parasite degrades host cell hemoglobin. This leads to the release of toxic heme which is immediately detoxified by its conversion into an inert, non-toxic hemozoin crystal. This is a key biological process in the parasite as drugs that bind to heme and prevent its conversion to hemozoin demonstrate potent anti-malaria activity. As a part of our functional genomics initiative aimed at identifying novel *Plasmodium* proteins involved in disease pathogenesis, we discovered a parasite-specific Heme Detoxification Protein (HDP) and demonstrated its role in hemozoin formation in the malaria parasite. HDP is a single copy gene that bound heme with high affinity and was extremely potent at converting the bound heme into hemozoin. Compared to lipids, both, native and recombinantly produced PfHDP were 2000-fold more potent in producing hemozoin *in-vitro*. Once expressed, the parasite uses an "outbound-inbound" circuitous trafficking route, by initially secreting the protein out into the infected red blood cell cytoplasm, followed by, a subsequent uptake along with endocytosis of the host cytosol and transport to the food vacuole, the site of hemozoin formation. HDP was highly conserved with 60 % sequence identity in the *Plasmodium* genus. The protein was found to be functionally conserved as recombinantly produced *P. vivax* and *P. yoelii* HDP was equally potent as its *P. falciparum* ortholog in producing hemozoin. Discovery of HDP fills an important gap in our understanding of the mechanism of hemozoin formation. Identification of new targets is vital for developing the next

generation of antimalarials, and with our discovery, drugs that specifically target HDP can now be developed.

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DEFINING THE INTERACTION BETWEEN *PLASMODIUM FALCIPARUM* SKELETON BINDING PROTEIN 1 AND THE MEMBRANE SKELETON OF MALARIA-INFECTED RED BLOOD CELLS

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During development inside red blood cells (RBCs), *Plasmodium falciparum* malaria parasites export a number of proteins beyond the confines of their own plasma membrane where they associate with the RBC membrane skeleton. Here they participate in protein-protein interactions with both RBC proteins and other parasite proteins and assemble into complex multi-component structures. These interactions cause profound changes to the biophysical properties of RBCs, particularly reduced cell deformability and increased adhesiveness, which underpin the severe and often fatal clinical manifestations that accompany the disease. *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) is one such exported parasite protein that plays a major role in parasite virulence since its exposure on the surface of parasitised RBCs (pRBCs) mediates adhesion to vascular endothelium. En route to the RBC membrane, PfEMP1 transiently associates with Maurer's clefts (MCs), parasite-derived membranous structures in the RBC cytoplasm. Since we have previously shown that a resident MC protein, skeleton binding protein 1 (SBP1) is essential for the placement of PfEMP1 onto the pRBC surface, we hypothesised that the function of SBP1 may be to target MCs to the RBC membrane and therefore set out to identify the putative SBP1 binding partner. Using a combination of biochemical and biophysical assays including solid phase binding, protein pull-down and surface plasmon resonance together with recombinant malaria and host protein fragments, we have defined the region of SBP1 that binds specifically to a defined sub-domain of a major phosphoprotein component of the RBC membrane skeleton. We propose that this interaction serves to anchor MCs to the RBC membrane skeleton and is critical for the translocation of PfEMP1 onto the RBC surface.

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BROAD-SPECTRUM ANTI-INFECTIVE DRUGS THAT TARGET METABOLIC PATHWAYS AND *ENTAMOEBA HISTOLYTICA* TROPHOZOITE GROWTH

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Amebiasis is the second leading parasitic cause of death worldwide and its causative agent is the anaerobic protozoan *Entamoeba histolytica.* Approximately 12% of the world's population is infected. Clinical symptoms manifest in nearly 50 million people annually, causing 100,000 fatalities worldwide. Enteric protozoa are included among the category B agents due to their potential for dissemination through compromised food and water supplies in the United States. Limited drug options, drug toxicities, and invasive forms of the disease complicate treatment. *E. histolytica* has cytosolic fermentation enzymes to survive within the human intestine. *E. histolytica* alcohol dehydrogenase 2 (EhADH2) is an essential fusion-protein with ALDH and ADH activities for the glycolytic fermentation of *E. histolytica*. This study identified potential anti-amebic therapies based on two strategies: (1) iron-starvation of EhADH2 which inhibited trophozoite growth; and (2) discovery of novel bioactive agents for anti-protozoan drug development. Growth of *E. coli*$_{pEhADH2'}$ *E. histolytica*, and EhADH2 enzymatic activities were modulated by iron and inhibited by zinc or chelators. Initial screening of 46 extracts from marine actinomycetes generated two diverse metabolites, echinomycin A and tirandamycin capable of inhibiting trophozoite growth and EhADH2 activities. Preclinical studies have shown that iron chelators have anti-malarial and anti-mycotic actiivities suggesting that iron-sequestration can be used therapeutically. Further studies with a FDA approved chelator are in progress. In an initial screening of 46 pre-fractionated extracts from chemically prolific marine actinomycetes we identified two metabolites that inhibit amebic growth and EhADH2 activities. Our results now support that actinomycete metabolites, especially those from the marine environment, are a rich resource for the discovery of potent and structurally unique anti-protozoan agents. Further studies with *E. histolytica* clinical isolates will be pursued.

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MOLECULAR CHARACTERIZATION OF FATTY ACID BINDING PROTEINS FROM THE HOOKWORM *ANCYLOSTOMA CEYLANICUM*

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Nematodes are unable to synthesize fatty acids de novo and must acquire them from the environment or host. It is hypothesized that two unique classes of fatty acid and retinol binding proteins that nematodes produce (fatty acid and retinol binding (FAR) and nematode polyprotein antigen/allergen (NPA)) are used to meet this need. The cDNAs corresponding to FAR and NPA proteins have been cloned from the hookworm *Ancylostoma ceylanicum*. The full length translated amino acid sequence of *A. ceylanicum* FAR-1 (AceFAR-1) shows homology to FAR proteins previously identified in the dog hookworm *Ancylostoma caninum* and *C. elegans*. A partial cDNA corresponding to 4 subunits of a putative *A. ceylanicum* NPA (AceNPA) has homology to those from *Ascaris suum* and *Ostertagia ostertagi.* The *AceFAR-1* and *AceNPA* mRNAs are transcribed in all hookworm life cycle stages, with the highest levels of transcript seen in developing larvae. Immunoblot experiments using polyclonal anti-AceFAR-1 and anti- AceNPA IgG reveal proteins of the expected sizes in adult excretory/secretory proteins and adult worm extracts. Immunohistochemistry experiments indicate localization of AceFAR-1 to the hypodermis, ovaries and testes of adult worms, and AceNPA to the cuticle and pseudocoelomic space, suggesting distinct roles for these proteins in hookworm biology. Using *in vitro* binding assays, both rAceFAR-1 and a single recombinant subunit of AceNPA (rAceNPAb) were found to bind the fluorescent fatty acids DAUDA, cis-parinaric acid, and retinol at equilibrium dissociation constants in the low micromolar range. *In vitro* data also show that the recombinant proteins (rAceFAR-1 and rAceNPAb) bind fatty acids with chain lengths of C_{12}-C_{22}, with AceFAR-1 demonstrating the greatest affinity for $C_{14\text{-}16}$ and arachidonic acid (C_{22}), and AceNPA demonstrating the greatest affinity for arachidonic, linoleic (C_{18}), and eicosapentaenoic (C_{20}) acids. These data suggest that AceFAR-1 and AceNPA play important and distinct roles in hookworm development and metabolism by mediating uptake of fatty acids *in vivo*.

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HOOKWORM SECRETED TISSUE INHIBITORS OF METALLOPROTEINASE: CLONING, CHARACTERIZATION AND FUNCTIONS

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Dogs vaccinated with irradiated *Ancylostoma caninum* third stage larvae obtained significant protection against infective larvae (L3) challenge. The immune dog sera were used to immunoscreen *A. caninum* larval cDNA library in order to clone protective antigens. Two tissue inhibitors of

metalloproteinase (*Ac*-TIMP-1 and *Ac*-TIMP-2) were found to be the major antigens recognized by the immune dog sera. *Ac*-TIMP-1 and *Ac*-TIMP-2 consists of 140 amino acids with molecular weight of 16 kDa, and 244 amino acids with 27.7 kDa, respectively, which share common N-terminus with other vertebrate and invertebrate TIMPs. Even though the mRNA of both TIMPs could be detected at larval and adult stage of hookworm, the native proteins were only found in adult hookworms and their excretory/secretory products, and *Ac*-TIMP-1 was the most abundant protein in the ES products (accounting for 6.3% of total proteins). Immunolocalization with specific antiserum showed that native *Ac*-TMP-2 was located in adult worm's esophagus and cephalic glands. *Pichia* expressed recombinant *Ac*-TIMP-1 could *in vitro* inhibit proteolytic activity of *Ac*-MTP-2, a metalloproteinase produced by adult *A. caninum*. Recombinant *Ac*-TIMP-2 were highly immunogenic and recognized by ir-L3 immunized dog immune sera. The recombinant *Ac*-TIMP-2 specifically inhibited the human matrix metalloproteinases, MMP-2, MMP-7 and MMP-13. As immunodominant proteins having a possible roles in the parasite-host relationship of canine hookworm infection, recombinant *Ac*-TMP-1 and Ac-TIMP-2 represent plausible targets for vaccine development.

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ASSESSING THE IMPACT OF INDOOR RESIDUAL SPRAYING ON MALARIA INDICATORS USING A SENTINEL SITE SURVEILLANCE SYSTEM IN WESTERN UGANDA

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Accurate assessment of the impact of malaria interventions is necessary to optimize control efforts. Indoor Residual Spraying (IRS) has been increasingly advocated as an effect method of malaria control in Africa, especially in areas of low-to-moderate transmission. However, there are limited contemporary data on the impact of IRS on key malaria indicators. We evaluated the impact of an IRS campaign in Kanungu District of Western Uganda, an epidemic prone area with an EIR measured to be 6 infective bites per person year in 2001. Approximately 45,000 households covering a population of 190,000 persons were sprayed with the short-acting, synthetic pyrethroid lambda-cyhalothrin between February and March 2007. We used an existing sentinel site surveillance system to measure the impact of the IRS campaign on key malaria indicators measured on all patients presenting to the outpatient department of a government health center. Pre and post IRS data were compared using time-series analysis controlling for temporal trends and rainfall. A total of 14,942 patients (23% under 5 years) were evaluated in the 36 weeks prior to IRS and 18,482 patients (33% under 5 years) were evaluated in the 48 weeks after IRS. The proportion of patients under 5 years referred for microscopy with a positive blood smear decreased from 33.2% prior to IRS to 6.2%, 4.1%, and 10.1% in the periods 1-16 weeks, 17-32 weeks, and 33-48 weeks after IRS, respectively ($p<0.0001$ for all pairwise comparisons). The proportion of patients 5 years or older referred for microscopy with a positive blood smear decreased from 27.4% prior to IRS to 12.3%, 15.5%, and 21.8% in the periods 1-16 weeks, 17-32 weeks, and 33-48 weeks after IRS, respectively ($p<0.05$ for all pairwise comparisons). IRS was associated with a significant decline in the proportion of patients suspected of malaria with a positive blood smear, especially in children under 5 years. However, there was some evidence that the benefit of IRS began to wane after 32 weeks. Sentinel surveillance systems are a useful method for evaluating malaria interventions and we recommend their use.

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THE RELATIONSHIP BETWEEN MALARIA TRANSMISSION INTENSITY, CLINICAL DISEASE AND MORTALITY IN AN AREA OF DECLINING TRANSMISSION

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The relationship between malaria transmission intensity, infection, and disease has been a subject of intense epidemiological investigation since the early 1900's. Over one hundred years later, it is still little understood apart from a handful of descriptive studies that compare disease incidence and age of clinical disease between multiple sites. As malaria control efforts are being expanded across the world, understanding the role of transmission intensity in determining the burden of clinical malaria is critical to predicting and measuring the impact of control programs that reduce transmission. Comparisons of hospital data between areas of differing transmission intensity suggest that the mean age of hospitalized clinical malaria is higher under relatively lower transmission, but the total number of episodes is similar until transmission drops below a threshold, where-upon the risks of hospitalized malaria decline. The proportion of severe episodes that manifest as cerebral malaria is greater under lower transmission, whereas severe malarial anemia dominates under relatively higher transmission. Taken together, these observations have led to speculation about a possible increase in malaria mortality as transmission declines. However, there has rarely been an opportunity to study these relationships longitudinally in a single community where transmission declines over time. We reconstructed 17 years (1990-2006) of pediatric hospital surveillance data and infection prevalence surveys from a malaria endemic area on the Kenyan coast. The incidence of clinical malaria remained high, despite sustained reductions in exposure to infection. However, the age group experiencing the clinical attacks of malaria increased steadily as exposure declined. Although the incidence of cerebral malaria increased, mortality declined as transmission declined due to a decrease in the overall case fatality of malaria admissions as age increased.

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INCREASING RISK OF TREATMENT FAILURE WITH ANTIMALARIAL COMBINATION THERAPY: PARASITE AND HOST FACTORS

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Clinical response to antimalarial therapy is dependent on both drug-parasite and host-parasite interactions. We prospectively measured response to treatment with amodiaquine + sulfadoxine-pyrimethamine (AQ/SP) within a cohort of children in Kampala, Uganda between 2004 and 2007. Response to therapy was measured as the risk of treatment failure (TF) by day 63 after therapy, adjusted by genotyping. Over a 29 month period, we observed a significant increase in the risk of TF, from 5% at the beginning of the study to 21% at the end (HR=2.4/yr, p=0.002). The prevalence of predicted molecular markers of drug resistance (DHFR 51I, 59R, 108N, 164L; DHPS 437G, 540E; PfCRT 76T; PfMDR1 86Y, 184F, 1034C, 1042D, 1246Y) did not increase over this time, and adjusting for these markers did not change the association between time and TF. Thus, decreasing efficacy of AQ/SP was not explained by known parasite factors. To establish whether differences in host immunity might contribute to the increasing risk of TF, we considered exposure

as a surrogate of immunity. Those living in the area of highest malaria incidence had a significantly lower risk of TF (5%) compared to those living in the area of lowest malaria incidence (18%) (HR=0.26, p=0.003). To test the hypothesis that declining immunity within subjects was responsible for the increase in TF over time, we used the presence of recent asymptomatic parasitemia (AP) as a time-dependent surrogate of immunity. We compared the association between time and the risk of TF in a model which included recent AP, markers of drug resistance, geography, and age, with a model which did not include recent AP. Including recent AP in the model reduced the association between time and risk of treatment failure (HR=2.8/yr, p=0.002 vs. HR=1.4/yr, p=0.38), suggesting that declining immunity in our subjects explained in large part the increase in TF over time. Our results suggest that effective malaria control interventions may lead to declining host immunity and subsequently an increased risk of TF, especially with suboptimal antimalarial therapy.

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POPULATION HEMOGLOBIN LEVELS: A NEW METRIC FOR DEFINING MALARIA ENDEMICITY

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The estimation of malaria endemicity in a given population is essential for implementation and monitoring of the effectiveness of national control programs. Tools traditionally used to define malaria transmission intensity include: entomological inoculation rates (EIR), incidence of disease, prevalence of infection (PR), rates of enlarged spleens in children <10 yrs (SR), or records of (presumptive) malaria diagnosed at health facilities. Using data from rapid, household based malaria prevalence surveys we investigated the association of population mean haemoglobin (Hb) levels with malaria endemicity in Papua New Guinea (PNG) where malaria transmission ranges from highly endemic in coastal regions to absent in highlands areas. In total 24,508 individuals from 171 different communities were surveyed at altitudes ranging from 5 to 2120m above sea level, representing a full spectrum of assumed transmission intensities. In non-epidemic surveys, overall PR (r^2 = -0.78), PR in children 2-10yrs (r^2 = -0.74), SR (r^2 = -0.68) and Hb (r^2 = 0.82) were all significantly correlated with altitude ($p < 0.001$). Correcting for differences in Hb levels with age, sex and altitude did not alter the association of Hb with altitude (r^2 = 0.76, $p < 0.001$). Population mean Hb was also significantly associated with PR (overall: r^2 = -0.79, $p < 0.001$, 2-10yrs: -0.80) comparable to SR associated with PR (r^2 = -0.82, $p < 0.001$). Both measures (Hb and SR) correlated better with altitude and with parasite rate than disease incidence or records of malaria treatment (r^2<0.30). These findings suggest that mean population hemoglobin is a valuable metric to estimate the burden of malaria in a given population. Population mean Hb, which is easy to measure, may thus be a useful alternative tool for monitoring effectiveness of malaria control programs, on its own or in combination with other, more traditional measures of malaria burden.

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EFFICACY AND COST-EFFECTIVENESS OF MALARIA PREVENTION IN PREGNANCY IN LOW AND UNSTABLE TRANSMISSION: RESULTS OF A RANDOMISED CONTROLLED TRIAL

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Pregnancy is a time of increased malaria risk for mother and foetus. Susceptibility to malaria infection during pregnancy, and the severity of clinical manifestations, are determined by the level of immunity prior to pregnancy, which depends on intensity and stability of malaria transmission. Most intervention trials to control malaria during pregnancy have been conducted in areas of intense transmission. Less work has been carried out in areas of low and unstable transmission, where malaria is less frequent but the risk of spontaneous abortion and stillbirth is very high in women of all parities due to lack of sufficient malaria immunity. Reducing exposure to infection through ITN use during pregnancy may thus be of particular importance in areas of low transmission. Routine chemoprophylaxis is generally not recommended in areas of unstable malaria transmission. However, intermittent treatment with an effective anti-malarial drug may be beneficial, especially if targeted during periods of malaria transmission. Data on the cost-effectiveness of malaria control in low transmission settings is also lacking. A randomised intervention study was conducted among pregnant women living in the highlands of SW Uganda, to compare the efficacy and cost-effectiveness of three strategies for the control of malaria during pregnancy in low transmission settings: 1) intermittent preventive treatment (IPT); 2) insecticide treated nets (ITNs); and 3) IPT combined with ITNs. A total of 5775 pregnant women were recruited into the study, randomised to one of the three interventions at time of first antenatal visit, and followed until delivery. Data on maternal and birth outcomes were recorded for 5341 (92%) births. The impact of the three malaria intervention strategies on maternal anaemia and low birth weight will be presented (primary trial endpoints). The incidence of abortion, stillbirth and neonatal death in this population will also be described. Data on the relative cost-effectiveness of the three interventions will also be presented.

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EFFICACY OF INTERMITTENT PREVENTIVE TREATMENT WITH SULFADOXINE-PYRIMETHAMINE IN PRIMI- AND SECUNDIGRAVIDAE IN RURAL BURKINA FASO: IMPACT ON PARASITAEMIA, ANAEMIA AND BIRTH WEIGHT

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Intermittent preventive treatment for pregnant women (IPTp) is one of the key components of prevention and control of malaria in pregnancy recommended by the World Health Organization (WHO). However, the effectiveness of this intervention largely depends on sufficient coverage with at least 2 doses of sulfadoxine-pyrimethamine. The impact of IPTp on peripheral and placental parasitaemia, anaemia and birth weight was analysed at individual level, in primi- and secundigravidae included in a large health centre randomized trial aiming at establishing whether a targeted community-based promotion campaign to increase antenatal clinic attendance and uptake of sulfadoxine-pyrimethamine could effectively improve pregnancy outcomes. Among 1,441 primi- and secundigravidae peripheral parasitaemia at delivery was reduced by 74% with one dose (OR 0.26 95%CI 0.07-0.91 p=0.037) and by 93% with ≥2 doses of sulfadoxine-pyrimethamine (OR 0.07 95%CI 0.02-0.27 p=0.001). Placental parasitaemia was reduced by 84% (OR 0.16 95%CI 0.02-1.4 p=0.09) and 95% (OR 0.05 95%CI 0.01-0.49 p=0.015) with 1 and ≥2 doses respectively. Mean packed cell volume increased by 1.1 (standard error 0.33) with each dose at 32 weeks (p=0.006) and by 1.5 (0.47) at delivery (p=0.011). Prevalence of anaemia at delivery (1 dose: OR 0.11 95%CI 0.05-0.23 p<0.001; ≥2 doses: OR 0.07 95%CI 0.02-0.25 p=0.001) and low birth weight (1 dose: OR 0.29 95%CI 0.13-0.65 p=0.006; ≥2 doses: OR 0.15 95%CI 0.09-0.23 p=0.001) were reduced only in primi- but not in secundigravidae. In conclusion, although primi- and secundigravidae are at increased risk of malaria infection during pregnancy, and this was efficiently reduced by IPTp with sulfadoxine-pyrimethamine, the impact on anaemia and low birth weight was restricted to primigravidae. The current Roll Back Malaria goal of 80% of

pregnant women receiving ≥2 doses of sulfadoxine-pyrimethamine may not be effective if high coverage is not achieved among primigravidae.

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GLUCOSE 6-PHOSPHATE DEHYDROGENASE (G6PD) DEFICIENCY GENOTYPE-PHENOTYPE CORRELATIONS IN MALARIA ASSOCIATION STUDIES

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We explored the relationship between G6PD genotype (G202A) and phenotype (enzyme assay) and the incidence of malaria in a cohort of 596 children, aged 1-10, recruited from a census population in Kampala and followed for 2 years. G6PD genotype was assessed based on the predominant East African allele (G6PD A-, G202A) and phenotype by quantitative spectrophotometric methods. The prevalence of G6PD deficiency was significantly higher by mutational analysis (99/591; 16.8%), than by enzymatic assay (62/599; 10.4%). In females, the prevalence of G6PD deficiency was three-fold higher when assessed by mutational analysis compared to enzymatic assay (64/284; 22.5% vs (20/285; 7.0%, respectively (p<0.01). Heterozygous females accounted for the majority (46/54) of children who had a mutant genotype yet normal enzyme; males accounted for the majority (12/16) of children who were wild-type at G202A, yet deficient by enzyme assay. To explore the impact of these discrepant measures upon the association of G6PD status and malaria incidence, we compared the two measures in a longitudinal cohort of children. A total of 695 episodes of uncomplicated malaria were diagnosed after 901 person years of follow-up. Multivariate analysis using generalized estimating equations was used to identify independent predictors of malaria incidence. Using the enzymatic assay, G6PD deficiency was associated with significant protection from uncomplicated malaria only in females (RR=0.48, 95%CI 0.31-0.75). This protection was comparable to the protection afforded by the usage of insecticide-treated bednets (RR=0.52, 95%CI 0.32-0.83). However, when G6PD status was assessed by genotyping the G202A allele, this association no longer was present (RR=0.96; 95%CI 0.69-1.33). Thus, there was substantial discordance between enzymatic and genotypic assays for G6PD deficiency. This observation may explain, in part, the heterogeneity of published association studies to date. Potential explanations and implications for these findings will be discussed.

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HIV-1 INFECTION INCREASES THE RISK OF SEVERE MALARIA IN SEMI-IMMUNE ADULTS IN ZAMBIA

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The impact of HIV-1 infection on malaria is probably driven by the incapacity of the suppressed immune system to control the parasite load, resulting in a higher parasite load, incidence of clinical malaria and treatment failure in immune- suppressed HIV patients. Reports on the risk of hyperparasitaemia or severe malaria in HIV- infected individuals are limited. In Luanshya, where malaria is meso-endemic, adults with suspected severe malaria were screened and possibly recruited between December 2005 and March 2007. For each confirmed severe malaria case 2 matched controls, an adult with uncomplicated malaria and a 'community' control with no evident sign/symptom of diseases, were selected within a pre-defined time period. Matching criteria were gender, age and area of residence. HIV-1 infection and the related immune suppression were explored as risk factors. HIV-1 prevalence was 93.1% (27/29) among severe malaria cases, 51.7% (15/29) among uncomplicated malaria cases and 44.8% (13/29) in 'community' controls. CD4 count was below 350 CD4/μL in 82.6% HIV-1 severe malaria cases, 78.6% in uncomplicated malaria cases and 8.3% in 'community controls'. HIV-1 was identified as a highly significant risk factor for severe malaria as compared to 'uncomplicated malaria' (OR:13.0; IC 95: 1.7-99.4; p=0.01) and 'community' controls (OR: 8.0; IC 95: 1.8-34.8; p=0.006).In conclusion, in malaria endemic areas, HIV-1 infection is an important risk factor for severe malaria in adults.

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IMMUNE RECONSTITUTION INFLAMMATORY SYNDROME IN THE FIRST SIX MONTHS OF ANTIRETROVIRAL THERAPY IN HIV-INFECTED UGANDAN CHILDREN

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There are few studies of immune reconstitution inflammatory syndrome (IRIS) in children receiving antiretroviral therapy (ART) in Africa. We characterized frequency and predictors of IRIS in HIV-infected Ugandan children. 84 Children (1-10 years) in an ongoing cohort in Kampala, Uganda with ≥ 6 months of ART were prospectively evaluated. Clinical data using standardized case definitions were collected during sick and monthly routine visits. IRIS was defined as an event within the first 6 months of ART caused by microorganisms or conditions previously reported as IRIS in patients having immunologic/virologic response to ART. Independent predictors of IRIS were identified using negative binomial model. At baseline, median age was 4 years, CD4 count was 340 cells/mm^3(12%) and HIV RNA was 5.4 log copies/ml. Children received either nevirapine (n= 38) or efavirenz (n= 46) based regimens. By 6 months, 85 % had <400 copies/ml HIV RNA; median CD4 count was 760 (23%). There were 60 IRIS events (0.71 events/person); median onset 22 days, including 9 cases of pustular rash, 8 oro-labial herpes simplex, 5 acute parotitis, 4 oral candidiasis, 4 herpes zoster, two each of chicken pox, tonsillitis, molluscum contagiosum and one abdominal tuberculosis. Of note, 6 of 84 children were receiving TB therapy at ART initiation and the remainder had received neither INH nor TB therapy. Risk of IRIS was associated with increase in CD4 count at 2 weeks (p= 0.001) and female gender (p=0.018) but not with baseline CD4 count or %, change in CD4% at 12 weeks. Though not considered classical IRIS, 49 cases of presumed bacterial pneumonia were reported (0.45 events/person), that resulted in 5 hospitalizations. In conclusion, in the first 6 months of ART, IRIS was common with a predominance of dermatologic manifestations; there was an unexplained low rate of TB IRIS in this population.

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DIARRHEAGENIC *E. COLI* IN PERUVIAN CHILDREN WITH HIV

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Diarrhea associated with HIV continues to be a common problem in developing countries. The diarrheagenic *E. coli* are an important cause of diarrhea in children from those countries; however, they are not routinely sought as stool pathogens in clinical laboratories worldwide. The aim of this study was to determine the prevalence of diarrheagenic *E. coli* in Peruvian children infected with HIV with and without diarrhea and to determine its association with viral load and immunosuppression. We conducted a prospective study in 3 hospitals in Lima, Peru. 101 HIV

children 1m to 18y of age with and without diarrhea were enrolled. Stools were analyzed for common enteric pathogens including *E. coli*. Five *E. coli* colonies/patient were studied by a multiplex real-time PCR to identify Enterotoxigenic (ETEC), Enteropathogenic (EPEC), Shiga toxin-producing (STEC), Enteroinvasive (EIEC), Enteroaggregative (EAEC), and Diffusely Adherent *E. coli* (DAEC). We have analyzed 48 diarrheal samples (4y ± 4y) and 70 control samples without diarrhea (7y ± 4y). Age-related immunosupression (CD4) was severe in 58% and 20% of children with and without diarrhea (p<0.001). The median viral load was 242,000 and 1,700 (p<0.001) and HAART was used in 73% vs. 99%, respectively (p<0.001). Among the diarrheal episodes, 23% were persistent, 4% dysenteric and 29% were associated with moderate or severe dehydration. The diarrheagenic *E. coli* were the most commonly isolated pathogens in diarrhea (21%, 10/48) and control samples (27%, 19/70), including EPEC 6% vs. 10%, EAEC 4% vs.10%, ETEC 6% vs. 3% and DAEC 4% vs. 4%, respectively. Among children with diarrheagenic *E. coli* there was a trend for more frequent severe immunosupression in children with diarrhea than controls (60%, 6/10 vs. 26%, 5/19), however the viral load (% with >400 copies/ml) was similar in both groups (80%, 6/10 vs. 84%, 5/19). In conclusion, HIV children with diarrhea had worse immunosuppression and viral load, and less frequently were on HAART, than HIV children without diarrhea. Diarrheagenic *E. coli* were the most commonly isolated pathogens in HIV children. Since diarrhea is treatable, molecular studies of *E. coli* should be part of the routine workup of children with HIV and diarrhea.

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DETECTION AND GENOTYPING OF *ENTEROCYTOZOON BIENEUSI* IN STOOL SPECIMENS FROM HIV-INFECTED RURAL KENYANS

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Microsporidia are obligate intracellular eukaryotic parasites, which cause a wide range of infections with distinct clinical features. *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis* are two species that cause diarrheal disease in HIV-infected persons, especially among patients with CD4+ cell counts < 200 cells/mm^3. At least 50 distinct genotypes of *E. bieneusi* have been described based on analysis of internal transcribed spacer (ITS). Some genotypes, (e.g., A and B) are considered anthroponotic, while other genotypes have a zoonotic potential (e.g., D, E, K). We assayed 150 stool specimens from 109 HIV-infected individuals from rural areas in Kenya (CD4+ cell count < 350 cell/mm^3) collected between February 2003 and November 2003. We screened these samples for *E. bieneusi* and *E. intestinalis* using species-specific 18 SrRNA conventional PCR primers EBIEF1/EBIER1, SINTF1/SINTR, respectively. No specimens were positive for *E. intestinalis*. Fifty-two specimens from 34 individuals were positive for *E. bieneusi*; 27 of these *E. bieneusi* PCR-positive individuals had CD4+ cell counts < 100/mm^3. Ten *E. bieneusi*-positive samples from 7 individuals were randomly selected and analyzed using primers that amplify the ITS of *E. bieneusi* genome. With this approach, we identified 2 distinct genotypes: A and K. Genotype K was detected in samples from 4 individuals and A in samples from 3 individuals. Considering previous reports in the literature about genotypes A and K, these preliminary results suggest that the population studied were mainly acquiring infections through person-to-person contact (genotype A) and contact with animals (genotype K), with neither route of transmission clearly predominating.

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CARING FOR THE MOTHER AND CHILD IN AN INTEGRATED HEALTH SYSTEM: THE UTILITY OF A POSTNATAL BRIDGING CARD

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Currently in South Africa, information about the infant/mother dyad is transmitted via the child's Road to Health chart. Previous qualitative research by Richardson and Etsane in southwest Tshwane district has demonstrated this to be an inadequate means of communicating patient information, especially in regards to PMTCT. Other studies have shown increased morbidity and mortality due to poor information transfer. To facilitate communication of information between hospitals and clinics and to promote increased uptake of health services via a postnatal bridging card. This pilot forms part of a larger study aimed at identifying and developing resources to assist managers and health care workers in the integration of services for HIV-infected mothers and their children. After obtaining consent, exit interviews were conducted on a sample of 100 mothers (positive and negative) on discharge from the regional hospital. A bridging card containing a discharge summary for both the mother and child was stapled to each child's Road to Health chart. Staff at each of the local clinics were briefed on the card and were asked to fill out the health details in the space provided for the one- and six-week postnatal visits. The cards were then collected from the various clinics to assess the utility of the intervention. 65% of the cards were retrieved after 6 weeks. This is significantly different from the 55% of controls who attended their 6 week visits (p <.001). Of those showing up at 6 weeks, only 51% attended their postnatal visit within 1 week. They did so at an average of 5.6 days. 27% of mothers were HIV positive. In conclusion, a postnatal bridging card has the potential to improve health care to mother and infants in several ways. First, it allows for improved communication between hospital and clinics by providing more detailed patient history. Second, it provides a checklist to nurses at the clinic to facilitate referral for common problems. Lastly, it increases uptake of postnatal services by giving mothers a reminder of their responsibilities after birth.

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BIOLOGY IS DESTINY OR SOCIAL STATUS MEETS SERO-STATUS?: DETERMINANTS OF HIV INFECTION IN AFRICA

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As a result of several successful clinical trials, male circumcision has been equated with a HIV vaccine "of high efficacy" and recommended among the existing arsenal of HIV prevention strategies in high-prevalence countries. However, evidence from population studies suggests that wealthier men and their partners are at increased risk for HIV in Africa despite the fact that they are more likely to be circumcised. Examining survey data with linked HIV testing from Tanzania's 2005 AIDS Indicator Survey, this study assesses the relative importance of male circumcision versus wealth in predicting HIV serostatus. Logistic regression and hypothesis testing was undertaken to examine the relationship among wealth status, male circumcision and HIV risk. Chi-square tests of significance yielded no significant relationship between male circumcision and HIV (X^2=.024(1), p>0.1). Wealthier men were both more likely to be seropositive (m[HIV+]=3.58, m[HIV-]=2.98, p<.01) and circumcised (m[HIV+]=3.29, m[HIV-]=2.55, p<.01). In the regression analysis adjusted for covariates, wealth was significantly positively associated with HIV status (B=.315, p<.01) and male circumcision negatively associated with HIV status (B=-.636, p<.01). While wealthier individuals had a higher mean number of sexual partners, poorer individuals were more likely to be in polygamous unions and to have a lower age at first intercourse. In conclusion, despite their increased likelihood of being circumcised,

wealthy individuals were still at increased risk for HIV. Circumcision's protective effect appears to be outweighed by risk associated with wealth. However, wealthy individuals' increased risk was only modestly associated with heightened sexual risk behavior. This study suggests that attention to circumcision status alone is not sufficient to stem the tide of generalized HIV epidemics in Africa without additional attention to the socio-economic causes of infection.

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IMPACT OF HIV-1 INFECTION ON THE HEMATOLOGICAL RECOVERY AFTER CLINICAL MALARIA

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Anemia is the most frequent cytopenia in HIV-infected individuals and is often associated with malaria. The objective of this study was to assess the impact of HIV-1 on the hematological recovery after a clinical malaria episode. In Ndola, Zambia, a region with high malaria and HIV prevalence, Hb was measured in 634 malaria patients 14 and 45 days after antimalarial treatment. Risk factors for hematological recovery were analyzed in a multivariate linear regression model. At enrolment, HIV-1 infected malaria patients had lower Hb compared to HIV-1 uninfected (122.7g/L vs. 136.0g/L;p<0.001). In both groups, mean Hb was significantly lower at day 14 post-treatment than day 0 (p<0.0001) and significantly higher at day 45 than at day 14 (HIV-1 negative: p=0.0001; HIV-1 infected: p=0.007). HIV-1 was a risk factor for a larger Hb decrease until day 14 (p<0.001) and slower recovery until day 45 (p=0.06). When considering the whole 45-day follow up period, mean Hb increased in the HIV-1 negative group (+3.45g/L; 95%CI, 1.36 to 5.54; p=0.0001) but not in the HIV-1 infected group (-1.2g/L; 95%CI, -4.22 to +1.71; p=0.66). HIV-1 infection as such (p<0.0001), not CD4 cell count (p=0.68), was an independent risk factor for a slower hematological recovery. In conclusion, HIV-1 infected malaria patients had a slower hematological recovery after successful parasite clearance. Malaria preventive measures should be targeted to this high risk group.

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THE USE OF HUMAN-MURINE CHIMERIC ANTIBODIES FOR TREATMENT OF YELLOW FEVER IN THE AG129 MOUSE MODEL

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Yellow fever virus (YFV) is a mosquito-borne flavivirus found in tropical regions of Africa and South America. Despite the availability of an effective vaccine, 17D, YFV is still responsible for an estimated 200,000 cases and 30,000 deaths annually. In addition, use of the live-attenuated YF 17D vaccine strain is contraindicated in certain individuals, and recently has been associated with an increase in adverse events. Because there is no approved antiviral treatment for YF, we are investigating immunoprophylaxis and immunotherapy as possible interventions for human YF. Our approach is to humanize murine monoclonal antibodies (MAbs) that previously have been shown to favorably alter the outcome of YFV infection in rodent models. Development of YF antivirals has been hindered by the lack of a good adult small animal model of YFV infection. We previously have developed an adult peripheral challenge mouse model for dengue virus infection in mice lacking receptors for interferons α, β, and γ (strain AG129). We have now determined that intraperitoneal challenge of AG129 mice with 10^5 plaque forming units (PFU) of YF 17D vaccine results in 100% morbidity beginning 7 days post-infection. This model permits us to test humanized YF MAbs in a system that requires only BSL-2 containment. The first humanized IgG MAb we developed was based on the YFV E protein-specific murine MAb 2C9. This murine MAb protects 6-week-old mice from lethal YF encephalitis when administered either 1 day prior to or up to 4 days following intracranial virus challenge with 10^6 PFU of YF 17D vaccine. A plasmid that encodes the human constant γ chain with inserted variable regions of the 2C9 murine MAb was created and used to transfect murine myeloma cells. Humanized 2C9 IgG produced by transfected cells has been shown by ELISA to react with YF 17D. The humanized 2C9 IgG is currently being evaluated for its utility in prophylaxis or therapy of YF using the YF 17D - AG129 animal model.

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YFV-INDUCED CYTOKINE EXPRESSION IN HUMAN HEPATOCYTES

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Yellow fever virus (YFV) is a mosquito-borne flavivirus that causes clinical illness ranging from febrile to fatal hemorrhage fever affecting an estimated 200,000 people a year (15-20% mortality rate). YF is an important medical threat since there are no therapeutics available to treat disease. Reported data for patients infected with either the Asibi-wild type or 17-D vaccine virus have indicated that certain pro-inflammatory cytokines (such as IL-6) have increased expression; as much as 21-30% difference in plasma concentrations between day 0 and 7. However, data is limited and, especially for wild-type or vaccine-adverse event patients, there is typically only one sample tested after the onset of clinical disease. Understanding a complete pro-and anti-inflammatory cytokine (PIC, AIC) response is important for understanding the mechanisms of early pathogenesis, as early and late cytokine production induces antiviral states, recruits inflammatory cells, and eventually activates adaptive immunity. The human hepatic system is the primary target of YFV and is greatly under-investigated. The aim of this study was to examine PIC and AIC production from Asibi and 17-D infected human hepatocytes. Results of this study identified several PICs that were significantly different in their production between 17-D or Asibi infected cells. IL-6, IL-12 (p70), IL-8, TNF-α and IL-1β all showed significant differences between infections during 24-72 hours post infection. Production of AICs IL-4 and IL-10 was also significantly different at some time points during infection, but collectively was not significantly different between cells infected with Asibi or 17-D. These data suggest that Asibi virus may preferentially target PIC production in order to avoid inducing an anti-viral state and that it either does not target or cannot control inflammatory feedback mechanisms that initiate AIC production.

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PHYLOGENETIC ANALYSIS OF WEST AFRICAN ZIKA VIRUS USING SEQUENCES OF PARTS OF E, NS5 AND NS5/3'NC

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Zika virus (ZIKV) belongs to the family Flaviviridae, genus flavivirus. ZIKV causes fever, arthralgia and maculopapular rash in humans and is widespread in Africa and Asia. In April 2007 a major outbreak have been reported in Micronesia. Entomological and virological surveillance program of arbovirus showed a permanent circulation of ZIKV in West Africa. However, genetic relationship between them is unknown. This study presents the first molecular characterization of ZIKV isolates from West Africa over a period of 40 years. The sequences of the envelope (E), polymerase (NS5), and junction NS5/3'NC gene of 37 strains isolated in Senegal, Ivory Coast, Burkina Faso and Central African Republic have been obtained using published primers while full genome sequencing was done using gene walking approach. The phylogenetic trees were constructed by MEGA (v3.1 Molecular Evolutionary Genetics Analysis). Phylogenetic

analysis of E, NS5 and NS5/3'NC sequence of the 37 strains showed two distinct main lineages.The major one includes old and recent strains isolated between 1968 to 2002 in Senegal, Ivory Coast, Burkina Faso and Central African Republic. The second lineage clustered isolates from Senegal between 1998 and 2001. Besides, analysis of full length genome sequences reveal 5 potential recombinants isolates in Senegal and Ivory Cost reinforcing the co-circulation of isolates. Alignment of nucleotide and amino acid sequences revealed deletion at the glycosylation site Asn-153, conserved among many flaviviruses, for 2 strains isolated in Senegal and Central African Republic. Our data showed a co-circulation of old and recent lineage of ZIKV in West Africa and potential recombinant strains. Comparison genomic sequence of ZIKV involved in the recent epidemic of Micronesia with those of West Africa would help gain insight to understand the emergence of ZIKV.

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INSECT-ONLY FLAVIVIRUSES DETECTED IN *CULEX* SPECIES MOSQUITOES FROM NORTHERN COLORADO

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It is important to understand the dynamics of vector-borne disease systems in order to make efficient use of arbovirus surveillance efforts. The genus *Flavivirus* contains many viruses that are arthropod-borne and are associated with vertebrate disease. In addition, there is a small outgroup in this genus consisting of insect-only viruses that have been found to replicate in mosquito cells, but not in vertebrate cells. We have detected two insect-only viruses in mosquitoes collected in Northern Colorado. Over 200,000 mosquitoes were collected from June through September, 2007 along a riparian transect extending from Thompson Canyon to Sterling, Colorado. *Culex* species mosquito pools were tested by RT (reverse transcription)-PCR, using universal flavivirus primers targeting the NS5 gene. *Culex* flavivirus, which was first described in Japan, has been isolated from *Cx. pipiens* mosquitoes and a new insect-only flavivirus has been found in *Cx. tarsalis* mosquitoes. Sequences from this putative new virus show approximately 75% homology to Kamiti River virus. These findings could have important implications in regards to co-infection with a heterologous flavivirus in vector populations. West Nile virus has continued to circulate along the Colorado Front Range since its introduction in 2002. It is hypothesized that primary infection with an insect flavivirus could modulate secondary infection with another flavivirus, like West Nile virus. This study describes this interaction by determining the prevalence of flavivirus superinfection in nature by testing individual field-caught mosquitoes and by challenging persistently infected cell cultures and mosquito colonies with mosquito-borne viruses.

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EVALUATION OF IGM CAPTURE ELISA ASSAYS FOR THE DETECTION ANTI-JEV IGM ANTIBODIES IN CEREBROSPINAL FLUID SAMPLES

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Japanese encephalitis (JE) is a major public health problem in Asia. As the clinical presentation in JE virus infection resembles several other common infections of the human nervous system, it is necessary to confirm the diagnosis by an appropriate laboratory test. Detection of JE virus-specific IgM antibodies in serum and CSF by the IgM antibody capture enzyme-linked immunosorbent assay (MAC ELISA) is currently the most widely used method. Three commercial JE MAC ELISA kits were recently evaluated in a panel of serum. However, the presence of cross-reactive IgM antibodies to other flaviviruses in serum and the high ratio of inapparent to apparent infections in JEV infection limit the diagnostic utility of serum based diagnostic assays in a surveillance system. A positive result in serum only suggests a recent infection and not necessarily an encephalitic illness due to JEV. Consequently, the detection of virus specific IgM antibodies in a single specimen of CSF assumes great diagnostic relevance in JEV infections. Two commercial JE IgM ELISA kits, JE-Dengue IgM Combo ELISA (Panbio Limited) and JEV CheX (XCyton Diagnostics Ltd), were evaluated using 60 well-characterized CSF samples obtained from patients with acute encephalitis syndrome (AES): 20 positive for JE IgM antibodies and 40 negative for JEV IgM antibodies, including 10 Herpes simplex encephalitis (HSE) and 10 subacute sclerosing panencephalitis (SSPE) confirmed cases. Sensitivity and specificity was 45% and 98% with the Panbio kit and 85% and 98% with the XCtyon kit. After modification of the cut-off calculation and addition of a ratio to the results interpretation, sensitivity of the Panbio kit increased to 80% and specificity decreased to 95%. Performance information on these commercial JEV ELISA kits in CSF, the preferred diagnostic specimen for JEV surveillance, should assist in laboratory-based JEV surveillance programs.

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FIRST CLINICAL TRIAL OF A VERO CELL DERIVED, INACTIVATED JAPANESE ENCEPHALITIS (JE) VACCINE IC51 IN PEDIATRIC POPULATION

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JE is the most common childhood viral encephalitis in Asia. More than 50,000 cases are reported annually, with a case fatality rate up to 35% and long-term sequelae up to 75%. JE is highly endemic in tropical and subtropical countries in Asia. Preventive vaccination is the single most important control measure. We report on the first clinical trial of IC51, an Al(OH)3-adjuvanted vaccine based on the purified, inactivated JEV strain SA14-14-2 in children. This clinical trial was designed to assess safety and immunogenicity of the standard and the half dose of IC51 compared to an active comparator, JENCEVAC® in Bangalore, India. In the open-label, randomized, controlled Phase 2 trial IC51 was administered to 48 children ≥1 to <3 years of age. 24 Children received the standard dose and a second group received half of the standard dose of IC51. Another group of 12 children received the commonly used JENCEVAC®. Immune response was assessed by PRNT50 (Plaque Reduction Neutralization Test) titers in comparison to baseline. SCR was defined by percentage of subjects with ≥1:10 anti-JEV antibody titer (PRNT). Solicited and unsolicited events were recorded for local and systemic tolerability. The primary analysis at Day 56 resulted in 95.7% SCR in the IC51 - 3 mcg group, in 95.2% SCR in the IC51 - 6 mcg group and in 90.9% SCR in the JenceVac™ group. Secondary Analyses revealed no significant differences of SCR at Day 28 as well as GMT at Days 28 and 56. No serious adverse events occurred during the entire trial period. Overall, in 12 children 13 adverse events were reported: 12.5% of children in the IC51 - 3 mcg group, 20.8% of children in the IC51 - 6 mcg group and 33.3% of children in the JenceVac™ group without significant difference. In conclusion, the standard and the half dose of the JEV vaccine IC51 and the active comparator are equally immunogenic and safe in children aged 1 to 3 years. Both IC51 groups appeared to have a lower rate of adverse events compared to JenceVac™.

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SIX MONTHS SAFETY OF A VERO-CELL CULTURE DERIVED JAPANESE ENCEPHALITIS VACCINE, IC51, ACROSS PHASE 3 TRIALS AND IN A LONG-TERM FOLLOW-UP COHORT

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An estimated 50.000 Japanese Encephalitis cases with 6.000 deaths are reported annually. Vaccination is an important control measure, but potentially serious adverse events had limited the recommendations for use of a former JE vaccine in travelers. IC51 is a Vero cell-derived, SA_{14}-14-2 based Japanese Encephalitis vaccine that has been proven immunogenic and safe when administered i.m. in a 0, 28 Day schedule in adults. Here we present an overview of safety data. 6-month safety data were pooled from five phase 3 trials in which subjects were vaccinated with IC51, JE-VAX®, HAVRIX® or Placebo; and two safety follow-up trials. Data collection by self-assessment of local (pain, itching, hardening, swelling, redness, tenderness) and systemic (muscle pain, flu-like symptoms, rash, excessive fatigue, headache, fever, nausea, vomiting) reactions and grading of Adverse Events Following Immunization (AEFI) were prospectively defined and comparable across studies. A cohort of subjects is followed up for long term immunogenicity and safety for at least 36 months. 4715 subjects were included in the analysis, 3558 subjects received IC51. 54.1% of them reported local symptoms vs. 56.1% in the placebo and 61.1% in the JE-VAX® group. Severe local symptoms were reported by 3.2% of IC51 subjects, comparable to placebo (3.1%) but significantly higher in the JE-VAX® group (13.8%). Systemic symptoms were reported in 39.8% of IC51 subjects, 40.0% in the placebo and 35.6% in the JE-VAX® group. 64.1% of subjects in the IC51 group experienced at least one AEFI and 5.8% of subjects at least one severe AEFI. Serious AEFIs were reported by 1.1% of subjects in the IC51 group, and treatment-related AEFIs by 38.3%. The AEFI rate was comparable across vaccination groups. Serious allergic reactions were not observed in any analysis group. The pooled analysis confirms the excellent safety profile of IC51 which is comparable to placebo and more favorable than JE-VAX® in terms of local reactions.

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PLASMODIUM PYRUVATE DEHYDROGENASE IS ONLY ESSENTIAL FOR LIVER STAGE DEVELOPMENT

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Plasmodium has a single pyruvate dehydrogenase (PDH) complex that is localized to the apicoplast. The PDH complex consists of the E1α, E1β, E2 and E3 subunits that catalyze successive parts of the overall reaction in the conversion of pyruvate into acetyl CoA, the primary precursor for de novo fatty acid biosynthesis. Recently we had undertaken a comprehensive study of the *P. yoelii* liver stage transcriptome and proteome and our data revealed that among the genes and proteins that are highly expressed in the liver stages are the four genes encoding the PDH subunits: *PY00819 (E1α)*; P*Y07062 (E1β); PY04573 (E2)*; and *PY00573 (E3*). Surprisingly these genes showed very low expression in the erythrocytic and mosquito stages suggesting that PDH may have an essential role only in liver stage development. To test this hypothesis, we constructed a *PDH E1α* knockout (*pdhe1α-)* by gene replacement of the *PY00819* locus with a pyrimethamine resistant gene marker. Our results indicate that deletion of the *E1α α* gene did not affect parasite blood stage replication or mosquito stage development. Naïve mice infected with blood stage *pdhe1α(-)* or wild type (WT) parasites showed no significant difference in the course of blood stage parasitemia when followed over fourteen days. Furthermore, the blood stage *pdhe1α(-)* parasites showed normal gametocyte development and male gamete exflagellation. Within mosquitoes, midgut oocyst development, formation of oocyst sporozoites and invasion of sporozoites into the salivary glands was similar to WT. However mice injected with *pdhe1α-* sporozoites did not became blood stage patent even when injected with one million sporozoites. The phenotypic defect during pre-erythrocytic development is under investigation. These results indicate an essential role of acetyl CoA, and thus possibly fatty acid biosynthesis, for liver stage development but not for mosquito stage or blood stage development.

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DISTINCT ROLES OF *PLASMODIUM* RHOMBOID 1 IN PARASITE DEVELOPMENT AND MALARIA PATHOGENESIS

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Invasion of host-cells by the malaria parasite involves recognition and interaction with cell-surface receptors. A wide variety of parasite surface proteins participate in this process, most of which are specific to the particular invasive form of the parasite. Upon entry, the parasite has to dissociate itself from the host cell receptor. One mechanism by which it does so is by shedding its surface ligands using specific enzymes. Rhomboid belongs to a family of serine proteases that cleave cell-surface proteins within their transmembrane domains. Here we identify and partially characterize a *Plasmodium berghei* rhomboid protease (PbROM1) that plays distinct roles during parasite development. PbROM1 localizes to the surface of sporozoites after salivary gland invasion. In blood stage merozoites, PbROM1 localizes to the apical end where proteins involved in invasion such as apical membrane antigen 1 (AMA1) are also present. Genetic analysis suggests that PbROM1 functions in the invasive stages of parasite development. Whereas wild type *P. berghei* is lethal to mice, animals infected with PbROM1 null mutants clear the parasites efficiently and develop long-lasting protective immunity. The results suggest that *Plasmodium* PbROM1 plays a non-essential but important role in host cell invasion and identify rhomboid proteases as potential targets for disease control.

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ISOLATION OF INVASIVE LONG LIVED *PLASMODIUM FALCIPARUM* MEROZOITES BY CELL SIEVING

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The *in vitro* cultivation of *Plasmodium falciparum* over thirty years ago was a milestone for research and development within the malaria community. Furthermore, around the same time period, the success of isolating viable merozoites from the simian malaria *P. knowlesi* also provided a rich source of material for many important observations related to the process of merozoite invasion of erythrocytes and even a possible blood stage vaccine. Despite these earlier successes, unpublished attempts by various investigators including ourselves to isolate viable *P. falciparum* merozoites failed. The basis for this difference in merozoite viability between *P. falciparum* and *P. knowlesi* is unknown. More recently we renewed the effort to isolate viable *P. falciparum* merozoites to facilitate research and development. Parasitized *P. falciparum* erythrocytes were irradiated prior to providing selective pressure by continuous motion suspension culture with limiting numbers of red blood cells (RBC) *in vitro*. After five months under selective pressure, the merozoites were observed to have an increased viability or long-life by about 4-fold greater than the parental strain, as reported previously. This selected parasite strain, identified as HH Suspension Select, was used in conjunction with a water jacked temperature controlled cell-sieve specifically designed for purification of merozoites. Cell-sieve HH Suspension Select merozoites were greater than 99.9% pure based on Giemsa stained preparations of merozoites and retained most of their ability to invade RBC for up to 30 minutes of storage at room temperature (23 C) but lost most of their invasiveness within about 10 minutes of storage at 37 C. During the

optimum period of merozoite isolation along with the addition of fresh RBC, ring parasitemias of 10 - 15% have been observed by flow cytometry and Giemsa stain. Evaluation of the parental strain following similar procedures showed only limited number of ring stage parasites (<1%). This new development will impact our understanding of RBC invasion by *P. falciparum* merozoites as well as possibly aid in the identification of novel vaccine candidate antigens.

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A COMPLEX FORMATION OF RHOPTRY NECK PROTEIN 2 WITH A MICRONEME PROTEIN, AMA1, IN *PLASMODIUM FALCIPARUM*

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Erythrocyte invasion is an essential step of the malaria parasites to establish the infection in human. Recent proteome analysis of the closely-related apicomplexa parasite, *Toxoplasma gondii*, revealed a panel of novel rhoptry neck proteins (RONs), and some of them have been shown to form a complex with a microneme protein, Apical Membrane Protein 1 (AMA1), at the interface of the parasite and the host cell (the moving junction) during invasion. Because most of the RONs and AMA1 are conserved among apicomplexa phylum parasites and *Plasmodium* AMA1 is a leading blood-stage vaccine candidate, RONs appears to have not only a fundamental role in the invasion mechanism of this parasite, but also a potential for the malaria intervention. Here we characterized *Pf*RON2, one of RONs in *Plasmodium falciparum*. *Pf*RON2 transcription peaked at the mature schizont and expressed at the neck portion of the rhoptry in the merozoite shown by immuno-electronmicroscopy. *Pf*RON2 possesses a region harboring a strong homology with a rhoptry body protein *Pf*RhopH1/Clag, a component of the RhopH complex, however, co-immunoprecipitation experiment indicated that *Pf*RON2 was not a component of the RhopH complex. Co-immunoprecipitation of *Pf*RON2 and *Pf*AMA1 was observed, suggesting that co-operative function of the rhoptry and microneme proteins during erythrocyte invasion.

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A PURINE TRANSPORTER IN THE ENDOPLASMIC RETICULUM OF *PLASMODIUM FALCIPARUM*

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Plasmodium falciparum, the agent of severe human malaria, is entirely dependent on exogenous purines for growth. One transporter, PfNT1, has been functionally characterized and shown to play an important role in the transport of physiologically relevant purine nucleosides and nucleobases across the parasite plasma membrane, as reported previously. Interestingly, transport assays on wild-type parasites indicated that some, but not all, purines are accumulated by the parasite. In addition, while the parasite is dependent on exogenous purines, high concentrations of certain purines are toxic to the parasite. These findings suggest that *P. falciparum* might have evolved a mechanism for intracellular transport, storage, detoxification or recycling of host purines. Interestingly, the sequencing of the *P. falciparum* genome revealed three other putative purine transporters. One of these, PfNT2, contains a putative trafficking motif. Together these findings led us to hypothesize that PfNT2 might be involved in the intracellular transport of purines in *P. falciparum*. Here we provide evidence that PfNT2 is a purine transporter localized to the endoplasmic reticulum. Furthermore, expression of PfNT2 complements a yeast strain otherwise deficient in nucleoside transport. This study provides new insight into purine transport and metabolism in malaria parasites and a novel biological function for the endoplasmic reticulum in eukaryotes.

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STUDY ON PREVALENCE, DISTRIBUTION AND BEHAVIORAL ASPECTS OF THE POTENTIAL VECTOR/S OF CUTANEOUS LEISHMANIASIS IN SELECTED AREAS OF SRI LANKA

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Cutaneous leishmaniasis, caused by *Leishmania donovani,* is an established parasitic disease in Sri Lanka with increasing number of patients detected since 2001. Even though potential vector Phlebotomine spp. has been recorded since early 1900s no confirmatory evidence of vector status is available so far. This study was carried out to determine the prevalence and distribution of various species of sandflies in selected areas of Sri Lanka and to determine their aggregation behavior. Two field sites were selected from north western and southern parts of Sri Lanka based on areas where there were confirmed cases of cutaneous leishmaniasis. Adult sandflies were sampled using cattle-baited net traps, light traps and manual methods. The species of sandflies were determined with the help of standard keys after dissection. Sub sets of sandflies were identified using a PCR-based method. An hourly collection of sandflies attracted to cattle traps was carried out for several consecutive nights (From 18.00 hr to 6.00 hr). Two species of sandflies, *Phlebotomus argentipes* and *Sergentomyia zeylanica*, were identified after dissection of a total of 3587 sandflies. There were only 88 (5%) identified as *P. argentipes* out of 1756 sandflies dissected from the sites of southern area. The number of *P. argentipes* was 1649 (90%) out of 1831sandflies identified in north western area sites of Sri Lanka. *S. zeylanica* was found to be 95% and 10% in above sites respectively. PCR studies on sandflies carried out confirmed the presence of *P. argentipes* and a single unidentified species which is compatible with the identification through manual methods. Peak aggregation of sandflies was observed during 20.00 hr 23.00hr with the maximum number seen between 21.00hr to 22.00hr. Male sandflies dominated females with a ratio of 7:1. A large number of sandflies was found within the cattle traps in north western area sites when compared to southern area sites. In conclusion, *Phlebotomus argentipes* was found to be the dominant species in north-western province of Sri Lanka where as *S. zeylanica* dominate in the southern areas. Studies are being continued to identify the true vector species in these areas. Total number of sandflies collected using cattle-baited net traps was higher in areas where *P. argentipes* is dominant.

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GENETIC STRUCTURE OF A HIGHLY *TRYPANOSOMA CRUZI*-INFECTED POPULATION OF *TRIATOMA SANGUISUGA* IN NEW ORLEANS, LOUISIANA, USA

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Chagas disease is the most significant parasitic disease and a leading cause of heart disease in Latin America. Yet historically, Chagas disease has been rare in the United States. However, an estimated 100,000-675,000 *Trypanosoma cruzi*-infected immigrants are believed to live in the United States and could possibly introduce new strains of *T. cruzi* into an already active sylvan cycle. AABB (formerly American Association of Blood Banks) has reported 1638 *T. cruzi*-repeat reactive blood donations (486 of these confirmed) in the U.S. so far in 2008. This data indicates that there is a population of infected individuals living in the United States. Furthermore,

six autochthonous infections of *T. cruzi* have been reported in the United States; our lab reported the last case in a rural area of New Orleans, LA in 2006. As a follow up of this autochthonous transmission, ~350 *Triatoma sanguisuga* were collected over a two-year period from the location of the index case and showed a *T. cruzi* prevalence of 56% by PCR. Such high prevalence is usually associated with endemic regions. Since little is known about the population structure of Chagas vectors in the United States we have chosen to study the genetic structure of this highly infected vector population. Analysis of DNA sequence including the mitochondrial gene (*CytB*) and a nuclear marker (ITS2) will be used to determine the genetic diversity and population structure of this population. The genetic structure of the population does not to appear to have changed over time.

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ANALYSIS OF THE SPATIO-TEMPORAL DYNAMICS OF HOUSE INFESTATION BY NON-DOMICILIATED *TRIATOMA DIMIDIATA* REVEALS AN HETEROGENOUS DISTRIBUTION OF CHAGAS DISEASE TRANSMISSION RISK AND POTENTIAL VECTOR MANIPULATION BY *TRYPANOSOMA CRUZI*

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Chagas disease transmission by non-domiciliated triatomines is emerging as a major challenge for vector control. These triatomines present a limited ability to establish permanent domestic colonies, but can occasionally infest domestic habitat by immigration from peridomestic and/or sylvatic habitats. A better understanding of this process is required for the optimization of novel vector control strategies. We examined here the spatio-temporal dynamics of house infestation by non-domiciliated *Triatoma dimidiata* in several villages in the state of Yucatan, Mexico. Triatomines were collected by community participation over an 18 months period, and infection by *Trypanosoma cruzi* was determined by PCR. All houses and bug collections were georeferenced. House infestation was highly transient (March-July) and occurred preferentially at the periphery (external 100 m) of the villages during most in the infestation period, in agreement with a sylvatic origin of the bugs. Male triatomines were found in houses closer to the center of the village than females. Infection by *T. cruzi* altered the distribution of both males and females triatomines, suggesting for the first time a possible manipulation of triatomine dispersal by the parasite. These data are important for the understanding of vector-parasite interactions and the optimization of vector control in the region.

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EFFECTS OF VIRAL INFECTION ON BLOOD FEEDING BEHAVIOR AND FECUNDITY IN *CULICOIDES SONORENSIS* (DIPTERA: CERATOPOGONIDAE)

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Culicoides sonorensis (Diptera: Ceratopogonidae) is the primary vector of bluetongue virus (BTV) in North America and a competent vector of vesicular stomatitis virus (VSV). Little is known about how viral infection of this midge affects its blood feeding behavior and fecundity. Blood feeding success of midges intrathoracically inoculated with either virus-infected or virus-free cell lysate was measured at 2, 3, and 4 days post inoculation (DPI) for a study of VSV and at 2, 4, and 7 DPI for a study of BTV. Viral growth curves were determined for each virus, and blood meal DPI were selected to include the normal optimal blood feeding time point (2 DPI) and peak virus titer in the insects. To determine effects of infection on feeding, midges were offered a non-infectious artificial blood meal for a short (10 minutes) or long period (60 minutes) and proportions of fed midges were compared using a generalized linear mixed model. Fecundity, egg viability, and the number of days from initial hatching to pupation were measured for egg clutches from individual females, and results were compared based on virus infection status and the number of DPI that a blood meal was taken. The potential consequences of altered blood feeding behavior and fecundity on virus transmission and epidemiology are discussed.

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CHARACTERIZATION OF TRYPSINS IN *LUTZOMYIA LONGIPALPIS*, THE MAIN VECTOR OF VISCERAL LEISHMANIASIS IN BRAZIL

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We are studying *Lutzomyia longipalpis*, the main vector of visceral leishmaniasis in Brazil. In sandflies of the Old World midgut enzymatic activity during blood digestion is one of the obstacles which Leishmania must surpass to succeed in establishing infection. In *L. longipalpis* little is known about the effect of the digestion process over parasites. Our preliminary results of enzyme activity assays using dissected guts suggest that *L. longipalpis* females at 48 hours after infection with *Leishmania chagasi* have lower trypsin activity than non-infected insects. We have previously described two trypsin cDNAs of *L. longipalpis*. One (Lltryp1) has a bloodmeal induced transcription pattern while the other (Lltryp2) is expressed constitutively. We have cloned and expressed a fragment of Lltryp2 cDNA, and the recombinant protein was used to produce a polyclonal anti-trypsin antibody. Western blot experiments using this antibody and whole insects showed a band of approximately 28 kDa, as expected, between 6 and 48 hours after blood ingestion. This profile is compatible with the appearance of Lltryp1, suggesting a cross recognition between the two trypsins. In order to improve the specificity of this recognition, we designed peptides specific for each of the two trypsin aminoacid sequences and these were used to produce polyclonal antibodies. We have used anti-Lltryp1-peptide antibody in Western blot experiments using insect midguts or carcasses. Bands of approximately 28 kDa were detected in midgut samples between 2 and 24 hours after blood ingestion, but not in carcass samples. Again, this is indicative of a blood meal induced enzyme. We are presently performing experiments with the anti-Lltryp2-peptide antibody. In-gel protease activity of insect midgut preparations at different times after feeding was also studied. Gel incubation in buffer solutions with different pH values showed pH 8.0 to be optimal for proteolysis. Gel polymerized with gelatin, hemoglobin, casein, or BSA showed a diverse pattern of bands for each substract, as well as different bands produced by midguts and carcasses. Gel incubation with specific inhibitors indicates that the major proteolytic activity is due to trypsin-like enzymes.

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RESPONSE OF *PHLEBOTOMUS PAPATASI* (DIPTERA: PSYCHODIDAE) TO COMMERCIAL MOSQUITO TRAPS IN SOUTHERN EGYPT

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Phlebotomus papatasi (Scopoli) is the primary Old World vector of the etiological agents responsible for cutaneous leishmaniasis from North Africa east into India and a vector of several phleboviruses. The World Health Organization has recently recognized leishmaniasis as an important