compared to North American controls. Frequencies of positive antigen-specific responses for these cytokines ranged from 31-55%. Cytokine responses to individual pre-erythrocytic peptides were less common, but levels of TRAP-specific IL-6 (38%, 7.6, 0-141, P=0.02), LSA-1-specific IL-6 (41%, 15.1, 0-336, P=0.03) and RANTES (34%, 4.3, 0-33, P=0.02), and CSP-specific TNF-α (31%, 0.7, 0-24, P=0.04) were also elevated in this population as compared to unexposed persons, with frequencies of positive responses ranging from 31-41%. In this population of unstable transmission, during a period of low transmission, IL-6 responses predominate, and more diverse responses are seen to blood-stage than pre-erythrocytic antigens.

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ANTIBODY MEDIATED BLOOD STAGE IMMUNITY AS MEASURED BY FUNCTIONAL GROWTH INHIBITION ASSAYS IS GREATER IN AREAS OF UNSTABLE AS COMPARED TO STABLE TRANSMISSION

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Antibodies that impair *Plasmodium falciparum* (Pf) merozoite invasion and intraerythrocytic development may mediate naturally acquired immunity to malaria. Attempts to correlate anti-malaria antibodies with risk of infection and morbidity have yielded inconsistent results. Functional assays may offer new insights into the development of antibody-mediated immunity. Plasma samples from children and adults residing in regions of Kenya with highland, unstable malaria transmission (n=150) and stable malaria transmission (n=197) were tested using two functional assays that quantify a) overall anti-malaria growth inhibitory activity (GIA) and b) invasion inhibition antibodies directed specifically against MSP-119 (MSP-119 IIA). Results were analyzed by age and correlated with time to acute disease (unstable region) or time to infection (stable region). Plasma samples from individuals in the unstable transmission region had greater overall GIA (median 19.46%) compared to that from individuals in the stable transmission region (median 8.7%, p<0.0001). Additionally, individuals in the unstable transmission region demonstrated greater MSP-119 IIA (median 9.56%) compared to individuals in the stable transmission region (median 0.85%, p<0.0001). Plasma from individuals in both unstable and stable transmission plasma exhibited age related decreases in overall GIA, but not MSP-119 IIA. Time to acute disease (unstable transmission area) was not correlated with GIA or MSP-119 IIA. Time to infection (stable transmission area) was correlated with GIA but not MSP-119 IIA. In conclusion, functional assays suggest that lower cumulative exposure to *P. falciparum* is associated with stronger GIA and MSP-1-specific antibody-mediated immune responses. However, among individuals with high cumulative exposure, and lowest level GIA responses as a group, higher GIA remains associated with protection from infection. Further studies are required to elucidate the mechanisms by which transmission intensity affects functional antibody activity.

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RESTRICTION OF SEROLOGICAL CROSS-REACTIVITY BETWEEN VARIANTS OF *PLASMODIUM VIVAX* DUFFY BINDING PROTEIN FOLLOWING SINGLE MALARIA INFECTION

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The Duffy binding protein of *Plasmodium vivax* (DBP) is a critical adhesion ligand that participates in merozoite invasion of human Duffy positive erythrocytes. A small outbreak of *P. vivax* malaria, in a village located in a non-malarious area of Brazil, offered us an opportunity to investigate the DBP immune response among individuals who had their first and brief exposure to malaria. Thirty-three individuals participated in the 5 cross-sectional surveys, 15 of then with confirmed *P. vivax* infection in the outbreak area (cases) and 18 who had non-experienced malaria infection (non-cases). In the present study, we demonstrate that although only 20% (5 out of 15) of the individuals who had experienced their first *P. vivax* infection develop antibody response to DBP; a secondary boosting can be achieved by the time of a recurrent *P. vivax* infection. DNA sequences from primary/recurrent *P. vivax* samples demonstrated that a single *dbp* allele was detected in the outbreak area. To investigate inhibitory antibodies to ligand domain of the DBP (cysteine-rich region II, DBP_{II}), we performed *in vitro* assays with mammalian cells expressing DBP_{II} sequences which were homologous or not to those from the outbreak isolate. The results of a 12-month follow-up period provided evidence that naturally acquired inhibitory antibodies to DBP_{II} are short-lived, and seem to be allele-specific.

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POLYMORPHIC VARIABILITY IN THE IL-4 -589T/C PROMOTER IS ASSOCIATED WITH INCREASED SUSCEPTIBILITY TO HIGH-DENSITY MALARIA PARASITEMIA

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Interleukin (IL)-4 is released as part of the innate immune response to *Plasmodium falciparum*. While lower production of IL-4 has been associated with malaria disease severity, associations between IL-4 -589T/C and malaria outcomes in holoendemic transmission areas remains undefined. In this study, the relationship between IL-4 -589T/C, presence of parasitemia, high-density parasitemia (HDP; ≥10,000 parasites/μL), and severe malaria anemia (SMA; Hb<6.0g/dL) were investigated in Kenyan children from a holoendemic *P. falciparum* transmission area. Functionality of IL-4 -589T/C was examined by determining the association between circulating IL-4 levels and malaria disease outcomes. Children (*n*=618) were enrolled at Siaya District Hospital in western Kenya. Genotyping was carried out using Taqman 5'-allelic discrimination assay. IL-4 plasma levels were assayed by a Human Cytokine 25-Plex assay. Prevalence of CC, CT, and TT genotypes were: 13%, 24%, and 63%, respectively. In a multivariate logistic regression model controlling for age, gender, bacteremia, HIV-1 and sickle-cell status, heterozygous individuals (CT) had a 64% increased risk of developing HDP relative to the TT group (OR; 1.64, 95%CI; 1.01-2.65, *P*<0.05). Polymorphic variability at -589T/C was not associated with either SMA or presence of parasitemia. However, variation at -589T/C was not significantly associated with circulating IL-4 levels. Results presented here demonstrate that IL-4 -589T/C is significantly associated with increased susceptibility to HDP.

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IDENTIFICATION OF *PLASMODIUM YOELII* RBC MEMBRANE PROTEINS INVOLVED IN ADHERENCE TO A MURINE ENDOTHELIAL CELL LINE

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Plasmodium falciparum invades normocytes and reticulocytes and causes most malarial fatalities. *P. vivax* is infrequently fatal and preferentially invades reticulocytes. These host cell preferences can be studied in rodent models using lethal *P. yoelii* 17XL parasites which infect both mature and immature RBCs, or non-lethal *P. yoelii* 17X parasites which primarily invade reticulocytes. We have previously shown that in the presence of

antibody-mediated immune pressure *P. yoelii* 17XL parasites preferentially invade immature RBCs. Gene expression changes associated with this switch reveal altered expression of genes predicted to be expressed on the surface of infected RBCs. We hypothesize that expression of these proteins promotes the localization of *P. yoelii* infected RBCs to reticulocyte-rich tissues such as bone marrow and spleen. This may limit exposure to merozoite neutralizing antibodies while providing access to target RBCs that are normally present in low numbers in circulation. Here we show that *P. yoelii* infected RBCs adhere to a mouse endothelial cell line. We have quantitated differences in the adherence of *P. yoelii* 17X and *P. yoelii* 17XL infected RBCs and can partially block adherence using sera obtained from animals immunized with membrane proteins prepared from *P. yoelii* infected RBCs. Adherent *P. yoelii* 17X infected reticulocytes can be released using heparin sulfate or dextran sulfate. We have used DNA microarrays to analyze gene expression in adherent and non-adherent populations of *P. yoelii* infected RBCs. We have identified a set of ~60 genes whose expression is consistently upregulated in the adherent population. This gene set has been further refined using *P. yoelii* 17X pRBCs obtained from mice on both day 9 and day 14 of infection, *P. yoelii* 17XL infected reticulocytes obtained from *P. yoelii* merozoite surface protein-8 (*Py*MSP8) immunized mice and *P. yoelii* 17X pRBCs from animals that have been immunized with the *P. yoelii* 17X RBC membrane proteins. We have defined a set of genes whose expression is consistently associated with the adherence phenotype and ranked genes of interest based on signal intensity, expression ratio, presence of a signal sequence and/or transmembrane domain, Pexel motif, etc. Currently, we are focusing our studies on the 10 top ranked genes to determine their localization of expression and to evaluate their role in mediating adherence of pRBCs to endothelial cells.

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MECHANISMS OF DRUG INDUCED GENE EXPRESSION IN *PLASMODIUM FALCIPARUM*

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The mechanisms that control gene expression in the malaria parasite are poorly understood with little information on the role of promoters, terminators and transcription factors. Observations from early studies suggested that they appear to conform to the classical eukaryotic bipartite structure consisting of a proximal promoter regulated by upstream enhancer elements (cis-acting elements), yet a classical regulatory motif, which can be identified in multiple unrelated genes, has yet to be identified. In stark contrast is the wealth of information available about the role of transcription factors and promoters in gene regulation in mammals: One such example is the regulation of Phase I and II drug metabolizing enzymes and drug transporters by members of the nuclear receptor super-family, such as the pregnane X receptor (PXR) and the constitutive androstane receptor (CAR), and their role in modulating drug resistance patterns in mammals. We have recently exploited this area of research and have shown that after a 48 h exposure to the anticonvulsant drug phenobarbitone (PB) the multidrug resistance protein, Pgh1, was significantly up-regulated compared to untreated controls. This increased expression of a P-glycoprotein is similar to that observed in mammals and clearly demonstrates that the parasite has the ability to alter its repertoire of controlled gene expression in response to external stimuli, presumably to combat the toxic effects of the drug. However, the factors that control this increased level of expression are currently unknown. The initial work highlighted above focused primarily on two genes that were known to be involved in parasite drug resistance, *pfcrt* and *pfmdr1*. There are at least 107 identified transporter genes in the *P. falciparum* genome with potentially many of them showing differential expression after exposure to PB. Here we have investigated the expression levels of a subset of these transporter genes after PB exposure by reverse transcriptase real-time-PCR with the aim to identify a transcriptional motif common to all transporters showing differential expression. Transporters were selected based on their potential involvement with parasite drug resistance and importance in cell cycle development- with the ultimate goal being to exploit them as targets for future drug development programs.

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MOLECULAR CHARACTERIZATION OF POLYMORPHISMS IN THE *PLASMODIUM VIVAX MDR1-LIKE* GENE *(PVMDR1)* FROM THE AMAZON BASIN AND THE NORTH COAST OF PERU

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Reports from South America have suggested the presence of isolated cases of chloroquine (CQ) resistant *Plasmodium vivax* malaria. To date, very few confirmed cases of *P. vivax* CQ resistance have been reported. The *pvmdr1* gene is characterized by a single ORF of 4392 bp, encoding a protein of 1464 aa. We report polymorphisms present in *pvmdr1* in isolates from the Amazon Basin of Peru analyzed using PCR and DNA sequencing. Two regions of the *pvmdr1* gene have been taken into consideration: one encompassing the *P. vivax* positions homologous to the polymorphic sites 86 and 184 in *P. falciparum,* and the other encompassing the *P. vivax* positions homologous to the polymorphic sites 1034 and 1042 in *P. falciparum.* Sequence analysis of the *pvmdr1* gene in samples from the Amazon Basin (n= 78) shows 99% of the samples harbor the mutant M908L allele and 49% exhibit the M908L/T958M double mutant genotype. We also found wild-type alleles at codons N91, Y189, S1071, N1079, and D1291. Less frequent mutant polymorphisms were observed in 12-16% of the samples at codons V221L, D500N, Y976F, F1070L, F1076L, and synonymous mutations at T529 (ACA →ACG), L1022 (CTA →TTA), and K1355 (AAA → AAG). In contrast, isolates from the North Coast region of Peru reveal a similar pattern of wild type alleles (N91, Y189, S1071, N1079, and D1291) but differ in their mutant genotype pattern. In 100% (n=12) of the samples mutations were observed in V221L, M908L, T958M, F1070L, T529, L1022, and K1355. These isolates exhibit unique genotypes compared to those previously reported and may contribute to CQ resistance patterns observed in *P. vivax* from Peruvian isolates.

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THE FREQUENCY OF DRUG RESISTANCE MUTATIONS IN *DHFR*, *DHPS*, AND *PFCRT*, ON THE PACIFIC COAST OF PERU

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Clinical studies have demonstrated that *Plasmodium falciparum* parasites from the Pacific Coast of Peru are generally resistant to Chloroquine (CQ), but still susceptible to Sulfadoxine Pyrimethamine (SP). Therefore, artesunate plus SP is used for primary treatment of *P. falciparum* malaria in the coastal region. In chloroquine resistance transporter (pfcrt) gene has been linked to CQ resistance. In the case of SP resistance, two genes, dihydrofolate reductase (dhfr) and dihydropteroate synthase (dhps) have been linked to pyrimethamine and sulfadoxine resistance respectively. Although the frequencies of CQ and SP resistant genotypes have been reported for the Amazon region of Peru, the frequencies of these genotypes in the coastal region are not well understood. In order to determine the frequencies of these mutations in the coastal region, we are currently genotyping a total of 151 samples from three different sites on the Peruvian coast to define the prevalence of mutations that confer antimalarial resistance in pfcrt, dhfr, and dhps,. Direct sequencing is being used to genotype mutations in pfcrt (codons 72-76) Pyrosequencing is being used to genotype mutations in dhfr (codons 50, 51, 59, 108, 164) and dhps (codons 436, 437, 540, 581, 613). All but one of the samples currently genotyped for pfcrt show the resistant genotype CVMNT

(n=104) with the single exception showing the SVMNT (n=1) genotype. Our preliminary data for dhfr suggests that only the S108N mutation is present (15 of 71 samples). These results suggest that the CQ resistant CVMNT genotype is fixed and mutations associated with clinical resistance to SP are not established on the Peruvian coast. This finding is consistent with the previously observed clinical sensitivities of *P. falciparum* infections to CQ and SP on the Peruvian coast.

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DOES *PLASMODIUM FALCIPARUM* INDUCE SPECIFIC GENE EXPRESSION? COMPARISON WITH OTHER PATHOGENS

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To relate gene expression in *Plasmodium falciparum* infection to gene expression in viral and bacterial infections, we compared microarray data for human peripheral blood mononuclear cells from patients with influenza and Gram-positive bacterial infection to data from patients with uncomplicated malaria. We compared public gene expression data from children with influenza or Gram-positive bacterial infections (GSE 6269) to data from children with acute *P. falciparum* infection. Total RNA was isolated, converted to cRNA and hybridized using similar protocols and platforms (Affymetrix GeneChip HG- U133 Plus 2.0 Array). The five groups of patients analyzed were: 1] Group 1: 10 patients with influenza virus infections; 2] Group 2 : 10 patients with Gram-positive bacterial infections; 3] Group 3: 10 patients with uncomplicated *Plasmodium falciparum* infections; 4] Group 4: 10 treated patients (recovery from malaria); 5] Group 5: 10 healthy controls. Microarray data analyses were performed using dChip software and genes were classified using GO terms. Each disease group was compared to both the recovery and healthy groups (2 fold change 1% false discovery rate, 100% p call). Based on these criteria, we identified 657 genes induced in Group 1, 519 genes induced in Group 2 and 25 genes induced in Group 3. The genes induced by uncomplicated *P. falciparum* infection included innate immune response, antigen binding, transcription factor-related genes that have been described previously in malaria. However, other genes identified have not been associated with malaria infection or its pathogenesis previously, and their potential roles are unclear. Detailed analyses of these genes may help to identify new transcriptional markers for human *P. falciparum* infection.

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GENETIC DIVERSITY STUDIES OF *PLASMODIUM FALCIPARUM* AND *P. VIVAX* ISOLATES CIRCULATING IN PANAMANIAN ENDEMIC AREAS

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Malaria continues to be an important public health problem in Panama, Central America. A molecular epidemiology study was conducted to evaluate the genetic diversity of among 50 field isolates of *Plasmodium falciparum* and 50 isolates of *P. vivax* from different malaria endemic regions in Panama. Allelic diversity was analyzed using PCR- based methods and direct amplicons sequencing to evaluate polymorphisms within three *P. falciparum* genes (MSP-1, MSP-2 and GLURP) and three *P. vivax* genes (CSP, MSP-1 and MSP-3). Results from the genetic analysis suggest that *Plasmodium* population circulating in the country is genetically homogenous as evidenced by the fact that only two different alleles from each *Plasmodium* species could be demonstrated with these molecular markers. Thus, multiple genetic markers are recommended to detect sufficient parasite diversity in Panama. The public health implications of the limited genetic diversity observed in the country are discussed.

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TRANSCRIPTIONAL ANALYSIS OF PUTATIVE FOLATE TRANSPORTER GENES IN *PLASMODIUM FALCIPARUM*

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The *de novo* biosynthesis of folic acid pathway in *Plasmodium* and the mechanism of resistance to anti-folates are now well defined but there is little attention on the search for the underlying molecular mechanisms involved in the inter-strain growth variability under different 'folate' nutrient conditions. The parasite has also evolved an intricate and adaptively-derived salvage capability of scavenging 'folate' molecules. Some isolates show excellent growth pattern in both folate deplete and replete media condition while others cannot do well in folate deplete conditions. There are speculations that this biochemical phenomenon is linked to the properties of the well characterised key folic acid enzymes; Dihydropteroate Synthase (DHPS) and Dihydrofolate Reductase (DHFR). Studies on the putative folate transporter genes that encode for proteins which mediate the salvage process could offer a clue to the 'folate-linked' inter-strain growth variability. We hypothesised that differences in the regulation pattern of these putative transporters could be the underpinning mechanism behind this physiological process. Using Real Time-PCR, we analysed the transcription pattern of the putative folate transporter genes in a number of isolates exhibiting distinct 'folate-linked' growth variability. The transcriptional regulation pattern and the gene responses to specific 'folate' substrates will be discussed.

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STRAIN SPECIFICITY IN THE REQUIREMENT FOR MITOCHONDRIAL ELECTRON TRANSPORT IN ERYTHROCYTIC STAGE *PLASMODIUM FALCIPARUM*

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Previous studies in our laboratory have revealed that *Plasmodium falciparum* D10 parasites become fully resistant to the mitochondrial electron transport chain (mtETC) inhibitor atovaquone when engineered to express the yeast dihydroorotate dehydrogenase (yDHOD) gene, presumably allowing the parasites to maintain pyrimidine biosynthesis in the absence of mtETC. However, we subsequently found that *P. falciparum* 3D7·yDHOD transgenic parasites, in contrast to D10·yDHOD, did not show full atovaquone resistance during long term growth. Since the yDHOD gene was episomally expressed, this inconsistency could be due to differing interaction of these lines with episomal plasmids. In order to investigate the different requirement for mtETC in distinct strains of *P. falciparum*, we are attempting to integrate a single copy of yDHOD into an identical chromosomal position in the cg6 locus of each of the strains D10, 3D7, Dd2, and HB3 using site specific integration mediated by mycobacteriophage Bxb1 integrase. In initial experiments, we achieved integration into two strains. After successful integration of yDHOD, we will assess the susceptibility of these transgenic cell lines to mtETC inhibitors and determine the atovaquone resistance in long term culture. If significant differences are observed between these strains, we plan to introduce the yDHOD gene into progenies of the HB3xDd2 and HB3x3D7 crosses. By correlating the phenotypic differences with genotypic markers, we hope to find the basis for the differing reliance on mtETC in different strains.

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PLASMODIUM FALCIPARUM ERYTHROCYTE BINDING ANTIGEN (EBA) 175 GENE DIVERSITY IN MALARIA ENDEMIC AREA WITH SEASONAL VARIATION IN BURKINA FASO

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The high polymorphism of *Plasmodium falciparum* genes is known to represent one of the main obstacles to the development of an effective malaria vaccine. The erythrocyte binding antigen 175 kDa (EBA-175) of *Plasmodium falciparum* one of the major ligand for red blood cell invasion by merozoites presents a well characterized dimorphism. The two variants of the EBA-175 dimorphism are evolutionarily conserved suggesting functional relevance with impact on malaria immunology. Although, previous studies have characterized the EBA 175 gene, the dynamic of EBA 175 genotypes in malaria endemic area where transmission is markedly seasonal, is not well known. The study was conducted in Saponé Health District, 50 km South-West of Ouagadougou the capital city of Burkina Faso. Blood samples were collected from children below five years to prepare filter papers for DNA extraction during the malaria low and high transmission season. Malaria smears were also prepared to identify *P. falciparum* positive samples. The DNA was extracted by Qiagen kit and analyzed by a nested PCR amplification of EBA 175 gene. 191 filter papers blood samples were extracted and analyzed. Prevalence's of CAMP strains (C-segment) and FCR-3 strains (F-segment) were respectively 17.3% (33/191) and 49.7% (95/191). No significant seasonal variation was observed. The prevalence of mixed CAMP/FCR3- infection was 33.0% (63/191). At any of the transmission season, the FCR3 strains were more prevalent than CAMP strains: 45.1% versus 17.1 %(p=0.001) during the low and 53.2% versus 17.4% (p<0.001) during the high transmission season). In conclusion, these results showed that FCR3 strains were more prevalent than CAMP in the study site and no seasonal variation has been observed. This finding must be taken into account when planning vaccine trial with this malaria vaccine candidate.

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GENETIC DIVERSITY OF THE CRITICAL BINDING MOTIF OF *PLASMODIUM VIVAX* DUFFY BINDING PROTEIN IN SRI LANKA

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Exploring the genetic diversity of candidate antigens is obligatory in vaccine development. The efficacy of a vaccine based on polymorphic parasite antigen is influenced by the local host immune response. Therefore, the characterization of diversity among the local strains is important in specific geographic settings. Genetic diversity of the critical binding motif of region II in *Plasmodium vivax* Duffy Binding Protein (PvDBPII), was assessed among field isolates from Sri Lanka. Single clonal infections were identified from two malaria endemic areas (EA) and from a non-endemic area (NEA), by assessing the nucleotide diversity of PvMSP-3α gene using PCR/RFLP techniques. Nucleotide sequence data of PvDBPII (aa 285 to 521) was obtained for 40 isolates (EA=24; NEA=16) by nested PCR amplification followed by direct sequencing. Thirteen different haplotypes consisting of 18 dimorphic sites were identified spanning a region of 678 bp in the protein. Genetic polymorphism in terms of pair wise diversity (π) and Tamura's three parameter model (*d*) were calculated to be 0.0090 and 0.0091, respectively, which are consistent with published data from world wide isolates. Sixteen of the 18 nucleotide polymorphisms were of non-synonymous (N) nature. The ratio of NS (0.0104) to synonymous (S) substitutions (0.0046) was >1, suggesting a positive selection acting on this motif. A significant departure from neutrality with a high frequency of NS mutations was also observed within *P. vivax* sequences (McDonald Kreitman test) when *P. knowlesi* DBP-α was considered as an out group. Although not significant, polymorphic sites (18) and π (0.0103) in EA were higher compared with that of NEA (N=15: π =0.0089). Of the nucleotide polymorphisms, NS mutations were markedly higher in both EA (16/18) and NEA (13/15) isolates where *d*N/*d*S was >1. Relatively high allelic diversity and positive selection acting on the PvDBPII critical binding motif was evident, possibly due to immune pressure, even under low and unstable transmission conditions prevalent in the island.

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EFFECT OF INSECTICIDE-TREATED BED NETS (ITNS) ON GENE POLYMORPHISMS OF *PLASMODIUM FALCIPARUM* VACCINE CANDIDATE ANTIGENS IN A MALARIA HOLOENDEMIC AREA OF WESTERN KENYA

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Insecticide-treated bed nets (ITNs) have been associated with 70-90% reduction in malaria transmission and significant health benefits in vulnerable populations. Although several studies have investigated the long term impact of ITNs on morbidity and mortality patterns, there is a paucity of data on their effect on parasite genetic diversity. The current study investigated the effect of high coverage (>70% household ownership and use) with ITNs on genetic polymorphisms in two leading *Plasmodium falciparum* vaccine candidate antigens, the pre-erythrocytic stage circumsporozoite protein (CSP) and blood stage Merozoite Surface Protein-1 (MSP-1). Sequencing was used to determine single nucleotide polymorphisms (SNPs) on the relatively conserved B-cell epitopes bearing 19 kilodalton region of the MSP-1 (MSP-1 $_{19kDa}$) and the highly polymorphic CSP Th2R and Th3R T-cell epitopes. Smear-positive blood samples collected from children aged less than 5 years old, prior to (year 1998), and post (year 2001) ITNs intervention (referred to as control and ITN periods, N = 71 and 76 respectively) in western Kenya were used. Preliminary data analysis show that the Q-KESNG-L and E-KESNG-L MSP-1 $_{19kDa}$ haplotypes corresponding to the FVO and FUP strains of *P. falciparum* were the most prevalent, range 26-37%. The frequency of haplotype corresponding to the 3D7 strain was less than 4%. There was no significant difference in the number of MSP-1 haplotypes between the two time periods (9 vs 10). However, we observed 4 new haplotypes post ITN intervention while 3 haplotypes had disappeared. The most prevalent CSP Th2R and Th3R haplotypes were PSDQHIEKYLKTIQNSLS and NKPKDQLDYEND corresponding to parasite isolates reported in The Gambia and Vietnam respectively, range 21-37%. There was an increase in the number of Th2R but not Th3R haplotypes between the two time periods (25 vs 32 and 13 vs 13 respectively). Whereas there were only 4 new Th3R haplotypes observed over the ITN period, there were 16 new Th2R haplotypes during the same period. Our preliminary data suggests that the Th2R region of CSP could be under strong selective pressure compared to the Th3R and MSP-1$_{19kDa}$ even after transmission reduction in an ITN trial site. This information may be useful in interpreting data on

the effectiveness of CSP-based malaria vaccines in endemic areas with or without ITNs.

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IMMUNOGENICITY OF TWO DOSES OF A MULTI-STAGE, MULTI-ANTIGEN ADENOVIRUS-VECTORED *PLASMODIUM FALCIPARUM* MALARIA VACCINE IN A PHASE 1 TRIAL

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To select a safe and immunogenic dose of the NMRC-M3V-Ad-PfCA adenovectored (serotype 5) malaria vaccine (CSP and AMA1 antigens) for testing for efficacy against *Plasmodium falciparum* (Pf) sporozoite challenge, we administered either 2x10<10 or 10x10<10 particle units to healthy, malaria-naïve, Ad5-seronegative, adult volunteers. Two groups of six individuals received a single injection followed by the collection of serum and PBMCs on day 10 and months 1, 4, 7, 10 and 13. Antibodies to PfCSP and AMA1 antigens were measured by ELISA and to sporozoite, hepatic and blood stage parasites by IFA. Antibody functional activity was tested by growth inhibition assay against Pf 3D7 (homologous) or FVO (heterologous) Pf strains. Responses peaked on day 28 post-vaccination; geometric mean titers against CSP were 1:692 and 1:930 and against AMA1 were 1:4395 and 1: 8478 in the low and high dose groups, respectively (difference not statistically significant). IFAs were weakly positive in both groups. No significant growth inhibitory effect was detected for either group when purified immunoglobulin was added to Pf cultures. Cell mediated responses were assessed by interferon-γ (IFNγ) ELISpot assay and by flow cytometry/intracellular cytokine staining for IFNγ, IL-2 and TNF following stimulation with pooled 15mer overlapping peptides spanning CSP (9 pools) and AMA (12 pools) proteins. As with antibody responses, IFNγ responses peaked day 28: for CSP, means of 522 and 226 spot forming cells/million PBMCs were recorded in the low and high dose groups respectively ($p<0.05$), while for AMA1, 1037 and 560 sfc/million were recorded (groups not significantly different). As IFNg and TNF may synergize in killing targets and IL2 may enhance the expansion of effector cells, we have analyzed profiles of antigen-specific CD4+ and CD8+ responses for these three cytokines to determine the degree of polyfunctionality. These results will enable down-selection of the optimal dose for the challenge phase of the clinical trial.

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DIFFERENT ASSESSMENT METHODS OF MALARIA MORBIDITY FOR FUTURE MALARIA VACCINE TRIAL IN A HIGH AND SEASONAL MALARIA TRANSMISSION AREA OF BURKINA FASO

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The incidence of clinical malaria is one of the key endpoints measures for malaria vaccine candidates' trials. These measures might be done either by active or by passive case detection (ACD, PCD) methods. This study aims to identify the most suitable method to be use in a site being prepared for future malaria vaccine candidates' trials. Two cohort studies were conducted within the same study area of saponé. The first cohort consisted (ACD cohort) of a group of 554 children aged 0-5 years followed up by biweekly home visit performed by trained study nurses. The second cohort (PCD) included 927 children of the same age as for the ACD, whose parents were encouraged to report to the nearest community clinic or hospital at any time should their child feel sick. Treatment was provided free of charge in both cohorts. At each visit of the study participants (home visit or child visit to the community clinic), a malaria smear was obtained if fever (child axillary temperature ≥ 37.5°C). Study duration was one year for both cohorts. A malaria episode was defined as positive *Plasmodium falciparum*- parasites density in presence of fever. In the PCD cohort, 3479 clinic visits were recorded over the year period with 1076 malaria episodes diagnosed. The incidence of clinical malaria was 1.17 episodes/child-year at risk (ci 95% [0.48 - 1.86]. In the ACD cohort, total 56716 home visits were performed. The children were seen during 49062 visits. A total of 381 malaria episodes were diagnosed. The overall incidence of clinical malaria was 0.78 episode/child-year at risk (95% ci [0.70-0.86]). In conclusion, these finding suggest that in our setting with a treatment provided free of charge PCD will be the efficient method to assess malaria morbidly in under five children.

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RANDOMIZED, CONTROLLED, DOSE ESCALATION PHASE 1 CLINICAL TRIAL TO EVALUATE THE SAFETY AND IMMUNOGENICITY OF WALTER REED ARMY INSTITUTE OF RESEARCH'S AMA-1 MALARIA VACCINE (FMP2.1) ADJUVANTED IN GSK BIOLOGICALS' AS02 VS. RABIES VACCINE IN 1-6 YEAR OLD CHILDREN IN BANDIAGARA, MALI

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The malaria vaccine candidate antigen FMP2.1 is a recombinant protein based on the 3D7 strain *Plasmodium falciparum* apical membrane

antigen-1 (AMA-1). The purpose of this randomized, dose escalation Phase 1 clinical trial (NCT: 00358332) was to evaluate the safety and immunogenicity of FMP2.1 formulated in GlaxoSmithKline's Adjuvant System AS02 in children in Bandiagara, Mali, West Africa. One hundred healthy children aged 1-6 were randomized to receive 3 doses 30 days apart of 10, 25 or 50 µg of FMP2.1 with 0.10, 0.25 or 0.50 mL of AS02, respectively, or rabies vaccine. Solicited symptoms were monitored actively for seven days following each vaccination and unsolicited adverse events were followed for 30 days following each immunization. Children were followed for one year after the last vaccination. Baseline demographic characteristics were similar in all groups, as was the incidence of solicited systemic reactions following vaccination. Solicited local reactions, especially swelling, were higher among those receiving the candidate malaria vaccine, but this was usually transient and did not cause functional impairment nor concern to the parents of participants. The incidence of unsolicited adverse events was similar in all groups. Anti-AMA-1 antibody titers were similar in all participants receiving malaria vaccine regardless of the dose, and were significantly greater in recipients of malaria vaccine when compared to rabies vaccine. All 3 dose levels of FMP2.1/AS02 had a good safety profile, were well-tolerated and elicited a significant rise in anti-AMA-1 antibody titers. Follow-up safety and immunogenicity analyses will be presented.

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A *PLASMODIUM FALCIPARUM* MULTI-ANTIGEN MULTI-STAGE PLASMID DNA PRIME/ADENOVECTOR BOOST VACCINE, NMRC-M3V-D/AD-PFCA, IS IMMUNOGENIC IN BALB/C MICE

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NMRC-M3V-D/Ad-PfCA prime/boost is a candidate malaria vaccine undergoing product development at the US Military Malaria Vaccine Program. The vaccine is a multivalent multi-stage vaccine designed to induce cellular and humoral immune responses against both pre-erythrocytic and erythrocytic stages of the *Plasmodium falciparum* parasite life cycle. The priming component (NMRC-M3V-D-PfCA) comprises a mixture of two plasmid DNA vaccines encoding either PfCSP (sporozoite/liver stage antigen) or PfAMA1 (sporozoite/liver/blood stage antigen). The boosting component (NMRC-M3V-Ad-PfCA) comprises a mixture of two recombinant adenoviral serotype 5 vectors expressing either PfCSP or PfAMA1. We have previously demonstrated that research stocks of this heterologous DNA prime/adenovirus boost vaccine are immunogenic in murine, rabbit, and swine models of malaria. Here, we confirmed in mice the immunogenicity of GMP-produced NMRC-M3V-D-PfCA and NMRC-M3V-Ad-PfCA vaccine drug products (VDP) and the improved immunogenicity associated with prime/boost administration. BALB/c mice were primed IM with a mixture of 50ug each plasmid (100ug NMRC-M3V-D-PfCA) 3 times at 4 week intervals and then boosted 4 weeks later IM with $1x10^8$ pu of each recombinant adenovector ($2x10^8$ pu NMRC-M3V-Ad-PfCA). Sera were collected for antibody assays pre- (day -2) and 14 days post each immunization. Splenocytes were harvested for T cell assays 14 days post adenovirus boost. Data establish that the NMRC-M3V-D/Ad-PfCA vaccine drug product is immunogenic in BALB/c mice, as indicated by the induction of *Pf*CSP- and *Pf*AMA1-specific T cell and antibody responses (IFN-γ ELIspot, intracellular cytokine staining, and ELISA). Antibody and T cell responses were enhanced by the heterologous prime/boost regimen as compared with homologous DNA or adenovector alone. Overall, GMP-produced NMRC-M3V-D/Ad-PfCA induces robust antigen-specific T cell and antibody responses, supporting clinical evaluation of NMRC-M3V-D/Ad-PfCA.

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PRODUCTION, CHARACTERIZATION AND IMMUNOLOGICAL EVALUATION OF AN *ESCHERICHIA COLI* EXPRESSED *PLASMODIUM FALCIPARUM* THROMBOSPONDIN RELATED APICAL MEROZOITE PROTEIN (PTRAMP), A PUTATIVE MALARIA VACCINE CANDIDATE

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The release of a number of *Plasmodium* genome sequences has opened up an extraordinary opportunity to discover new proteins that play crucial roles in development and invasion of red blood cells. The *P. falciparum* thrombospondin related apical merozoite protein (PTRAMP) is an example of such a novel protein. PTRAMP is expressed in the late stages of the parasite's asexual red blood cell cycle where it is located within the micronemes, organelles critical for erythrocyte invasion. PTRAMP relocates to the surface of the merozoite prior to schizont rupture. PTRAMP is a cysteine rich protein with a type I thrombospondin structural homology repeat (TSR) domain. The TSR domain is an evolutionary-ancient motif found in some cellular signaling proteins such as CSP which is a leading malaria vaccine candidate. We are evaluating whether PTRAMP is a target for inclusion in a recombinant protein based blood stage malaria vaccine. PTRAMP has been expressed in *Escherichia coli* using an *E. coli* codon optimized gene which also contains an in frame His_6 affinity tag at the carboxyl end of the protein. Recombinant PTRAMP was captured, refolded and purified using anion exchange and size exclusion column chromatography. Purified recombinant PTRAMP was characterized biochemically and biophysically for purity, integrity as well as protein folding by circular dichroism. Purified recombinant PTRAMP appears as a single peak by reverse-phase HPLC analysis under non-reduced conditions. Currently rats are being immunized using an oil-in-water adjuvant for the generation of antibodies against PTRAMP for immunological and parasitological analysis including *in vitro* parasite growth inhibition. The production and characterization results as well as the biological findings will be presented.

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PHASE 1 SAFETY AND IMMUNOGENICITY TRIAL OF A BLOOD-STAGE MALARIA VACCINE AMA1-C1/ISA 720 IN AUSTRALIAN ADULTS

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A Phase 1 clinical trial was conducted in malaria-naïve, Australian adults to evaluate the safety and immunogenicity of a recombinant protein, blood-stage vaccine based on *Plasmodium falciparum* apical membrane antigen 1 (AMA1) formulated in Montanide ISA 720 (SEPPIC, France), a stable, water-in-oil adjuvant. In order to induce immune responses that cover >50% of antigenic polymorphisms, the AMA1-FVO and 3D7 forms of the antigen were mixed (AMA1-C1). The vaccine was stable and potent over the course of the study. 24 subjects were enrolled to receive either 5 or 20 µg protein at 0 and 3 months. 24 received the first vaccination and 20 received the second. Withdrawals prior to 2nd vaccination were not due to vaccine related AEs. After first vaccination, nearly all subjects experienced

mild to moderate local injection-site reactions and 5 subjects experienced transient, delayed local reactions (mild to moderate pain) occurring at day 9 or later. After second vaccination 3 subjects experienced transient grade 3 (severe) local reactions; the remainder experienced grade 1 or 2 local reactions. All related systemic reactogenicity was grade 1 or 2, except 2 instances of grade 3 malaise. Antibody levels to the FVO and 3D7 forms of AMA1 were measured by ELISA at baseline, 8 weeks after first vaccination, at second vaccination and 4 weeks after second vaccination. Anti-AMA1 antibody responses were seen following each vaccination. A dose response was observed with geometric mean antibody levels 2 fold higher at each time point in the 20 µg group. In vitro *P. falciparum* growth inhibition assays (GIA) were performed using purified IgG isolated from week 16 sera. GIA activity increased with increasing anti-AMA1 antibody titer. The blood-stage vaccine AMA1-C1 formulated in Montanide ISA 720 is immunogenic in malaria-naïve Australian adults. It is reasonably well tolerated, though some transient, severe, local reactions are seen.

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RATIONAL DESIGN OF A PAN-REACTIVE APICAL MEMBRANE ANTIGEN-1 BASED MALARIA VACCINE USING SEROTYPES AND EPITOPE MAPS

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The malaria parasites prevailing in endemic areas comprise a continuum of diverse Apical Membrane Antigen-1 (AMA1) protein sequences. Immune response to AMA1 in animal models and in humans is highly strain-dependent. There is an urgent need to address the diversity problem as vaccines based on AMA1 are being tested for efficacy in human trials. Using inter-strain chimeric proteins we have previously mapped the polymorphic residues that lie within the most important inhibitory epitopes of AMA1. These polymorphic sites were shown to play an important role in escape from invasion inhibitory anti-AMA1 antibodies *in vitro*, and were therefore termed as Antigenic Escape Residues (AER) of AMA1. We hypothesize that global AMA1 sequences can be divided into 9 sequence families based on their respective AER genotypes and that these families may also represent AMA1 serotypes. Growth inhibition assays are being performed to test this hypothesis. We are also testing novel protein engineering strategies to dampen the immune responses against the AER and to focus the immune response towards the cross-reactive inhibitory epitopes. Results from ongoing epitope mapping studies using immune sera against AMA1 and serotying analyses using a diverse array of *Plasmodium falciparum* strains in growth inhibition assays will be discussed.

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HEMATOLOGICAL PARAMETERS CHANGES IN CHILDREN LESS THAN SIX YEARS LIVING IN MALARIA ENDEMIC AREA: IMPLICATION FOR FUTURE MALARIA VACCINE TRIALS

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Hematological parameters can be affected by nutritional status, hemoglobinopathy background, immunity and helminthiasis. In malaria endemic area, almost any children are infected by plasmodium which targeted red blood cells and could have some impact on hematological parameters. In order to describe these variations in a future malaria vaccine candidate trials site, we examined hematological profiles of children less than 6 years living in an area where malaria transmission is endemic and seasonal. Community cross sectional survey has been conducted at the peak of malaria transmission season in rural area of Saponé Health District located at 50 km South-west of Ouagadougou, the Capital city of Burkina Faso in September 2007. After a short history taken and clinical examination, a blood sample was obtained from all participants for malaria blood film and full blood count. Blood films were examined by microscopy using validated SOP; the full blood count was performed with a semi-automated haematology analyzer (Pentra 60). A total 414 children were recruited. From them, 192 were malaria negative and 222 children were infected with *Plasmodium* parasites at any density. The mean age of infected children was 41.8 ±15.4 months vs. 38.8±16.4 months (P>0.05) for non infected children. No significant differences were observed between the two groups in term of hemoglobin level (10.8g/dl vs. 10.4g/dl p =0.06), leucocytes count (9623±3262/µL vs. 9520±3297/µL p=0.75). However, parasitemic children tend to have significant lower lymphocytes count (4592±1999/µL vs 5141±1846/µL, p<0.001), platelets count (266149±114995/µL vs. 379540±163263/µL; p<0.001), red blood cells count ($4.388.10^6$±535647/µL vs. $4.158.10^6$±563356/µL; p<0.001) and higher monocytes count (1402±666/µL vs.1192±534/µL; p<0.001) as compared to non infected children. There was a significant difference of the platelets count between healthy children, those with asymptomatic malaria infection and those with acute malaria. In conclusion, these findings suggest that malaria parasites may affect the haematopoiesis of children living in malaria endemic area and special attention should be applied when interpreting haematological parameters in children living in this area and enrolled in vaccine trials.

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EXPRESSION AND LOCALIZATION OF *PLASMODIUM FALCIPARUM* MEROZOITE SURFACE PROTEIN 8 IN BLOOD STAGE MALARIA PARASITES

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An effective malaria vaccine remains elusive despite concerted research efforts. Current vaccine approaches target extracellular merozoites, which invade and replicate within RBCs and cause clinical disease. Studies indicate that immunization with a 42 kDa recombinant merozoite surface protein 1 (MSP-1_{42}), the lead vaccine candidate, induces suboptimal levels of antibodies against the conformational EGF-like domains of MSP-1_{19} known to inhibit the *in vitro* growth of *Plasmodium falciparum*. Merozoite surface protein 8 (MSP-8) also contains two C-terminal EGF-like domains similar to MSP-1, and is well conserved among plasmodial parasites. Using a murine model, we previously showed that immunization with a chimeric MSP-1_{19}+MSP-8 vaccine induced nearly complete protection against lethal *Plasmodium yoelii*, well beyond that achieved by immunization with an admixture of MSP-1_{42} and MSP-8. We have initiated studies to further evaluate *Pf*MSP-8 as a fusion partner for a chimeric MSP-1 based vaccine. *Pf*MSP8 is predicted to be a ~597aa protein with an N-terminal signal sequence and C-terminal membrane anchor sequence and is recognized by sera from *P. falciparum* infected individuals. Unlike *Py*MSP-8, *Pf*MSP8 contains additional tracts of Asp/Asn residues (~170aa) in an N terminal domain. Reports in the literature on the localization of *Pf*MSP-8 expression vary and differ somewhat with that observed for *Py*MSP-8. Using a bacterial expression system, we have expressed and purified two recombinant antigens, *Pf*MSP-8N (aa 24-344) and *Pf*MSP-8C (aa 336-578) against which we have raised high titer polyclonal rabbit antiserum. The anti-*Pf*MSP-8C sera poorly recognized parasite associated antigens in immunofluorescence analysis of *P. falciparum* infected RBCs and exhibited little or no growth inhibition activity *in vitro*. In contrast, use of the anti-*Pf*MSP-8N sera permitted localization of *Pf*MSP-8 expression to the surface of blood-stage parasites. The functional activity of the anti-*Pf*MSP-8N sera in growth inhibition assays is under evaluation.

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IMMUNIZATION WITH RECOMBINANT PROTEINS OF A GAMETOCYTE PROTEIN PFS230 EXPRESSED USING WHEAT GERM CELL-FREE SYSTEM SUCCESSFULLY INDUCE TRANSMISSION-BLOCKING ANTIBODIES AGAINST *PLASMODIUM FALCIPARUM*

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The aim of the malaria transmission-blocking vaccine (TBV) is to block the development of malaria parasites in the mosquito and prevent the following infection to the host. A gametocyte/gamete surface protein Pfs230 is one of the TBV candidate molecules for more than a decade. This molecule has seven cysteine motif domains, predicted to form complicated tertiary structure. Hence, the difficulty of producing the correctly folded recombinant protein made it difficult on the progress of Pfs230 study as TBV candidate. Here, we succeeded in producing recombinant Pfs230 proteins using wheat germ cell-free expression system without any codon optimization. Four overlapping recombinant proteins corresponding cysteine motif domains I-III (Pfs230c), domains I-IV (Pfs230D1), domains III-VI (Pfs230D3), and domains VII-X (Pfs230D7) among 14 cysteine motif domains, as predicted previously, were produced using this cell-free system. Rabbit antisera against these recombinant proteins were generated by immunization with Freund adjuvant and characterized. All antisera react on the surface of falciparum gametocytes isolated from Thai patients and also react on the surface of cultured gametes from the Thai isolates. Western blot analyses using cultured *Plasmodium falciparum* NF54 parasites revealed that all antisera recognized the 360-kDa form of parasite-produced Pfs230. Antisera against Pfs230c and Pfs230D7 reduced the infectivity of NF54 parasites to *Anopheles stefensi* mosquitoes by membrane feeding assay. Moreover almost complete transmission-blocking activity was induced by anti-Pfs230c serum in presence of the complement. Our data support that the wheat germ cell-free expression system can provide correctly folded recombinant Pfs230 protein and Pfs230c is the promising TBV candidate of *P. falciparum*.

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NOVEL ANTIGENS AT *PLASMODIUM FALCIPARUM* SCHIZONT-MEROZOITE STAGES AS POTENTIAL VACCINE CANDIDATES

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The reference genome sequence of *Plasmodium falciparum* has opened the way to discovering novel malaria vaccine candidates. There are several lines of evidence from studies of naturally acquired immunity that immune response to the blood stage antigens is associated with protection against either malaria infection or clinical malaria, which makes the development of asexual blood stage vaccines feasible. Here we took steps towards getting schizont-merozoite stage antigen proteins: creating a set of genes for cloning according to the transcriptome and proteome data, and synthesizing recombinant proteins in small reaction scale (~0.1mL) using a wheat germ cell-free system. The recombinant protein products were then screened for reactivity to immune sera from malaria exposed individuals, which identified ~10 positive molecules. Besides these immuno-screened ones, extra ~30 with < 2Kbs coding sequence were added from the same recombinant schizont-merozoite molecules source, and large-scale cell-free translation (>1mL) were conducted to obtain enough amount of purified proteins to immunize animals. Indirect immunofluorescence assay (IFA) using eventual ~25 antiserum specimens identified a surface or punctate staining pattern from approximately half the molecules tested. The approach should help to prioritize the antigens for detailed study including protection capacity, leading to novel asexual blood stage vaccine candidates.

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PLASMODIUM FALCIPARUM MEROZOITE SURFACE PROTEIN 6 AS A BLOOD STAGE VACCINE CANDIDATE: ASSESSING GENETIC DIVERSITY AND ANTIBODY SPECIFICITY

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The spread of drug-resistant *Plasmodium falciparum* parasites has made the search for an effective *P. falciparum* vaccine all the more urgent. The merozoite is the sole extracellular stage of the *Plasmodium* blood cycle, and the blood stages are responsible for the symptoms and pathology of malaria. Merozoite antigens that are exposed to the antibody-mediated system have therefore historically been an important focus for vaccine research. By necessity, the merozoite antigens that have proceeded the furthest towards vaccine development are those that were identified first. However, genomic and proteomic tools have now identified many additional surface antigens that have not yet been rationally assessed for their inclusion in a blood stage vaccine. One such antigen is *P. falciparum* Merozoite Surface Protein 6 (PfMSP6), which associates with the C-terminal fragment of PfMSP1 on the merozoite surface. Little is currently known about how much the PfMSP6 gene differs between *P. falciparum* field isolates, nor whether it is a target for antibody-mediated immunity. We have begun to address these questions using samples from an ongoing longitudinal cohort study near Iquitos, Peru. The hypoendemic transmission dynamics at this study site mean that *P. falciparum* infections are relatively genetically simple and are well spaced in time. We have genotyped PfMSP6 from more than 500 *P. falciparum* samples over 4 transmission seasons, and show that although PfMSP6 allele frequencies do change over time, intra-allele diversity is limited with few SNPs. We have also generated recombinant antigens comprising both the N- and C-terminal domains of PfMSP6, and used them in ELISAs with sera samples from individuals whose *P. falciparum* infections have already been genotyped for their PfMSP6 sequence. In this manner we are able to quantify both the presence and cross-reactivity of anti-PfMSP6 antibodies generated by a *P. falciparum* infection. These results are important for establishing whether PfMSP6 should be advanced any further along the *P. falciparum* vaccine development pipeline.

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ASSESSING PARASITE BURDEN IN *PLASMODIUM KNOWLESI* / RHESUS MONKEY SPOROZOITE CHALLENGE MODEL BY QUANTITATIVE REAL-TIME PCR AND HISTOLOGY

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The development of malaria vaccines would be facilitated by establishing an animal model predicting efficacy in humans. To this end, we have assessed the *Plasmodium knowlesi* / *Macaca mulatta* model by testing a variety of vaccine approaches including DNA plasmids, poxvectors, adenovectors, and irradiated sporozoites (irr spz). The most protective regimens for delaying or preventing parasitemia following sporozoite challenge have been (i) DNA prime + pox or adeno boost; (ii) irr spz. Recently, we considered an alternative approach to assessing protection, which is to sacrifice the animals following sporozoite challenge and perform quantitative real-time PCR (RT-qPCR) and histology on liver

samples to quantify parasite burden. In this preliminary study, we examined three monkeys, one challenged after immunizing with pox vectors (generating immunity targeting liver stage parasites), one challenged without immunization, and one not challenged as a negative control. Multiple liver biopsies were obtained and assessed by RT-qPCR and histology. There was no correlation between the two measures, with the pox-immunized monkey showing widely variable parasite burdens by PCR depending on biopsy location but no or few tissue schizonts on histology. The unimmunized, challenged monkey, in contrast, showed relatively consistent parasite burdens by PCR among biopsies and a range of schizont numbers on histology. We reasoned that both measures provide useful information, PCR readily detecting poorly developed parasites not visible by histology, and histology detecting normally developing parasites, which cannot be distinguished from moribund parasites by PCR. We concluded that if used together, the two measures allow lobe-specific assessments of protective responses and additionally will provide information on inflammatory cells associated with liver stage parasites. This approach opens the door to cannulating individual liver lobes with an arterial catheter, administering quantified local sporozoite injections and assessing lobe-specific responses.

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DIRECT ALUM FORMULATION IMMUNOASSAY (DAFIA): AN IMMUNOFLUORESCENT ASSAY THAT DIRECTLY DETERMINES THE CONTENT, IDENTITY AND INTEGRITY OF ANTIGENS FORMULATED ON ALHYDROGEL

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Currently, the only adjuvants approved for human vaccine use in the US are aluminum (alum) containing adjuvants. Regulatory authorities require that vaccines be tested to quantitate the antigen content in the final vaccine product. In particular, it is essential to determine the amount as well as the quality of antigens bound to alum following formulation. Presently, the amount of antigens on alum is determined by the o-Phthalaldehyde (OPA) Fluorescent Protein Assay (OPA assay) through reactions between OPA and primary amines of protein antigens, and antigen identity and integrity are determined by Western blot and SDS-PAGE. However, the OPA assay is non-specific and limited to detection total protein content only, and it is often not sufficiently sensitive to detect antigens in low dose formulations. Furthermore, antigens used in identity and integrity tests must be extracted from the formulated vaccines using an extraction procedure which is time-consuming and may not recover sufficient amount of antigens for analysis or alter the structures of antigens. The present study developed a Direct Alum Formulation Immunoassay (DAFIA) which was designed to directly (without antigen extraction), accurately, and sensitively determine the antigen content, identity and integrity on alum with only 5 hr. The AMA1-C1/Alhydrogel formulation was used as a model vaccine in assay development and validation. Briefly, vaccine formulation was added to 96-well plates and washed and followed by addition of a primary antibody/wash and a secondary antibody-fluorescein/wash which allowed detection by a fluorometer. The results showed that the DAFIA is highly antigen-specific, accurate (86-100%), sensitive (0.08µg/ml), reproducible, and simple with a linear detection range of 0.08-10µg/ml. These results demonstrate that DAFIA is an excellent assay to determine antigen content, identity and integrity of antigens bound to alum. This assay has proven to be superior in our laboratory to test vaccines formulated with single or combination antigens and may complement/replace the applications of OPA assay, Western blot and/or SDS-PAGE/silver staining in routine vaccine quality control.

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REDUCTION IN THE BURDEN OF MALARIAL ANEMIA: CONFIRMATION OF AN ANTI-VECTOR APPROACH... SOMETIMES

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Malaria is a principal cause of at least one-fifth of all young child deaths in Africa), and is thought to be the primary cause of severe anemia (Hb < 7 g/dl) in at least 50% of people living in malaria-endemic areas. With increasing levels of chloroquine resistance, the Roll Back Malaria partnership goal to halve the malaria burden by 2010 suggests the need for integrated approaches to combat malaria and reduce its consequences. Various projects and controlled trials have found improvement in anemia levels with the use of bednets, particularly insecticide treated nets. However, the efficacy of bed net use has not been evaluated with national survey data. We use nationally representative Demographic and Health Surveys (DHS) data from Benin, Uganda, and Mali from 2 points in time (2001 and 2006), as well as data from Cameroun (2004), Congo Brazzaville (2006), and Tanzania (2004). Our study sample in each country consists of children age 6-23 months. Our dependent variable is the anemia status of the child: whether the child suffers from severe anemia or not, with severe anemia being defined alternately as <7g Hg/dl and <8g Hg/dl. The key independent variable is bednet use among all children in the household the night before the interview was conducted. We use logistic regression to analyze the data. This study found a 50% reduction in the prevalence of anemia in children who used bed nets compared to children who did not in Benin and Uganda at both data points (2001 and 2006). The significance of the findings remains after controlling for household wealth and stunting in children, both of which have a significant relationship with anemia. Using a cut-off of <7g Hg/dl results in a better model fit than using a cut-off of <8g Hg/dl. In the remaining countries, while the association is generally in the expected direction, bednet use does not have a significant negative association with anemia status. We conduct additional analyses in an effort to discern the factors associated with the "bednet success stories" found in Benin and Uganda.

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METABOLIC AND TARGET SITE INSECTICIDE RESISTANCE IN WILD *ANOPHELES VAGUS* IN CAMBODIA

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The use of insecticide is a common vector control measure for malaria. In some parts of the world, resistance of the vector to the insecticide has emerge. The objective of this study was to detect the resistance mechanism of the secondary malaria vector *Anopheles vagus* against DDT and pyrethroids in Cambodia. Female *An. vagus* was collected by cattle-landing from three strategically located malarious areas in Cambodia. WHO susceptibility test was used with 4%DDT and 0.05% deltamethrin. The control mosquitoes from the susceptibility test were transported to Liverpool School of Tropical Medicine for biochemical (GST activity) and molecular assay (kdr). The mean 24h mortality of this population against DDT and pyrethroid was in the limit of resistance (86.98% and 88.60%), respectively. Their GST activities were significantly lower than either the two laboratory-bred susceptible strains (*An. arabiensis* Durban and *An. albimanus* Panama). The primer of the *An. vagus* was designed and a putative sequence of it was used to compare the *kdr* sequence of *An. gambiae* and *An. vagus* from gene bank. The assay showed no common *kdr* mutation substitution leucine-phenylalanine or leucine-serine in this population. In conclusion, the resistance mechanism indicated by an elevated GST to the substrate CDNB and *kdr* mutation was not seen in this mosquito population. However, the findings do not allow us to draw a firm conclusion about resistance in this population given the limit of phenotype resistance and limited sample size. A predicted correlation

between observed GST activity and actual resistance to DDT may be misleading.

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THE EFFECT OF BREASTFEEDING ON THE RISK OF MALARIA AMONG CHILDREN BORN TO HIV-INFECTED MOTHERS

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Breastfeeding protects infants against some infectious diseases, but data are limited on whether it reduces the risk of malaria. We assessed whether breastfeeding is protective against malaria among a cohort of HIV-exposed children (HIV-uninfected children born to HIV-infected mothers) and HIV-infected children in an area of high malaria transmission in Uganda. HIV-exposed and HIV-infected children all taking trimethoprim-sulfamethoxazole (TS) prophylaxis and given ITNs were enrolled between 1.5 and 9 months (mo) of age and followed to 15 mo of age. Malaria was diagnosed when a child presented with a fever and a positive blood smear. Date of breastfeeding cessation was determined using monthly questionnaires. Among 189 HIV-exposed children, 137 (72%) had stopped breastfeeding at a median age of 7.0 mo (IQR: 6.1-8.8). Among 45 HIV-infected children, 8 (18%) had stopped breastfeeding at a median age of 4.4 mo (IQR: 2.6-8.5). Generalized estimating equations were used to model the association between breastfeeding and the daily risk of malaria adjusting for repeated measures. Due to significant effect modification, data was stratified into two age groups (6-<9 mo and 9-<15 mo) while adjusting for age within each stratum. Among 6-9 mo old HIV-exposed children, the incidence of malaria was similar among those who were breastfeeding and those who were not (1.30 vs 1.37 episodes PPY; RR=1.17; p=0.63). HIV-exposed children 9-15 mo old who were breastfeeding had a lower risk of malaria compared to those not breastfeeding (0.77 vs 2.75 PPY; RR=0.32; p=0.004). Among 6-9 mo old HIV-infected children, the incidence of malaria was similar among those who were breastfeeding and those who were not (1.05 vs 1.49 PPY; RR=0.75; p=0.76). HIV-infected children 9-15 mo old who were breastfeeding had a lower risk of malaria compared to those not breastfeeding (1.09 vs 3.86 PPY; RR=0.31; p=0.03). These findings suggest that breastfeeding is associated with a reduced risk of malaria among HIV-exposed and HIV-infected children taking TS prophylaxis between the ages of 9 and 15 mo.

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LONG-LASTING INSECTICIDAL HAMMOCK NETS (LLIHN) FOR CONTROLLING FOREST MALARIA IN VIETNAM

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The aim of this study was to evaluate the effectiveness of LLIHN as a new strategy for malaria control in forested areas in central Vietnam. A community based, clustered randomised trial on LLIHN was carried out from March 2004 to December 2006 in the south-central coastal province of Ninh Thuan, Vietnam. Twenty clusters (about 1000 inhabitants each) situated in the forested area of the province were identified. The year before the intervention, a census of the study population was done. Passive detection of clinical cases was set up at village and commune level; moreover, two yearly malariometric surveys (one at the beginning and one at the end of the transmission season) were carried out throughout the study period. After the distribution of LLIHN in 10 clusters, the population was followed for 2 additional years. Before the intervention (2004), malaria prevalence was about 18% and the incidence 69/1,000/year. After the intervention, malaria prevalence decreased by 82% (from 22% in December 2004 to 4% in December 2006) in the intervention and by 71% (from 14% to 4%) in the control group. Similarly, the malaria incidence decreased by 83% (from 103/1000/year in 2004 to 17/1000/year in 2006) in the intervention group and by 62% (61/1000/year to 23/1000/year) in the control group, i.e. 48 malaria cases/1,000 person-years prevented by LLIHN. In conclusion, at the beginning of the study, malaria morbidity was higher in the intervention clusters. The surveillance system for detecting clinical cases probably had an impact also in the control clusters, where malaria prevalence and incidence decreased as well. Nevertheless, such decrease was more important in the intervention clusters suggesting that LLIHN had some impact on malaria prevalence and incidence. LLIHN may be a useful additional tool for controlling malaria in remote and forested areas in Southeast Asia and possibly South America. Their effectiveness should be evaluated in other settings.

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FIELD PERFORMANCE OF A WASH RESISTANT INSECTICIDE TREATMENT KIT FOR MOSQUITO NETS IN THREE DIFFERENT SETTINGS IN UGANDA AND MOZAMBIQUE

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The objective of this study was to test the performance of a longer-lasting insecticide treatment kit for mosquito nets under field conditions. New and used mosquito nets where treated with KO-Tab123® (BAYER Environmental Science) and given to households for regular use. Nets were assessed for number of washes, chemical residue and/or bio assay results after 6, 12, and 18/24 months. Total number of nets tested at end-point was 294. Bio assays (A. gambiae s.s. or A. arabiensis) were done using the WHO cone test with a 3 minute exposure and 60 minute knock-down and 24 hour mortality rates as outcome. Chemical residue was measured by gas chromatography. Nets were tested in three settings: 1. new nets, single dose, mass treatment in warehouse, washing frequency 3-4 per year, short rains (Inhambane, Mozambique); 2. new nets, individual dipping (single or double dose), washing frequency 1-2 per year, long rains (Kyenjojo, Uganda); 3. used nets of various materials with new polyester nets as control, mass dipping in plastic bags by volunteers, washing frequency 5-7 washes per year, long rains (Kayunga, Uganda). Only chemical residue was done in Kayunga. At base line all nets had sufficient deltamethrin (31-34 mg/m² for new and 19-41 mg/m² for used nets) and knock-down and mortality of 92-100%. At the Kayunga site (setting 3) all nets, used and new, rapidly lost insecticide and after 18 months only had on average 1.1-2.6 mg/m² (loss of 92%-96% of baseline). In contrast, in Kyenjojo (setting 2) the loss after 24 months was only 70-73% and median deltamethrin 8.9 mg/m² (single dose) and 20.0 6 mg/m² (double dose) with knock-down and mortality exceeding 90%. In Inhambane (setting 1) median deltamethrin was 16.6 mg/m² after 12 months (loss 54%) with 92% knock-down and 100% mortality (24 months samples currently under evaluation). In conclusion, the KO-Tab123® performed very differently in the various settings. Under high use and high washing frequency the nets had lost effectiveness after 18 months while under more moderate conditions they were still effective after 24 months.

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TARGETING SCHOOL CHILDREN FOR THE PREVENTION AND CONTROL OF COMMON ENDEMIC DISEASES IN SOUTHEAST NIGERIA

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Diarrhea, helminthic diseases, schistosomiasis, malaria, and tuberculosis are common endemic diseases in Nigeria. The potential of the school as a setting, and the pupils as champions for the prevention and control of these endemic diseases has not been explored in spite of the fact that the school setting is likely to provide a cost efficient setting for health information because of sheer size of school population, their distribution in every family and their role model potential. Two thousand one hundred primary six pupils were selected by systematic random sampling from twenty one schools in the Anambra, Nigeria. The knowledge, attitude and practice related to the five listed endemic diseases were studied through interviewer-administered questionnaires. The school physical environment and relevant curriculum were also studied. Awareness of the diseases was high (over 90%) except schistosomiasis (less than 10%). Knowledge of cause and prevention was generally low (less than 50%). Most of the school children (92%) thought tuberculosis was caused witchcraft, and did not associate BCG vaccination and hygienic disposal of cough with prevention or control of tuberculosis. Of those who knew about schistosomiasis, 74% thought that passing blood in urine by boys was male menstruation. Over 65% thought that some types of food cause malaria. Less than 25% of pupils knew that poor disposal of faeces and poor personal hygiene were key factors in the spread of diarrhea and helminthic diseases. About 80% of pupils thought that worms are needed in the gastrointestinal tract for food digestion. Many pupils (84%) admitted not washing hands with soap and water always after using the toilet. All the pupils were willing to learn more about the diseases and indicated they discuss what they learn at school with families. An assessment of the school physical environment and curriculum revealed unfavorable environment and inadequate curriculum for endemic disease prevention messages. In conclusion, although results of this study showed a defective school environment and curriculum which manifested in poor knowledge, attitude and practice in regards to prevailing endemic diseases, the children showed interest and potential to learn and act as role models in endemic disease control. Their sheer number, distribution and potential should be tapped in control of endemic diseases in Nigeria.

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IS MALARIAL PARASITEMIA RELATED TO THE NUMBER OF INSECTICIDE TREATED NETS IN A HOUSEHOLD? RESULTS FROM A NATIONAL POPULATION-BASED SURVEY IN ANGOLA

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The use of insecticide treated bednets (ITNs) to reduce the transmission of malaria is a key intervention across Africa. Studies show that ITN use can reduce malarial episodes by approximately 50% and save more child lives than any single intervention except breastfeeding and oral rehydration therapy. While the use of any type of bednet has significant health outcomes for individuals, the possession of ITNs in households is also known to have a protective effect on the individuals within the household. However, the effect of having multiple ITN of any type on malarial parasitemia has not been examined on a large scale. The aim of this study is to examine the relationship between the household possession of multiple ITN and parasitemia. Data come from the Angola Malaria Indicator Survey, a nationally-representative household survey that tested all children age 6-59 months for the *Plasmodium falciparum* in 2006-07. The analysis uses multivariate logistic regression to estimate the effects of the number of ITN in a household on parasitemia, controlling for household and individuals factors. Approximately 63 percent of children 6-59 months live in a household without a net, while 19 percent and 18 percent of children live in a household with 1 and 2 or more nets respectively. In the first model, which includes all children in the household, children who live in a household possessing any nets are significantly less likely to have parasitemia than those who live in a household with no nets (OR 0.64, CI 0.46-0.90) and that possessing 2+ nets reduces the risk further (OR 0.60, CI 0.42-0.82). The second model excludes children who slept under an ITN to control for the individual protection of sleeping under a net and estimate the protective effect of having an ITN in the house. The results were similar to the first model. These results suggest in a dose-response effect of having multiple ITN in a household regardless of the user.

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TESTING COMPREHENSION AND ACCEPTABILITY OF PARASITE SYMBOLS TO STRENGTHEN ADHERENCE TO ANTIMALARIAL TREATMENT IN TANZANIA AND UGANDA

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Patients stop taking antimalarials (AMs) when they feel better - treating symptoms, not disease. Providers often tell patients to finish treatment, most do not give reasons. The purpose of this study was to assess comprehension, acceptability and usefulness of two versions of AM packs with focus on potential instructional value for improving patient adherence to treatment. 104 and 116 respondents from rural, malaria-endemic areas of Tanzania and Uganda respectively were selected, 80% were women 15-45 years. 12% had no schooling and 75% had primary education. Respondents assessed two packs: Pack 1 displaying user, symbols of sun, moon and tablets over 3 days, showing how to take the treatment. In pack 2, round symbols of malaria parasites diminishing under each tablet provided connection between taking full course and eradicating parasites/getting cured, showing how and *why* to complete full course of treatment. Packs were tested with respondents using semi-structured questionnaires and observation. Images of parasites and connection to full cure was seen immediately by 67% in Tanzania, 27% in Uganda. After explaining these were parasites, another 20% in Tanzania and 63% in Uganda saw connection, 13% and 10% respectively did not understand. In Tanzania, all but two would select pack 2 to explain reasons for adherence to a neighbour, and in Uganda, all but one (of 45). Many commented on usefulness of pack, some were scared of parasites but still preferred this pack to explain reasons for adherence. In conclusion, instructions on use of AMs which give users visual logical reason to finish course may influence adherence.

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BED NET COVERAGE, USAGE AND CONDITION IN FISHING VILLAGES OF SUBA DISTRICT, WESTERN KENYA

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Past studies showed that bed nets can reduce child mortality. Currently, the Kenyan government is subsidizing the price of bed nets, and several non-governmental organizations (NGOs) are distributing nets with little charge. However, information on coverage, usage and condition of nets is lacking, particularly in the remote areas. We investigated bed net coverage and condition in seven fishing villages on the shore of Lake Victoria, Western Kenya. Usage of bed nets was examined through direct observation in early morning. As locals have started to replace traditional papyrus mats with bed nets for drying small fish, we also investigate how widely bed nets were used for capturing and drying fish. Coverage was 71.9% and 41.1% for mainland and island villages, respectively. We

recognized 262 bed nets in early morning, and 151 (57.6%) of them were in use. However, 51 nets in use were not hanged properly. Thirty four nets were found in boxes, and the others were hanged, but not in use. We observed three types of nets; insecticide treated bet nets (13.7%), long-lasting treated bed nets (LLTNs, 47.8%), and untreated bed nets (38.5%). Nearly 90% of the observed nets had at least one hole more than 1cm. We found 234 bed nets being used for drying fish on beaches, and 194 (82.9%) of them were LLTNs. Forty one bed nets had been used for capturing fish. Locals preferred LLTNs for drying or capturing fish because they were stronger and fish dry faster and straighter. An NGO distributed 150 LLTNs in one village eight months before this survey, and we recognized 52 (36.7%) of them were being used for either drying or capturing fish. Over 95% of interviewed residents did not have any training on proper usage and maintenance of nets. In addition to pursuing high coverage of bed nets, an education component should be included in the ITN distribution.

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DIFFERENTIAL INSECTICIDES SUSCEPTIBILITY OF THE MALARIA VECTOR *ANOPHELES ARABIENSIS* IN RURAL/ URBAN SITES AT KHARTOUM CITY (SUDAN)

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An effective malaria vector control demands a good management of public health insecticides. Insecticides resistance of *Anopheles* mosquito in malarious countries is a threat to the success of national controls programs. However, heterogeneity of situation in large african cities should be considered in developing a policy on the management of insecticides resistance. In order to elucidate the susceptibility situation of *An. arabiensis*, the sole malaria vector at Khartoum City, to eight types of insecticides, a baseline survey was carried out in 2007. Specimens were collected from the field as larvae, eggs and pupae reared in trays on rice powder, and then transferred to Adult cages as pupae. Emerged females and males were fed only on sucrose 10% solution until the performance of the tests. Following WHO standard protocols, susceptibility tests were carried out at six sentinel sites resembling the urban/rural strata of the capital city. Overall, *An. arabiensis* at Khartoum city is susceptible to the Carbamate: Bendiocarb 1% (%mortality rate=98.1%; n=1365); Pyrethroids: Deltamethrin 0.05% (96.88%; n=1212) and Lambdacyhalthorin 0.05% (97.88%; n=1290); two Organophosphorus insecticides: Fenitrothion 1% (100.00%; n=1182) and Propoxur 0.1% (100.00%; n=1230). While *An. arabiensis* is tolerant to DDT 4% (96.88%; n=1212) and Permethrin 0.75% (97.88%; n= 1290), it is suggestively resistant to Malathion 5% (69.11%; n=1225). However, *An. arabiensis* at urban sentinel sites was susceptible to the later three insecticides, compared to being resistant or tolerant at the rural ones: Malathion ($t=13.52$, d.f. = 22, $p<0.001$); DDT ($t=5.23$, d.f. = 22, $p<0.001$); and Permethrin ($t=2.68$, d.f. = 22, $p=0.014$); respectively. Likewise, significant differences on knockdown between rural and urban sites are obtained for these three insecticides. Resistance of *An. arabiensis* to Malathion, and its tolerance to DDT and Permethrin at rural sites of Khartoum city is mainly attributed to the use of pesticides in vegetable farms at these areas. Therefore, stratification of Khartoum city by insecticides resistance should be done with respect to the agricultural practice of the population (i.e. areas under pesticides pressure). In conclusion, management of public health insecticides should consider divergence between urban and rural districts in large african cities like Khartoum.

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EVALUATING ULV MOSQUITO CONTROL APPLICATIONS IN A SOUTHERN CALIFORNIA DESERT HABITAT

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Current U.S. military operations call for insect control technology that will function in extremely hot, dry, and dusty field conditions to protect deployed personnel. We used a large date palm plantation in Coachella Valley, California, to evaluate efficacy of ultra-low volume (ULV) insecticide applications under these extreme desert conditions. We applied treatments of malathion at the label rate to two 400 ft x 400 ft grids in this plantation, one with ULV technology and the other for comparison with thermal fog technology. Using a combination of caged *Culex quinquefasciatus* bioassays dispersed equally across the grids, suction traps deployed before and after the sprays to sample wild *Psorophora* species, and control bioassays located at a distance from the spray zones, we evaluated the relative efficacy of the ULV technology. Weather stations were deployed at ground and canopy levels to precisely and accurately characterize conditions during ULV applications. Suction traps were also deployed at different canopy heights prior to the experiment to target the ULV spray in regions of the air column used by mosquitoes in the plantation. Finally, results were compared to a similar pilot study performed in warm, moist conditions in northern Florida. We discuss the outcomes of these experiments in the context of developing spray protocols that have maximum efficiency and efficacy in desert conditions.

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MONOTORING OF PYRETHROID INSECTICIDES AND DDT AND THE KDR GENE ASSOCIATED IN *ANOPHELES GAMBIAE* S.L. IN FOUR VILLAGES OF BURKINA FASO

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Insecticide treated materials are used as a major tool for reducing vectors bites and subsequently malaria morbidity and mortality. However, the resistance of malaria vectors to the pyrethroid insecticides may compromise the use of such a tool. To learn more about this phenomenon, we have investigated on the resistance of *Anopheles gambiae* s.l to pyrethroids and DDT and the knock down resistance (kdr) gene associated. A two years survey was carried out in 4 rural villages, where insecticides are used in rice and cotton cultivation (Boromo, Zampa) and in vegetable gardens (Saaba, Nongremasson). Mosquito larvae were collected in the field and reared in the insectarium until the adult stages are obtained. To assess susceptibility to insecticide using the standard WHO (1998) procedures, two to five days old female mosquitoes were used. The 50% and 95% knock down time (KDT_{50} and KDT_{95}) and the 24 hours mortality rate were determined. All specimens identified as *An. gambiae* s.l. were PCR tested for species and the molecular forms identification and detection of the Leu-Phe kdr mutation. Data were analyzed using XLSTAT software. 1151 and 1132 *An. gambiae* s.l. were tested respectively in 2005 and 2006 for susceptibility to DDT and pyrethroids. The mortality rate for permethrin varied between 93% and 100% in 2005, while in 2006, it varied between 98% and 100%. Mosquitoes sensitivity increased as confirmed by KDT_{50} values between 11.46 min and 17.33min and between 5.16 and 12.07 min respectively in 2005 and 2006. DDT results showed a decrease of mortality rate from 2005 to 2006 with increased KDT_{50} values. Mortality rate varied between 90 and 99% in 2005 and between 56 and 96% in 2006, which confirmed vectors resistance to

DDT. Vectors population remained deltamethrin-sensitive during the two years with a mortality rate between 99 and 100%. Kdr gene was found in all *An. gambiae* s.l. members including *An. arabiensis* and the M and S molecular forms of *An. gambiae* s.s. Moreover, we showed that the frequency of the Kdr gene was higher in Boromo (0.029) and Zampa (0.030) in 2005 and increased in 2006. Vectors resistance to DDT is actual but they seemed sensitive to pyrethroids. However the increased Kdr gene frequencies in the study area should be taken into account for decision-making.

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FREQUENCY OF *ACE*-1 ET *KDR* MUTATIONS WITHIN THE *ANOPHELES GAMBIAE* POPULATION COMPLEX IN WEST BURKINA FASO

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From August 2006 to January 2007, we monitored the frequency of *kdr* and the *ace*-1 gene dispersion in *An. gambiae* sl. population in the West part of Burkina Faso. The aim of the study was to update the exiting data on the occurrence of insecticide resistance genes in an area marked by an important utilization of pesticides in agriculture. Residual fauna capture followed by morphologic and PCR identification were used for specimen sampling. The results showed that the frequency of *kdr* did stayed stable since 7 years in our study area and in with the molecular form S of *An. gambiae* sl. (60% vs 62% reported in 2000). However, we noticed an increase of 27 % its frequency within the M molecular form compared to previews surveys. Our results showed also for the first time that the frequency of the gene *ace*-1 was relatively high within the molecular S form population (30%) in comparison with the values observed within the M form (17.5%). Taken together this study highlighted the impact of the agricultural utilization of insecticide of the progression of resistance genes in our study areas.

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DIFFERENTIAL EXPRESSION OF GENES IMPLICATED IN TEMEPHOS AND PERMETHRIN RESISTANCE ON MOSQUITO STRAINS OF *AEDES AEGYPTI*

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The mosquito *Aedes aegypti* is the principal vector of dengue virus worldwide. Many vector control campaigns had failed as a result of insecticide resistance on mosquito populations. We are using an *Ae. aegypti* detoxification-chip in order to identify potential genes implicated in insecticide resistance. Five strains from Mexico and one from Peru have been selected with an LC_{50} for the organophosphate temephos and the pyrethroid permethrin separately. The LC_{50} for each mosquito generation was recorded and the *Iso*1,016 allele implicated in knockdown resistance was obtained. Gene expression from the unselected strains was first compared with the susceptible strain from New Orleans. The initial unselected strain was also compared with the F6 offspring after selection. Four unselected strains from Mexico showed resistant ratios more than 30x compared with the susceptible New Orleans strain. These strains also showed high frequencies of the *Iso*1,016 allele indicating the presence of the knockdown resistance mechanism. Array comparisons between New Orleans and the unselected strains showed that some genes appear to be differentially expressed in unselected strains. Some of these genes GSTe3, GSTe4, GSTe6, GSTe7, CCEae1C, CCEae2C, CCEae5C, CYP9J26 and CYP9M9 appear frequently in the mosquito strains. These data will be helpful to develop simple insecticide resistance diagnostic assays in order to support vector control campaigns.

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SEQUENTIAL DEVELOPMENT OF INSECTICIDE RESISTANCE MECHANISMS IN LABORATORY SELECTED DELTAMETHRIN *ANOPHELES ALBIMANUS* RESISTANT STRAIN

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Malaria vector control and prevention policing in Guatemala includes long lasting deltamethrin treated bed nets, aiming to give full vector coverage. These bed nets had been used for the past tree years. Due to the constant insecticide selecting pressure *Anopheles* populations may develop insecticide resistance. According to the Guatemalan Ministry of Health, insecticide resistance in *Anopheles albimanus* Wiedeman has been reported in Guatemala since 1956. Biochemical mechanisms of resistance such as insensitive acetylcholinesterase activity, elevated oxidase and elevated non-specific esterase had been reported in organophosphate, pyrethroid and carbamate resistant *An. albimanus* mainly from areas with high agricultural activity. Cross resistance has also been reported, as high oxidase levels have shown to confer resistance of *An. albimanus* to organochlorines (DDT) and permethrin. The main objective of this study is to document the sequential development of insecticide mechanisms, form a parental generation to an F_{10}, in a laboratory selected deltamethrin resistant *An. albimanus* strain. We selected an *An. albimanus* deltamethrin resistant strain using insecticide coated 250 ml Wheaton bottles. With the selection of each resistant generation we have done biochemical bioassays in order to identify the biochemical mechanisms that are selected in each generation. The insecticide parental selecting concentration (LD_{90}: 0.3µg/ml), was determined by testing several deltamethrin concentrations and analyzing the mortality data with a probit analysis. Each generation was exposed with the LD_{90} of the previous generation. Deltamethrin resistance ratios and frequencies were determined for each resistant generation. F3 resistant strain percent mortality decreased a 43.31% in comparison with the parental strain. Additionally we are doing histological cross sections of deltamethrin resistant *An. albimanus*, in order to determine if there is an association between deltamethrin resistance and thickness of mosquito cuticle.

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SEARCH FOR MUTATIONS IN THE SUPER *KDR* REGION OF PARA IN *AEDES AEGYPTI* FROM LATIN AMERICA

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Pyrethroid resistance in the mosquito *Aedes aegypti* (Diptera: Culicidae) is increasing worldwide and an insensitive sodium channel is likely to be the major mechanism of resistance. Most of the point mutations conferring knockdown resistance in the insect sodium channel gene occur in hydrophobic segment 6 of domain II of *para* and are postulated to prevent the rapid paralytic and lethal actions of all known pyrethroids, but do not diminish the efficacy of other insecticides. The hydrophobic segment 5 of domain II of *para* is called "super kdr" and can also carry mutations that confers much greater resistance to DDT and some pyrethroids. We screened exon 19 of *para* in *Ae. aegypti* that encodes hydrophobic segment 5 of domain II. We examined 1,110 mosquitoes in 37 strains from Latin America and found a transversion in the second position of *Leu*946 that instead encodes *Gln*946. We developed a melting curve SNP-PCR assay for these mutations that can be read either on an agarose gel or melting curve. These results will provide new insight into the mechanisms by which pyrethroids modify the function of voltage sensitive sodium channels.

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DIFFERENTIAL SUSCEPTIBILITY OF PERMETHRIN-RESISTANT *ANOPHELES GAMBIAE* TO INDIVIDUAL TOXINS OF A NEW ISOLATE OF *BACILLUS THURINGIENSIS* SUBSP. *ISRAELENSIS*

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The efficacy of several traditional chemical compounds such as organophosphates, carbamates and pyrethroids is threatened as mosquitoes gain resistance to such chemicals. For example, permethrin-impregnated nets are used widely for malaria control. However, increasing resistance by mosquitoes to permethrin is becoming widespread. Bioinsecticides based on the bacterium, *Bacillus thuringiensis* subsp. *israelensis* (Bti), are quite effective against *Anopheles* as well as *Culex* and *Aedes* mosquitoes. Importantly, no field resistance to Bti has been reported for these mosquitoes. Bti produces four Cry proteins, Cry4A, Cry4B, Cry10A and Cry11A, each of which exhibits different levels of toxicity against the three mosquito groups. For example, Cry4B is highly toxic to *Aedes* and *Anopheles* but not to *Culex*. Cry4A is quite effective against *Culex* and *Aedes* but not to *Anopheles*. M1 is a new isolate of Bti that is more toxic to permethrin-resistant *An. gambiae* (strain MRA-334) than any commercially available BTI-based larvicide. To determine the efficacy of the individual M1 Cry proteins against MRA-334, the *cry4A*, *cry4B*, *cry10A* and *cry11A* genes were PCR-amplified and cloned for high level expression in *Escherichia coli*. The expressed Cry proteins were purified and their larvicidal activity as well as their ability to act synergistically was assessed. Cry4B toxin was the most toxic to MRA-334 whereas Cry4A was the least toxic. Interestingly, Cry4A significantly synergized Cry4B toxicity. The Cry10A and Cry11A toxins showed no lethality at all and, in fact, both toxins antagonized the activity of Cry4B. The results demonstrate that Cry4B is primarily responsible for the extreme effectiveness of M1 against the pyrethroid-resistant mosquito strain MRA-334. Why M1 is the most efficient Bti strain is not known. Possibly, the binding affinity of the M1 Cry4B toxin for its cognate receptor is higher than that of other Cry4B toxins for their corresponding receptors, or the expression level of the *cry4B* gene is greater in M1 than in the other strains.

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ACTIVITY OF ORAL INSECTICIDAL DRUGS AGAINST *AEDES AEGYPTI* AND *ANOPHELES GAMBIAE*

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Oral insecticidal drugs offer an under-explored, yet potentially powerful, method to control certain mosquito-borne diseases. The advantages in getting insecticide into a bloodmeal are many: 1) it is a much more targeted way to shorten the life span of mosquito vectors as compared to environmental insecticide spraying, 2) the method would cross-target many mosquito species and sub-species and thus would also target all species of pathogen (all dengue serotypes, and all *Plasmodium* species), 3) the method is immune to behavioral adaptations of the vector, 4) the method targets the most critical variable in vector capacity equation (vector survival) but could be applied selectively enough to preferential kill-off the oldest and frequently biting mosquitoes, thus it would reduce transmission but would not easily select for resistant mosquitoes. We have been testing the activity of the oral insecticidal drugs ivermectin, nitenpyram and lufenuron, to reduce the survival of the malaria vector *Anopheles gambiae*, and the dengue vector *Aedes aegypti* after drug is titrated into an artificial bloodmeal. Unrealistically high concentrations of ivermectin and nitenpyram were needed to kill *Ae. aegypti* mosquitoes (LD_{50}s >200 ng/ml), but *An. gambiae* is particularly sensitive to ivermectin at pharmacokinetic-relevant concentrations (LD_{50} = 2.6ng/ml). Neither of these two drugs affected egg development in surviving mosquitoes at relevant concentrations. Lufenuron, at any dose, had no affect on the survival of adult mosquitoes, egg development or larvae development, but it did affect larval development when directly added to water. Our data suggest that ivermectin in particular, could be effective in reducing the survival of *An. gambiae* in the field and thus in controlling malaria transmission in sub-Saharan Africa if applied properly.

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AEDES AEGYPTI MONITORING IN PUBLIC AND PRIVATE BUILDINGS USING OVITRAPS, GPS AND A SIMPLE COMPUTER SYSTEM IN THE CITIES OF CHETUMAL AND PLAYA DEL CARMEN MEXICO

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Ovitraps have been place in governmental, private and public buildings in the cities of Chetumal and Playa del Carmen in the state of Quintana Roo in Mexico. The neighborhoods were the ovitraps have been place were selected by using retrospective 5 year Dengue case data. Ovitraps have been constructed out of 500ml black plastic containers and 5 x 25 cm white filter paper. At least one ovitrap set (exterior and interior) was place at one household per block. Among the selected public places there are bus stops and terminals, schools and cinema theaters. Governmental buildings include schools, health clinics and administration offices. The total number of selected building is 60 households and 50 government/public. Ovitraps are checked one per week trying to collect adults and immatures if found. Collected specimens are identified at the Public Health State Laboratory of the State of Quintana Roo. All ovitraps are georeferenced and data is being recorded using the Dengue Decision Support System (DDSS). The traps placement started in November 2007 and their inspection will continue through 2008. A school was the first to became positive in the third week of November and has remained positive since. An old marketplace has been found positive in most weeks. Public places with large number of visitors seam to follow this behavior. In private residences a week but significant spatial signature was detected (Moran's I = 0.35, $p < 0.05$) with some houses continuously producing positive traps. The current work is also being used for the refinement of the DDSS.

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THE RISE OF A KDR MUTATION IN *AEDES AEGYPTI* (L) IN MÉXICO

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Pyrethroids are commonly used as mosquito adulticides and evolution of resistance to these compounds is a major threat to public health. 'Knockdown resistance' to pyrethroids (Kdr) is frequently caused by nonsynonymous mutations in the voltage-gated sodium channel transmembrane protein (para) that reduce pyrethroid binding. Early detection of Kdr is critical to the development of resistance management strategies in mosquitoes including *Aedes aegypti*, the most prevalent vector of dengue and yellow fever viruses. Seven novel mutations in hydrophobic segment 6 of domain II of para in *Ae. aegypti* were described previously. Assays on larvae from strains bearing these mutations indicated reduced nerve sensitivity to permethrin inhibition. Two of these occurred in codons Iso1011 and Val1016 in exons 20 and 21 respectively. A transition in the third position of Iso1011 encoded a Gly1016 replacement. While the Gly1016 allele was never detected in Latin America. We found two new mutations in these same codons. A transition in the first position of codon 1011 encodes a Val replacement while a transition in the first position of codon 1016 encodes an Iso replacement. We developed PCR assays for these four mutations that can be read either on an agarose gel or as a melting curve. The present study demonstrates that the Iso1011

mutation has increased dramatically in frequency over the last 7 years in 14 states of Mexico

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GENETIC TECHNOLOGY FOR CONTROL OF DENGUE AND CHIKUNGUNYA

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Oxitec has developed an innovative and safe technology for the control of insect pests, including the Aedes mosquitoes that spread dengue fever and chikungunya. RIDL® insects are sterile in the absence of a specific diet component. RIDL males will seek out wild females and mate with them. The females lay eggs which do not develop into adults, leading to a decline in the target pest population. This is an improvement on the well-established Sterile Insect Technique (SIT) which uses radiation to sterilise insects, and which has been used around the world to control a number of agricultural pests, including the Mediterranean fruit fly. The WHO Collaborating Centre for Vectors at the Institute of Medical Research in Malaysia has independently tested the lead strain, OX513A, in confined "semi-field" conditions (a purpose built fully-contained field house simulating the natural habitat of this anthropophilic mosquito). The results, which were very promising, have been submitted to Malaysia's Director General of Health and relevant regulatory and ethical committees for review. Separately, Oxitec is working with an FNIH-administered consortium funded by the Bill and Melinda Gates Foundation Grand Challenges for Global Health initiative, and led by the University of California at Irvine, to develop further strains in which sexes may be separated by genetic instead of manual methods. WHO-TDR has awarded a grant to an international consortium led by Imperial college, and including Oxitec, to develop guidance for deployment of this new genetic approach.

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MOLECULAR BASES OF POST-MATING BEHAVIOUR IN *ANOPHELES GAMBIAE*

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In *Anopheles gambiae* mosquitoes, similar to other insects, mating induces a series of behavioural and physiological responses in females including changes in the flight activity rhythm, enhanced ovulation and oviposition, and induced refractoriness to further mating. In the model insect *Drosophila melanogaster* it has been clearly demonstrated that such post-mating responses are triggered by proteins produced by the male accessory glands (MAGs) and transferred to females during copulations, and a wealth of information is available on the nature of these male factors and on their effects on female behaviour. However, very little is known in *Anopheles* on the molecular mechanisms regulating the cascade of events triggered by the introduction of male factors during copulation, and leading to the induction of post-mating responses. Recently, we have identified in a genome-wide analysis a large number of *An. gambiae* MAG proteins (collectively named Acps) that are likely to represent important modulators of female behaviour. The *An. gambiae* genes identified include the putative orthologues of *Drosophila* Acps that are essential for male fertility and that induce female post-mating responses. While many *An. gambiae* Acps belong to the same functional classes reported for *Drosophila*, indicating a conserved functional role for these proteins in mosquitoes, some represent novel lineage-specific Acps that may have evolved to perform functions relevant to *An. gambiae* reproductive behaviour, e.g the life-long mating refractoriness of most *An. gambiae* inseminated females, compared to the resumption of mating behaviour observed in *D. melanogaster* 7 days post-mating. Here we report on our progress on the analysis of the interactions between male and female reproductive proteins and on our studies of the molecular changes that female mosquitoes undergo upon mating. An interesting picture is emerging which is allowing us to correlate behavioural and physiological post-mating changes to specific sets of genes and interactions. The analysis of genetic variability of some important candidates is also showing that at least one of these genes shows signatures of positive selection, suggesting a likely relevant functional role in *An. gambiae* post-mating behaviour.

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IDENTIFICATION AND MOLECULAR CATALOGING OF HEMOCYTE SPECIFIC IMMUNE GENES FROM MALARIA VECTOR *ANOPHELES GAMBIAE*

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Mosquito hemocytes have been proposed to contribute in cellular immune responses during microbial infection. However, so far there is limited information are available on the gene expression profile of hemocytes in the malaria vector *Anopheles gambiae*, particularly due to limitation of collecting threshold quantity of starting material viz RNA/Protein from small volume of mosquito hemolymph. In order to overcome these limitations we have optimized a hemocyte collection method and initiated a project to characterize hemocyte specific molecular transcriptome. To generate hemocyte specific EST catalogue, currently we are constructing large scale cDNA libraries. Preliminary sequence analysis of randomly selected EST clones indicates that at least 20-30% of the genes that were previously unknown, may code for some important molecular factors specific to hemocyte immune function. Ongoing high-throughput comparative analysis of *Plasmodium* infected vs uninfected hemocyte transcriptomes should provide a detailed overview of gene expression in hemocytes during *Plasmodium* infection and development.

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DISSECTING *AEDES AEGYPTI* INNATE IMMUNE RESPONSES TO DENGUE VIRUS INFECTION

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While the knowledge on the insect innate immune system has advanced considerably in the past decade, mosquito immune responses against dengue virus infection are still largely unknown. We have used functional genomics approaches to assess the activation of *Aedes aegypti* innate immune system upon dengue virus infection, at 7 days after mosquitoes were fed on dengue (DENV-2) infected blood. High density microarray analysis identified 28 up-regulated and 35 down-regulated genes in the midgut, and 240 up-regulated and 192 down-regulated genes in the carcass. This analysis revealed a strong bias in the transcriptional response toward genes that have been linked to the Toll and JAK-STAT immune pathways. Transient activation of Toll pathway through RNAi silencing of the negative regulator Cactus led to an approximately four-fold decrease in dengue infection level compared to the GPF dsRNA treated mosquitoes, suggesting the implication of the Toll pathway in the anti-dengue defense. We also silenced a variety of up-regulated genes that encode effectors or components of innate immune pathways and found that knockdown of Lysozyme C decreased virus titer in about three-fold. Conversely, silencing of the JAK-STAT pathway receptor, Domeless, provoked an increase of approximately three-fold in viral infection in the midgut, revealing that the JAK-STAT pathway is also involved in impairing DEV-2 replication. We are now silencing several JAK-STAT components such as negative regulators

SOCS (Suppressor Of Cytokine Signaling) and PIAS (Protein Inhibitors of Activated STAT) and signal transducer and activator of transcription STAT. We hope this will help us to understand how the mosquito's innate immunity acts in the anti-dengue defense system. These findings will open the road for new studies on dengue-mosquito interaction and will allow the creation of new strategies for dengue control.

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MEIOTIC DRIVE SYSTEM GENE EXPRESSION PROFILING IN *AEDES AEGYPTI*

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Meiotic drive in *Aedes aegypti* has potential as a mechanism for driving trangenes for pathogen resistance into natural *Ae. aegypti* populations. The native meiotic drive system found in some *Ae. aegypti* populations causes the female determining chromosome to fragment during spermatogenesis. Mating between a driving carrying male and a drive sensitive female results in highly male biased sex ratios. The molecular basis for the drive mechanism is presently unknown. We conducted a whole transcriptome analysis of testes from a meiotic drive carrying strain (T37) in comparison with a drive sensitive strain (RED) using microarrays based on the complete annotated *Ae. aegypti* gene set. Testes dissected from each strain were used for RNA extraction; cDNA labeling and hybridizations were performed by a commercial vendor (NimbleGen). We hypothesized that genes putatively involved in the meiotic drive system would show up-regulation in T37. Transcripts positively significant for T37 expression were identified by Significance Analysis of Microarrays (SAM) using a 5% false discovery rate. Functional classifications were determined using BIOMART (www.biomart.org/biomart/martview/). Because the meiotic drive gene is located on chromosome 1, genes located on known genome scaffolds for chromosomes 2, and 3 were excluded from detailed analysis. Screening and validation assays of potential candidate genes is ongoing.

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A PUZZLING PATTERN OF INTROGRESSION IN THE *CULEX PIPIENS* COMPLEX IN EAST ASIA

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Hybridization, through adaptive trait introgression, can increase local adaptation and invasiveness and lead to the emergence of vectors in new environments. Hybridization has long been thought to be important in shaping the species in the *Culex pipiens* complex. In this group hybrid zones appear to have been created independently across the world and have been implicated in providing bridge vectors for some important diseases. In east Asia *Cx. quinquefasciatus* and *Cx. pipiens pallens* have an overlapping distribution. Using microsatellite analysis, extensive classical hybridization was demonstrated between these two species. However, at the sex linked ACE locus an interesting pattern was found; All males of *Cx. pipiens pallens* displayed a hybrid signature at this locus, irrespective of the signature displayed by the neutral markers. Thus, it appears that a segment of the *Cx. quinquefasciatus* genome has introgressed asymmetrically into the males of *Cx. pipiens pallens*. In this group, sex is determined by a single autosomal gene rather than sexually dimorphic sex chromosomes and the sex ratio is affected by a meiotic drive system and *Wolbachia* infection. Using the recently sequenced genome of *Cx. quinquefasciatus* we have developed markers and investigated the region around the sex locus where the introgression has occurred in order to characterize how much of the genome has been transferred and which genes where involved.

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QTL ANALYSIS OF DENV-2 DISSEMINATION IN A FERAL POPULATION OF *AEDES AEGYPTI* FROM TRINIDAD, WEST INDIES

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Aedes aegypti is the most important vector of dengue fever in humans. In order to transmit dengue viruses (DENV), the virus must overcome barriers preventing infection and escape of the virus within the midgut. Quantitative trait loci (QTL) affecting the propagation of dengue viruses have been identified on all three chromosomes in *Aedes aegypti*. However, the applicability of QTL results from lab based studies to field populations is presently unclear. Herein we studied the genetic basis of DENV-2 dissemination in a natural population of mosquitoes. Feral female mosquitoes collected in Trinidad were mated with DENV refractory MOYO-R males. The MOYO-R strain was originally derived by selecting for refractoriness to *P. gallinaceum* in the Moyo-In-Dry strain and also shows high refractoriness to DENV. Field collected females were orally challenged with DENV-2 JAM1409 and scored by RT-PCR for DENV dissemination. Susceptible females were used in the development of F_2 half-sib families, which were subsequently challenged and scored for DENV-2 dissemination. DNA was extracted from individual carcasses and DNA genotype was determined using a panel of microsatellites and SNPs. Associations between DENV susceptibility and genotype were determined using QTL Cartographer.

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GUILT BY ASSOCIATION: GENE EXPRESSION DIFFERENCES IMPLICATED IN MATE RECOGNITION IN *ANOPHELES GAMBIAE* M AND S FORMS

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A speciation process is ongoing in the primary vector of human malaria in sub-Saharan Africa, *Anopheles gambiae*. Based on fixed nucleotide differences in X-linked ribosomal DNA genes, *An. gambiae* comprises two molecular forms: M and S. Strong assortative mating exists between forms. Only ~1% of sperm transfer in natural populations show evidence of cross-form mating. *An. gambiae* swarming and mating is crepuscular, beginning about 10 min after dusk and continuing for about 20 min. No measurable behavioral or sensory modality differences have been found that serve as reliable cues to the assortative mating between forms. Global gene expression differences between M and S were recently examined using oligonucleotide microarrays for three day old virgin female and male mosquitoes sampled mid-dusk, a time when virgin adults are likely exhibiting 'mate-seeking' behaviors. A larger than expected number of genes differentially expressed between forms were putatively involved in sensory pathways, with little overlap in gene expression differences between the sexes. Further, a preponderance of differentially expressed sensory genes are implicated in olfaction and potentially mate recognition. To further elucidate whether these putative olfactory genes are involved in mate-seeking behavior, quantitative rt-PCR was performed on both mated and virgin RNA samples from laboratory colonies at seven discrete time points over a two hour window encompassing the dusk cycle. Seven target genes were chosen under the expectation that mated and virgin samples from the same laboratory colony will have a different pattern and magnitude of expression for genes involved in mate-seeking behavior. Our preliminary results indicate that the majority of genes examined can be presumed implicated in mate-seeking behavior specific only to the up-regulated molecular form.

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POPULATION STRUCTURE OF COLLECTIONS OF THE MOSQUITO *AEDES AEGYPTI* (DIPTERA: CULICIDAE) FROM COSTA RICA

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Aedes aegypti is the main vector of dengue disease, and the most important tropical disease vector in Costa Rica. Population structure of this mosquito in Costa Rica is not well known, and previous studies of *Ae. aegypti* from Central America have not been clear about mosquitoes from. For this reason, a genetic characterization of laboratory collections from different regions of the country could explain the dynamics of populations of this mosquito. For the establishment of the collections, we obtained mosquito larvae and pupae from 19 localities of Costa Rica, in each locality were sampled five different points, and each one of these five sample sites were georeferenced. The mosquito colonies obtained from larvae collected in each locality were maintained in the insectary of the laboratory of Medical Arthropodology of the University of Costa Rica, and generation F_2 was obtained by locality. Genetic characterization of collections was performed with 50 mosquitoes of these F_2 generations. Five different molecular markers were used for the characterization: one mitochondrial marker (the subunit 4 of the NADH gene) and four nuclear markers. Nuclear markers included the microsatellites associated with the locus 19 (a homologous of membrane viteline protein), the locus GA (a subunit of the mRNA of the GABA receptor), and the locus C (mRNA of the ecdysteroid receptor). The fourth nuclear marker was the early trypsin gene, but it was analyzed only for eight localities. Data obtained from the genetic characterization were analyzed using Analysis of Molecular Variance (AMOVA) for comparison of haplotypes or allelic variables within and between mosquito collections and the calculation of F_{ST} value. Hardy Weinberg equation for allelic equilibrium in populations was used to determine the frequency of allelic variants. With these analyses, different haplotypes and allelic variants were obtained from *Ae. aegypti* populations of Costa Rica.

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POPULATION STRUCTURE OF THE MALARIA VECTOR *ANOPHELES ALBIMANUS* IN THE ATLANTIC AND PACIFIC REGIONS OF COLOMBIA BASED ON SEQUENCES OF THE MTDNA *COI* GENE

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Anopheles (*Nyssorhynchus*) *albimanus* is recognized as an important malaria vector in Colombia and is distributed along the Atlantic and Pacific coasts and on San Andres Island. Prior Colombian studies of *An. albimanus* using enzyme electrophoresis demonstrated low divergence and no differentiation among populations from both coasts; however, populations from the Atlantic coast had higher heterozygosity. Recent studies of this species using microsatellite (MS) loci and a mitochondrial DNA (mtDNA) marker reported differentiation between Central and South America based on geographical barriers. We used sequences of the mitochondrial cytochrome oxidase subunit I (*COI*) gene to investigate the population structure of *An. albimanus* in Colombia. A neighbor-joining tree, analysis of molecular variance, and F_{ST} pairwise comparisons consistently divided the data between the localities from the Atlantic and Pacific coasts; however, populations within each group showed very low divergence. Furthermore, a star-shaped minimum-spanning network and 5/5 significant neutrality tests suggested a past population expansion or a selective sweep in *An. albimanus* from the Atlantic coast. Although populations from the Pacific coast depicted a star-shaped minimum-spanning network, the neutrality tests were not significant. Our preliminary findings are in contrast with previous studies in detecting higher genetic diversity, and possibly a different demographic history, in Pacific coast *An. albimanus*. Importantly, only *An. albimanus* from the Pacific has been positively incriminated as a malaria vector. Further analysis of mosquitoes from Turbo, located in between these two regions, and sequences of a more conserved mitochondrial DNA marker (*Cyt b*) will extend our preliminary findings and provide more insight into the population genetic structure of *An. albimanus* in Colombia.

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DEVELOPMENT OF A HIGH-DENSITY SNP GENOTYPING ARRAY FOR THE VECTOR MOSQUITO *ANOPHELES GAMBIAE*, BY THE AGSNP CONSORTIUM

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Understanding the population structure and genetic mechanisms underlying disease transmission by malaria vectors continues to be hindered by the complex biogeography and reproductive biology of Anopheline mosquitoes, which impede classical genetic approaches to their investigation. The original *Anopheles gambiae* genome sequencing project and the recent completion of two additional genome sequences of the M and S chromosomal forms of *An. gambiae* have provided the genomic information required to develop a pilot single nucleotide polymorphism (SNP) genotyping array for *An. gambiae* (the "AGSNP array") and thereby extend the power of modern genomic approaches to this key malaria vector mosquito. We have initiated formation of a multi-center AGSNP Consortium that will develop, validate, and pursue initial use of high-density pilot AGSNP arrays in Affymetrix format. With the mosquito genome sequences in hand, and using two independent SNP callers, we have identified over 641,000 candidate SNPs, from among a total of more than 5 million primary SNPs, that survive filtering criteria based on distance from insertion/deletion (indel) polymorphisms and neighboring SNPs. These candidate SNPs are currently undergoing evaluation for probe design for a pilot array in the Affymetrix 49 format. We will summarize key questions in vector biology and disease transmission amenable to in-depth investigation using this vector mosquito genotyping tool, describe development of the probe set for the pilot AGSNP array, discuss prospects for pooled probing of Affymetrix-format arrays for analysis of population structure and genome-wide association studies of traits underlying malaria transmission by vector mosquitoes, and present progress on use of the AGSNP array for analysis of population structure in Africa.

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GENETIC ASSOCIATION AND LINKAGE DISEQUILIBRIUM IN *ANOPHELES GAMBIAE* IMMUNE GENES

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Understanding the molecular basis of natural mosquito immunity to the malaria parasite will give essential insights for novel malaria control strategies. Genetic variation in the mosquito is known to have drastic effects on immune competence. Understanding this genetic control of refractoriness is fundamental to future control via this route. Association

studies have proved to be useful tools in uncovering specific genetic loci with effect on disease outcome. Linkage disequilibrium (LD) data can help decipher components of molecular pathways and help to piece together the mosquito immunity puzzle. Moreover, as genome wide association studies in *Anopheles gambiae* are rapidly being developed, LD data will be key to determining the necessary resolution of genetic markers in such studies. This study aims firstly, to uncover single nucleotide polymorphisms (SNP's) involved in mosquito susceptibility/refractoriness to malaria in one geographical region. A targeted approach has been applied to select specific SNP's within known mosquito immunity genes for inclusion. Anopheles gambiae M form mosquitoes from Cameroon were experimentally infected with a sympatric *Plasmodium falciparum* isolate. The number of oocysts/midgut at day 8 post blood meal were counted to give a quantitative phenotype. Genotyping was then carried out on these mosquitoes for the selected SNP's and statistical tests applied to determine association. Secondly, the LD was measured for immune gene SNP's in *An. gambiae* populations. Data analysis will be fully presented.

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INTEGRATE MOSQUITO FORAGING IN ENVIRONMENTAL MANAGEMENT OF AQUATIC HABITATS FOR MALARIA CONTROL

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Malaria campaigns in tropical Africa are concentrated on management of *Anopheles gambiae* s.l., the major vector species, by using insecticide-treated bednets and indoor residual spray. Exploitation of alternative strategies is needed for reducing reliance on chemical insecticides. A new perspective of malaria control is developing based on compromising mosquito foraging for food and oviposition. With limited energy reserve and flight ability, mosquitoes only flourish in environments where foraging movement between hosts and aquatic habitats are facilitated. Reduced availability of oviposition sites by environmental management can prolong the ovipositional cycle and mitigate malaria transmission. In this paper, we develop an agent-based model to characterize foraging behaviors in response to change in the availability of aquatic habitats. In grid-based landscape, we compared intervention scenarios (3 coverages of targeted source reduction, 3 coverages of non-targeted soruce reduction, 3 coverages of insecticide-treated bednets ITN). Simulations show that interventions targeting ovipositional foraging by selective removal of aquatic habitats proximal to houses are more effective than insecticide-treated bednets in reducing malaria incidence and prevalence. In conclusion, spatial connectivity between human hosts and oviposition sites provides a promising target for malaria control.

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IDENTIFYING COVARIATES OF *ANOPHELES GAMBIAE* S.L. (DIPTERA: CULICIDAE) AQUATIC HABITAT DISTRIBUTION USING A POISSON REGRESSION MODEL, WITH A NON-CONSTANT, GAMMA-DISTRIBUTED MEAN

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We present a geostatistical approach that accounts for spatial correlation in malaria mosquito habitats in two East African urban environments. Multispectral Thermal Imager 5m data encompassing visible bands and the near infra-red bands were selected to synthesize images of *Anopheles gambiae* s.l. Giles aquatic habitats. These bands were used to determine which ecological covariates were associated with urban larval habitat development in Kisumu and Malindi, Kenya. Univariate statistics, correlations and regression analyses were perfomed on all field and remotely sampled data. Global autocorrelation statistics were generated from georeferenced *Anopheles* aquatic habitats. The results suggest that the geographic distribution of *An. gambiae* s.l. larvae in the study sites exhibit weak positive autocorrelation; similar numbers of log- larval count habitats tend to cluster in space. A negative binomial regression was used to decompose aquatic habitat data into positive and negative spatial autocorrelation eigenvectors. The principal finding is that synthetic map pattern variables, which were represented as eigenvectors computed for a geographic weights matrix, furnished an alternative way of capturing spatial dependency effects in the mean response term of the regression model. The results suggest the presence of approximately 13% to 32 % redundant information in the larval count samples. Additionally coefficient estimates were used to define expectations for prior distributions for a Bayesian analysis. By specifying the coefficients in a Bayesian framework, we successfully account for depth as being significantly associated with urban *An. gambiae* s.l. aquatic habitats.

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ECOLOGICAL BASIS OF SWARMING AND MATING BEHAVIOUR IN NATURAL POPULATIONS OF *ANOPHELES GAMBIAE* S.S., IN BURKINA FASO

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Recent advances in the molecular genetics of *Anopheles gambiae* have increased the prospects of using genetically modified mosquitoes in malaria control. However the use/release of such genetically modified vectors requires a proper understanding of potential interactions with naturally occurring populations. In many species of insect, larger males are more successful in obtaining mates. Since mating of *Anopheles gambiae* mosquitoes occurs in swarms, we attempted to characterize the swarming behaviour and determined whether size affects mating success of *An. gambiae* males. Swarming behavior of *An. gambiae s.s*. were investigated between July 2006 and December 2007 in Vallée du Kou and Soumousso two villages located in West Burkina Faso. Environmental parameters such as light intensity, temperature and humidity were also monitored. Wing lengths of males collected swarming and mating were measured then compared between both groups. The swarms of *An. gambiae* s.s. were represented by males aggregation in fly. The swarming began generally 1-10 min after sunset and stopped 9-35 min after the onset of darkness. Most of the swarms were observed between 0.6 to 4 m above a variety of markers. The luminosity, temperature and humidity variation had no fundamental impact on the swarming behavior along the months. We measured the wing of 654 swarming males and 152 copulating males. Copulating males were significantly larger than the other males sampled from the swarms ($F = 63.98$; $P = 0.001$). Indicating that the size affect mating success of *An. gambiae* mosquitoes.

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HOST-FEEDING PATTERNS OF *AEDES AEGYPTI* AND *AEDES ALBOPICTUS* IN NEW ORLEANS, LOUISIANA, 2006

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Field studies targeting *Aedes aegypti* and *Ae. albopictus* in New Orleans, Louisiana from August though October of 2006, yielded 4,027 *Ae. aegypti* and 1,573 *Ae. albopictus* adults. Collections were made with diurnal dry-ice baited light traps and Nasci aspirators in areas moderately to heavily flooded 12-15 months earlier following Hurricane Katrina. The purpose of this study was to evaluate mosquito host-feeding patterns given the drought conditions and varied degree of human repopulation

present in these areas in 2006. Preliminary results indicate that of the 162 fully or partially blood-engorged females tested, 53% of *Ae. aegypti* (47 of 88) and 49% *Ae. albopictus* (36 of 74) fed on mammalian hosts as determined by PCR amplification of the cytb gene. We are currently working to determine the proportion of hosts that were human, avian or non-mammalian vertebrates. Improved knowledge of the feeding patterns of container-inhabiting *Aedes* in urban areas after a large-scale disaster (e.g., hurricanes) should provide novel insights regarding these species' potential for arbovirus transmission. The implications of these findings and the results of on-going studies are discussed in the context of emerging arboviruses such as dengue and chikungunya.

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LARVAL ECOLOGY OF TWO CHROMOSOMAL FORMS OF *ANOPHELES FUNESTUS* IN WEST OF BURKINA FASO: LARVAE TRANSPLANTATION EXPERIENCE

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Cytogenetic investigations in Burkina Faso have shown within *Anopheles funestus* populations the existence of two chromosomal entities called "Kiribina" and "Folonzo". These two chromosomal should have a different bio-ecology suggesting incipient phenomena. In order to evaluate the parameters associated with the phenomena, we conducted larval transplantation experiments of these two chromosomal forms in two ecologically different areas in West Burkina Faso. The study took place during the rainy season and the cold and dry season in 2006 and 2007 at Bama a locality with an irrigated rice culture and at Soumousso a savanna classic village. Our results showed that the overall emergence rate was higher at Soumousso than in Bama. With regard to the chromosomal forms, the emergence rate of "Kiribina" was higher at Bama while that of "Folonzo" was higher at Soumousso. However the emergence rate of the two forms is similar in the rice fields. In puddles, the emergence rate of "Folonzo" was higher in Soumousso while it was higher for "Kiribina" in Bama. The emergence rate of "Folonzo" was higher than "Kiribina" in rainy season and conversely "Kiribina" would have an emergence rate higher during the dry and cold season. The larval development of "Folonzo" was faster than "Kiribina" whatever the locality, larval habitat and period. The overall larval development has been faster in the rainy season and slower in the cold and dry season for the two chromosomal forms. Taken together our results confirm that the two chromosomal forms should have different bio-ecology. This could explain the natural structure of their populations already proven by previous studies reinforcing the hypothesis of reproductive isolation between these two forms.

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THE ROLE OF THE RNAI PATHWAY IN THE MIDGUT OF *AEDES AEGYPTI* MOSQUITOES ON VECTOR COMPETENCE FOR ARBOVIRUSES

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Aedes aegypti mosquitoes are vectors of dengue (DENV), chikungunya (CHIKV), and Sindbis (SINV) viruses. Upon infection, these RNA viruses start replicating in the mosquito midgut, which appears to be the major organ that determines mosquito vector competence for arboviruses. Here, viruses have to overcome the RNA interference (RNAi) pathway. *Ae. aegypti* that were genetically engineered using double-stranded RNA (dsRNA) to silence DENV2 in the midgut are refractory to the virus. The RNAi pathway genes of *Ae. aegypti* such as dicer2 (*Aa-dcr2*), r2d2 (*Aa-r2d2*), and argonaute (*Aa-ago2*) have been identified and cloned. Transient gene silencing of the RNAi pathway using dsRNA significantly enhanced viral replication. However, this approach is not tissue-specific and could negatively affect mosquito survival. We hypothesize that the RNAi machinery in the mosquito midgut has a critical innate immune response against arboviruses. To test the hypothesis, we used a transgenic approach to silence RNAi pathway genes such as *Aa-dcr2* in the midgut. We generated 10 lines of transgenic *Ae. aegypti* harboring a transgene driven by the carboxypeptidase promoter to silence *Aa-dcr2* gene expression in the midgut of blood-fed females. *Aa-dcr2* gene expression was quantified using qRT-PCR. We are infecting one or two lines that have considerable reduction in *Aa-dcr2* gene expression with DENV2, CHIKV, and SINV strains that have different infection patterns in *Ae. aeqypti*. Here we present the effects of *Aa-dcr2* silencing in the midgut on arboviral replication, midgut infection, midgut escape barrier and mosquito fitness.

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COMPARATIVE POPULATION GENETICS: *CULEX RESTUANS* VS. *CX. PIPIENS* IN THE EASTERN US

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Culex restuans is an abundant local species that appears to occur seamlessly from North to South in the eastern US. This species starts its activity earlier in the year than *Cx. pipiens* (May/June vs. July) so it has been proposed that it is an important vector of WNV early in the transmission season. Although during the breeding season *Cx. restuans* are often collected together with *Cx. pipiens* in urban areas in New York State often making their relative importance as vectors difficult to resolve, during the winter diapausing populations found in human associated structures like sewers and basements are uniquely comprised of *Cx. pipiens*. In contrast, we do not know where *Cx. restuans* survive the winter. One possibility is that *Cx. restuans* overwinters in rural areas (possibly in the leaf litter) and reinvades cities every spring. We have made a comparison of genetic diversity of *Cx. pipiens* and *Cx. restuans*. We currently have 19 molecular markers (microsatellites) optimized for *Cx. restuans*. Preliminary analyses indicate the absence of winter driven bottlenecks in *Cx. restuans,* which supports the hypothesis that this species diapauses in very large numbers possibly in a myriad of small isolated natural cavities.

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EFFECTS OF SINGLE HOST ODORS AND ODOR COMBINATIONS ON FLIGHT CHARACTERISTICS OF *AEDES AEGYPTI* AND *AEDES ALBOPICTUS*

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Host location by mosquitoes is heavily influenced by olfactory cues emitted from hosts. These include a range of compounds such as carbon dioxide, lactic acid, acetone, 1-octen-3-ol and dimethyl disulfide. Preliminary observations indicated that flight activity of *Aedes aegypti* appeared to differ between compounds used in an olfactometer. We examined responses of individual compounds and compounds in combinations in a dual-port olfactometer. Responses of mosquitoes were recorded by a video camera, digitized and analyzed using behavioral analysis software. For both species, responses were highest to a hand which emitted an array of compounds. Individual odors only elicited up to a third of the response, however, some combinations of compounds significantly enhanced responses over those of individual compounds. Responses to host odors clearly differed between compounds for parameters such as time to fight initiation, duration of flight, directness and altitude of flight. Several combinations of odors reduced the duration of flight, increased

the directness of flight and altitude of flight with effects stronger in *Ae. aegypti* than *Ae. albopictus*. Data generated by this study will increase our understanding of how mosquitoes utilize cues for in-flight host orientation and can provide a basis for optimization of trap design for enhanced collection of attracted mosquitoes and for optimized odor delivery.

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RISK FACTORS RELATED TO THE NUMBER OF *AEDES AEGYPTI* PUPAE IN THE DISTRICT OF COMAS, LIMA, PERU

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The presence of *Aedes aegypti* was detected in Lima in 2001; currently it is present in 23 of the 32 districts. The first outbreak of dengue in Lima was reported in April 2005. We evaluated risk factors associated to the number of *Ae. aegypti* pupae in Lima. We conducted a cross-sectional study to assess the number of mosquito pupae found in households in Lima, Peru. All water containers were examined and the pupae were collected and transported to the laboratory for rearing. We then determined the species and the number of *Ae. aegypti* pupae per house. The association between the average number of pupae per house and the potential risk factors was assessed using bivariate and multiple negative binomial regression. A total of 8,585 houses were surveyed from April to May 2006; 524 (6.1%) had *Ae. aegypti* pupae with the average number of pupae per house being 0.825 (SD=6.88). The bivariate negative binomial regression showed an association between the average number of pupae and the presence of three container types (flowerpots, cylinders, low cement tanks) storage of water, house location (urban area, periurban area), water storage type (permanent public network, intermittent public network, other public network), and number of people per house. Conversely the variables presence of plastic water containers, drainage system, larvicidal control with temephos, housing area and construction material were not associated with the number of pupae. In the multiple negative binomial regression model, these variables were associated with the number of pupae: presence of cylinders (IRR=4.7, 95% CI=3.0-7.1), presence of low cement tanks (IRR=4.7 95% CI=2.8-8.0), periurban area, and the interaction between storage water and using flowerpots ($p<0.001$). The increase in the average number of pupae associated with having with flowerpots was 3.1 and 78.3 in houses that did or did not store water, respectively. In conclusion, the presence of flowerpots is a risk factor strongly associated with an increased presence of *Ae. aegypti* pupae. Flowerpots increase the number of pupae substantially more in households that do not store water. More studies evaluating the role of flowerpots are needed to adequately target vector surveillance campaigns.

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OVIPOSITION SITE SELECTION IN THE DENGUE VECTOR, *AEDES AEGYPTI*

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Oviposition site choice directly affects female reproductive success by influencing egg hatching, larval development, and offspring survival. Theory predicts that females should deposit their eggs in sites that maximize offspring performance, as has been demonstrated for numerous parasitoid and herbivore insect species. We tested the hypothesis that female *Aedes aegypti* actively select containers into which they oviposit that are most suitable for larval development. We surveyed *Ae. aegypti* egg distribution among 221 water-filled containers in households in Iquitos, Peru. For each container, we recorded the daily number of *Ae. aegypti* eggs laid over a 3 day period, along with characteristics such as size, shape, material, location, fill method, lid presence, solar exposure, and presence of conspecific larvae. We observed that 133 containers (60%) received *Ae. aegypti* eggs. Our data revealed that females most commonly deposit eggs in containers already holding conspecific larvae (odds ratio = 3.89, 95% CI = (2.13, 7.12)), particularly those with 3rd instars (odds ratio = 5.67, 95% CI = (2.28, 14.07)) and 4th instars (odds ratio = 3.59, 95% CI = (1.81, 7.12)). The average daily number of eggs that containers received was positively correlated with the abundance of conspecific larvae present (Spearman's rho = 0.41, P-value < 0.001). Suitability of 33 of these containers for larval development was assessed by conducting starvation assays on samples of 3rd instar larvae already present in containers. The average number of days that larvae survived starvation per container ranged from 1.5 to 13.7 days (mean = 9.0, SD = 2.5). We found no association between the number of eggs laid in containers and the length of time larvae from these containers survived without food ($R^2 < 0.01$, P-value = 0.69). This survey is ongoing and we are continuing to analyze data as it is collected. In additional experiments, we are testing in the field whether female oviposition container choice is associated with immature survival rates and size of emerging adults.

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DETERMINING FACTORS THAT PREDICT WEST NILE VIRUS POSITIVE MOSQUITO POOLS IN THREE LOUISIANA PARISHES

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West Nile virus (WNV) first appeared in the United States in 1999 and has since become a pathogen of interest to public health authorities across the country. To this end, state and local programs have been established to track the activity of mosquito species known to be capable of transmitting WNV, as well as their infection rates. These arbovirus surveillance programs are especially well established in the following parishes in Louisiana: East Baton Rouge, St. Tammany, and Calcasieu. Using mosquito sampling and environmental data from abatement districts in these three parishes, and test results from the Louisiana Animal Disease Diagnostic Laboratory, we have assembled a dataset that we are currently using to inform a predictive modeling effort. We seek to model arbovirus activity in these parishes based on the proportion of positive mosquito pools associated with entomological, environmental, temporal and spatial variables. Logistic regression will be used to model this proportion and probability of mosquito pool positivity based on several variables, such as location, month, and mosquito species. Using PROC GLIMMIX in SAS®, we will model both predictors and random effects to maximize the precision of the model; this will be compared and contrasted with the traditional logistic regression model. Additionally, we will report what trends exist in the positive pools between the years 2002 and 2004 for the three parishes specified, and anticipate model validation using arbovirus surveillance data from subsequent seasons.

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SIMULATION MODELS WILL INFORM SITUATION-SPECIFIC DENGUE PREVENTION STRATEGIES

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Current efforts to reduce dengue focus on universally prescribed vector control guidelines, which fail to consider spatial and temporal variations in transmission dynamics. Quantitative models can be used to assess the epidemiological significance of such variation. The Innovative Vector Control Consortium is developing a computer program for location-specific simulation of *Aedes aegypti* populations and dengue virus transmission in order to improve dengue prevention. Two

models completed in the mid-1990s (CIMSiM and DENSiM) have been incorporated into a Windows™-based graphical interface for enhanced ease of use. Major updates include resolution of coding bugs and implementation of model structural changes, such as age-dependent adult vector mortality, oviposition site selection, and human density calculations. The new program can be used to compare the impact of single or integrated vector control interventions based on locally-derived entomological, serological and climatic data. For example, space spraying (80% efficacy) every 6 months over a 5 year period was predicted to reduce the adult *Ae. aegypti* population in a 1 hectare simulation area from a baseline of 199.2 down to 186.0 females/day. Resultant reductions in human infection rates were minimal. Conversely, space spraying plus larviciding of 90% of large containers (90% efficacy) every 6 months effectively reduced mosquito populations by a further 66.0%, to 63.3 females/day. The resultant total number of human dengue infections within the simulation area dropped to 7.1-8.0 from a baseline of 18.5-19.0. Sensitivity analyses are being undertaken using a Latin hypercube sampling scheme to identify the most epidemiologically significant parameters. Validations are based on comparison of model outputs to longitudinal pre- and post-intervention entomological and serological field data from the Amazonian city of Iquitos, Peru. The program will be made available in multiple languages, free of charge. We anticipate that it will be utilized by vector control personnel, public health practitioners, and government officials to support the formulation of locally-adapted dengue control strategies.

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RAINFALL AND THE *CULEX PIPIENS* COMPLEX: HOW MUCH IS TOO MUCH?

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Mosquitoes in the *Culex pipiens* complex are important worldwide as pests and vectors of human pathogens, including several flaviviruses and filarial worms. *Cx. pipiens* s.l. utilizes storm drains and other organically charged larval habitats associated with human development. Although precipitation or anthropogenic water inputs are essential for creation of urban larval habitats, field and lab studies have suggested that intense rainfall flushes larvae out of these habitats, often leading to a reduction in adult abundance. We tested the hypotheses that (1) a moderate level of rainfall, enough to create breeding sources but not so much as to cause flushing, results in larger numbers of adult *Cx. pipiens* s.l. and (2) the precipitation-abundance relationship depends on the condition of the underground drainage system. Predictor variables considered were the amount and frequency of rainfall, combined with urban development dates and household income as surrogates for the condition of drainage systems. These were tested in spatially and temporally explicit Bayesian regression models fitted to a 10-year statewide dataset including 856 sites. In urban areas, high levels of late-winter precipitation were associated with reduction of adult female trap counts by > 50% during spring.

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CHARACTERIZATION OF IMMUNOGENIC PROTEINS IN *ANOPHELES GAMBIAE* SALIVARY GLANDS AND THEIR POTENTIAL USE AS A MARKER OF EXPOSURE TO MALARIA

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During blood feeding, the mosquito injects saliva into the vertebrate host. This saliva contains bioactive components which may play a role in pathogen transmission and in host-vector relationships by inducing an immune response in the vertebrate host. The evaluation of human immune responses to arthropod bites might also represent a research direction for assessing individual exposure to the bite of a malaria vector. We have previously described that IgG response specific to whole salivary extracts from *Anopheles gambiae* may be a marker of the risk of malaria transmission in young children exposed to malaria in Senegal. The aim of the present study is then to characterize immunogenic salivary proteins of *An. gambiae* by an immuno-proteomic approach. 2DE -blotting were performed with sera from children living in different malaria endemic area. Ten immunogenic proteins were revealed in uninfected saliva after 2DE-blotting with sera from exposed children. Among these proteins, seven were identified by mass spectrometry, several are involved in sugar meal (amylase) and other ones are involved in blood meal (5' nucleotidase, D7 long form and D7-r4). Several of these proteins are now produced under their recombinant form. The next step will be to test which protein has the better potential as a marker of exposure to *Anopheles* bite by ELISA technique on large cohort of malaria exposed individuals. The second step will be to characterize immunogenic proteins present in *Plasmodium falciparum* infected salivary glands. This work opens the way to design new epidemiological tools, to evaluate malaria exposure in the field, similarly to the study of the risk of transmission for other vector-borne diseases.

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MALARIA VECTOR BREEDING SITES AND ASSESSING THEIR IMPACT ON LOCAL MALARIA RISK: PRELIMINARY DATA ON THE RISK FACTORS FOR MALARIA INFECTION

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The study aims to investigate the importance of different vector breeding site types on malaria infection risk. The study was carried out in 3 villages in the Lower Shire Valley in southern Malawi. In an on-going process, longitudinal surveys of malaria parasitemia and mosquito abundance were carried out in the three study villages every 4 months beginning August 2006. Parasitemia was determined using microscopy of field collected thick films in children less than 10 years of age. Adult mosquitoes were collected by pyrethrum knockdown (PKD) to determine mosquito abundance/ density. Here we present preliminary results from the first two surveys carried out in September 2006 (dry season) and April 2007 (end of wet season). The odds ratio of having malaria was 27.79 higher in April 2007 (end of wet season) than in September 2006 ($p<0.001$). The finding was as expected since malaria transmission peaks towards the end of wet season. The odds ratio of having malaria was 0.08 higher for children living at Nkata village than those living at Nkhwazi ($p<0.002$). Similarly children living at Kela showed a higher odds ratio (0.14) of having malaria than those living at Nkwazi ($p<0.013$). No significant differences were found in children living either at Nkata or Kela ($p<0.411$). The difference in malaria prevalence between the study sites despite the short distance between them could be explained by heterogeneities in malaria transmission but also socio-economic factors. The odds ratio of having malaria was 6.69 higher for children not sleeping under an insecticide treated bed net ($p<0.020$) compared to children that were sleeping under a treated net. These results were also expected since treated nets have been shown to reduce all cause mortality and morbidity in children and communities using them. Parent education, child age or sex and mosquito abundance were not significant risk factors to malaria infection ($p>0.5$). It is too early to say that these are not risk factors to malaria infection as the results here are indeed only preliminary. In conclusion, this study has shown that time of survey (season), site or location (village) and use of ITNs were important factors to malaria infection.

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THE IMPACT OF IMPREGNATED SUDANESE THOBS ON HUMAN / VECTOR CONTACT OF *ANOPHELES ARABIENSIS* IN ENDEMIC AREA OF MALARIA - SUDAN

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An intervention trial using deltamethrin-impregnated Sudanese thobs as a control method for malaria, was conducted over a period of sixteen months (1998 - 1999) in two villages one as study and the other control, in Kordofan State. The area is surrounding a large man-made water body called '*Turda*', with many small agricultural schemes. Malaria was common in the area with prevalence of 30-37% in school children from previous surveys (1995-1997). Entomological data revealed two species of anpheline mosquitoes, *Anopheles arabiensis* and *An. rufipis* prevailing in the area. Data of monthly pyrethrum/spray catches of adult female *An. arabiensis* showed seasonal fluctuation. The mosquitoes totally disappeared during dry season (March - June) and then reappeared in the wet season (July - October) to an average of 1.0 female mosquito/room in the control village and 0.4 female mosquito / room in the study village in October. The cycle of human - biting rate showed that *An. arabiensis* was strongly endophilic, as about 60 % were captured while trying to bite human baits indoors. The earliest period of activity of *An. arabiensis* was found at 6: 00 p.m. indoor and 8: 00 p.m. outdoor. Comparing the human - vector contact before and after using treated *thobs*; there was considerable reduction of 54.3% (1.38 -0.63 bite /human/hr) indoor, and 76% (0.5 - 0.12 bite /human/hr) outdoor. A 62.2% of the contact of biting by *An. arabiensis* took place within one- hour stay indoors during the period 6:00 - 10: 00p.m., 53.2% of contacts biting outdoor during the same time. The present study found a reduction of human/ vector contact, and overall population decreases on mosquitoes densities. This suggested that the reduction in human - vector contact and *Plasmodium falciparum* infection in our study were probably caused by repellent effects of insecticide treated thobs that reduced mosquitoes from biting and picking up infection.

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ENTOMOLOGICAL SURVEY ON DENGUE VECTORS AS FOR BASIS ON PREVENTION AND CONTROL IN BARANGAY POBLACION, MUNTINLUPA CITY, 2008

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Dengue is a vector-borne disease that is of public health importance. The disease cause deaths and hospitalization of children in Asia. There is no available vaccine for dengue hemorrhagic fever hence prevention and control of the mosquito vector is a must in every community. The study aimed to conduct entomological survey of dengue hemorrhagic fever in Magdaong, Poblacion, Muntinlupa City as basis for prevention and control specifically in Barangay Poblacion, Muntinlupa City. The study involved a descriptive method using structured entomology survey form of the Department of Health to 100 households selected thru systematic sampling. Entomological investigation was carried out in Magdaong, Poblacion, Muntinlupa City, Metro Manila, Philippines on the presence of productive breeding sites of *Aedes aegypti* mosquito,vector of Dengue / Dengue Hemorrhagic fever. The breeding of *Aedes aegypti* mosquito was mainly found in drum, used automobile tires, soft drink case, pails, jar and jug. The drums were found to be the most preferred container for *Aedes* breeding. The percentage positive rate of drums (37.3 %), used tires (40 %), soft drink case (100%) pail (2.9 %), jar (2.8 %), and jug (8.3 %). An analysis of data revealed that the House Index (23 %), Container Index (63 %), and Breteau Index (63 %) indicating thereby the high receptivity of the area to DF/ DHF transmission. The results concluded that the presence of productive breeding sites indoor and outdoor in Magdaong, Barangay Poblacion revealed that possible outbreak can occur in the future if no vector control plan is adapted and implemented. A vector control prevention measures was formulated for the community affected. It is recommended that both the national and local government should cooperate and support each other in the prevention and control of dengue hemorrhagic fever.

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CALCIUM ALGINATE FORMULATIONS OF BACTERIA FROM PLANT INFUSIONS PRODUCE OVIPOSITION ATTRACTANTS AND STIMULANTS FOR GRAVID *AEDES AEGYPTI* AND *AEDES ALBOPICTUS*

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Oviposition attractants and stimulants for gravid females of *Aedes aegypti* and *Ae. albopictus* are known to be produced by bacteria in infusions made from senescent leaves of bamboo (*Arundinaria gigantea*) and white oak (*Quercus alba*). A standard calcium alginate encapsulation method was used to produce a novel formulation of bioactive bacterial species that were cultured from the two plant infusions. The alginate beads containing bacteria were highly active against gravid mosquitoes in behavioral bioassays. Formulated bacteria produced oviposition semiochemicals at biologically active levels over a 3-week period. Alginate encapsulated bacteria would augment the effectiveness of oviposition traps used for surveillance and control of *Ae. aegypti* and *Ae. albopictus* populations.

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MOSQUITOES, CATCH BASINS, HYDROLOGY, AND RISK OF WEST NILE VIRUS IN ILLINOIS

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In 2002, an outbreak of West Nile virus illness occurred in the state of Illinois, with a markedly high concentration in Cook County and DuPage County. Since then, these two counties have reported hundreds of positive mosquito pools and over 300 cases of human illness. Environmental conditions related to housing age, vegetation, and population density help to explain some of the local variability in WNV infection, but the complex interplay among mosquito populations, hydrology, climate, catch basin biology and WNV risk in humans is still largely unexplored. In this project, we describe associations among these factors as a key step in creating a more comprehensive spatially explicit predictive model of WNV infection in an urban setting. We developed a sensor system to measure light, temperature and water level in urban catch basins in suburban Chicago and assessed the relationship between catch basin biology, larvae production, precipitation and surface water hydrology. From these data, we determined the degree to which the environment of catch basins in different types of neighborhoods with known historical WNV mosquito infection rates differentially support populations of *Culex* species mosquitoes. Hypotheses tested: (1) Neighborhoods where WNV infection in mosquitoes and humans is higher will exhibit different catch basin biology characteristics than those that have lower infection rates. (2) Precipitation is a key control in determining local enzootic WNV amplification. We measured spatial and temporal trends in mosquito infection rates from three years of mosquito testing data from the Illinois Department of Public health surveillance database and developed

statistical models using landscape variables and precipitation to develop risk maps of the region across three years. Hydrological modeling provided variables of discharge, soil moisture, soil saturation, and water level depth. Catch basin characteristics as measured by the sensors were then analyzed in their context of WNV infection risk and neighborhood hydrological characteristics.

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VIRAL ETIOLOGY OF ACUTE FEBRILE ILLNESSES IN SOUTH AMERICA, 2000-2007

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To better characterize the etiologic agents associated with human febrile illnesses in South America, a clinic-based syndromic surveillance system was implemented at 30 sites in Ecuador, Peru, Bolivia, and Paraguay. Serum samples were collected from febrile patients reporting to local health clinics and examined for virus by immunofluorescence assay or RT-PCR, and for pathogen-specific IgM antibodies by ELISA. Evidence for infection by a specific viral pathogen was detected in 41% of all febrile cases. Dengue virus (*Flaviviridae*) was the prominent diagnosis, but no one pathogen was the source of greater than 20% of all febrile cases. In addition to the *Flaviviridae*, we detected serological evidence for infection by viruses from the *Togaviridae*, *Bunyaviridae*, *Arenaviridae*, and *Picornaviridae* families, and isolated dengue, Ilheus, yellow fever, Venezuelan equine encephalitis, Mayaro, Oropouche, Group C, and Encephalomyocarditis viruses from patient specimens. Considering the significant overlap of symptomologies, laboratory diagnosis is necessary to accurately define the relative disease burden of these circulating viral pathogens. Results from this study have been used to inform public health response and will provide a baseline for analyzing future epidemiological trends.

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REEMERGENCE OF BOLIVIAN HEMORRHAGIC FEVER IN BOLIVIA, 2007-2008

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Outbreaks of Bolivian hemorrhagic fever have occurred since 1960s and early 1970s, including large epidemics that affected hundred of individuals. Although no cases were reported from 1976 to 1992, possibly due to effective host control, a small outbreak was reported in 1994 and sporadic fatal cases have been observed since then. We report here a small outbreak of Bolivian Hemorrhagic Fever (BHF) in Beni, a department located in the northeastern region of Bolivia. Four arenavirus isolates were obtained from patients suffering with febrile hemorrhagic illness, three of these patients succumbed infection 6 to 10 days after the onset of symptoms. Another two cases were confirmed as Machupo virus infection based on IgM seroconversion, nine presumptive Machupo cases were also observed. Sequencing and phylogenetic analyses based on the S segment genome region of one of the isolates identified the virus as a New World Tacaribe Complex Machupo Arenavirus. Future studies will determine whether all four cases were caused by Machupo virus, the newly identified Chapare virus or a related Arenavirus. Our investigations have highlighted the necessity of constantly monitoring for Arenavirus disease in Bolivia and the Americas and more importantly, the significance of developing effective measurements to control human infections due to the health threat possessed by these viruses.

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RESISTANCE TO ADAMANTANES AND NEURAMINIDASE INHIBITORS AMONG INFLUENZA VIRUSES ISOLATED IN CENTRAL AND SOUTH AMERICA IN 2005-2007

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Recent influenza antiviral resistance studies reveal an alarming increase in both Adamantanes (M2) and Neuraminidase Inhibitors (NAIs) resistant viral strains in Asia, Europe and the United States. In this study, Influenza viruses, isolated from symptomatic patients throughout Central and South America in 2005-2007 were screened for inhibitor resistance. The M2 and NA genes of influenza viruses were sequenced and resistance patterns were inferred by comparison with published sequences. Our results indicate that there have been a) three major influenza A/H1N1 strains in circulation, the majority of the isolates were susceptible to M2 and to NAIs, only one isolate was found to be resistant to M2 inhibitors, b) two Influenza A/H3N2 strains, one resistant (95.5% of all A/H3N2 isolates) and one susceptible to adamantanes but all susceptible to NAIs and c) none of the influenza B viruses examined were resistant to NAIs.

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DERMATOLOGIC CONDITIONS IN "HEALTHY HTLV-I CARRIERS"

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Norwegian scabies and infective dermatitis are conditions that occur more often in HTLV-infected people than in the general population. However, the presence of other dermatologic conditions - infective vascular or autoimmune - could be early indicators of HTLV infection. The objective of this study was to determine if dermatologic conditions were common in female sex workers with asymptomatic HTLV-I infection. All female sex workers (FSW) who regularly attend STI Clinic "Alberto Barton" and were previously identified as HTLV-I seropositive, were invited to participate; five seronegative age-matched controls were enrolled for each seropositive subject. All women underwent an initial dermatologic exam, and at least 3 dermatologic follow-up examinations to evaluate for new dermatologic conditions. 104 FSW were enrolled, 18 of whom were HTLV-I seropositive. Dermatologic examination detected 19 findings suggestive of autoimmune conditions, and 36 of vascular conditions. In univariate analyses, significant relationships were found between HTLV-I seropositive status and presence of telangectasia, varices, melanosis, vitiligo, keratosis, cutaneous and seborrheic dermatosis, xerosis, eczema and onychomycosis. On logistic regression (adjusted for HIV status, diabetes and time working as a FSW),

HTLV infection was significantly associated with dermatologic vascular conditions (OR=7.79, p=0.01) particularly varices (OR=12.34, p=0.006); telangectasia (OR=11.47, p=0.01); immunologic skin disease (OR=16.39, p<0.001), particularly vitiligo (OR=7.91, p=0.03); keratosis (OR=90.37, p<0.01); and onychomycosis (OR=5.86, p=0.01). In conclusion, we detected dermatologic conditions in 12 of 18 (67 %) HTLV-infected FSW. Several unreported dermatologic conditions were detected during our screening examinations. Presence of one or more of these dermatologic conditions may alert physicians to the presence of HTLV infection in healthy individuals and provides additional evidence for a more systemic effect of HTLV-I infection.

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ANDES VIRAL RNA LOAD IN CHILEAN PATIENTS WITH HANTAVIRUS CARDIOPULMONARY SYNDROME

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Andes virus is endemic in Chile and is unique among hantaviruses in that it can be transmitted person-to-person. The role of the viral load in body fluids in the transmission and pathogenesis of Andes hantavirus cardiopulmonary syndrome (HCPS) is not well defined. We enrolled 66 patients with acute hantavirus infection confirmed by an IgM anti-hantavirus ELISA. Blood and urine in 66 cases and tracheal fluid in 17 cases were collected at admission and days 2, 3, 4, 5,14 and 28. Viral load was measured by a quantitative reverse transcription-polymerase chain reaction (RT-PCR) for Andes virus S segment RNA, based on amplification of a 234-bp fragment (LightCycler™, Roche Molecular Biochemicals). Assay sensitivity was 1,900/ml. Neutralizing antibodies were measured on 37 patients by plaque reduction neutralization test. Of the 66 patients, 42% were classified as mild, 17% as moderate and 41% as severe; the case-fatality rate was 12%. Overall 58% had detectable viral load in plasma. Mean copy numbers were 98,800/mL in RT-PCR positive cases at admission and decreased during the first 5 days of hospitalization. In 2 cases, plasma viral load was still detectable on day 28. Andes virus RNA was detected in urine in 18 (28%) cases; copy numbers fluctuated between 15,000-61,000/ml during the first 5 days of hospitalization. In tracheal fluids, 11 of 17 (65%) cases were positive and RNA copy numbers were higher (100,000 and 300,000 copies/ml). On the day of admission, no association was found between viral load and disease severity, but those with severe disease had significantly lower neutralizing antibody titers. No association was found between neutralizing antibodies titer and viral load in plasma. In conclusion, there was no significant association between plasma viral load at admission and disease severity. Plasma viral load may not adequately reflect viral replication, and further studies on PMBC-associated virus are necessary. High viral loads in urine and trachea fluids suggest possible routes for person-to-person transmission. Our data showing low Andes virus neutralizing antibody titers on the day of admission in Chilean patients with severe HCPS support the findings of studies of Sin Nombre virus neutralizing antibody titers in HCPS patients in North America.

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DETECTION OF HAMSTER CYTOKINE RESPONSES BY REAL-TIME PCR

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Hantavirus cardiopulmonary syndrome (HCPS) is associated with a pronounced inflammatory cytokine immune response that is thought to substantially contribute to its pathology and high fatality rate. Currently, only one suitable small animal model for HCPS has been developed; the Syrian golden hamster (Mesocricetus auratus) infected with Andes or Maporal hantaviruses. In this model, the gross histopathology is similar to that found in human HCPS; however, assessment of the role of the immune response has not been conducted because of a lack of methods for assessing such responses in hamsters. To address this deficiency, we have developed a multiplex real-time PCR assay to detect hamster T cell cytokine gene expression response to antigenic stimulus. Hamsters were immunized with antigen and assessed for cytokine gene expression upon recall challenge *in vitro*. RNA was extracted, converted to cDNA and used as template for real-time PCR using gene specific primers for (list cytokines here). The development of immunological methods will be essential for validation of the hamster model of HCPS, and development of new therapies and vaccines.

1105

FIRST CHARACTERIZATION OF CYTOKINE GENES FROM A BAT, USING SEBA'S SHORT-TAILED BAT (*CAROLLIA PERSPICILLATA*)

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Bats represent one fifth of the nearly 5,000 known species of mammals. In recent years, bats have been identified as reservoirs and possible reservoirs for several important human viruses, including Nipah virus, Hendra virus, SARS coronaviruses and Ebola virus. Little is known about how the immune system of bats engages viruses, nor how these viruses might evade sterilizing immunity. We have initiated a project to develop immunologic methods in two bat species, *Carollia perspicillata* (Seba's short-tailed fruit bat) and *Artibeus jamaicensis* (Jamaican fruit bat), from which Nepuyo and Tacaribe viruses have been isolated respectively. Our initial work has focused on the cloning of cytokine genes, and we have succeeded with partial cDNA clones of tumor necrosis factor (TNF), interleukin-10 (IL-10), Interleukin-23 (IL-23), and granulocyte macrophage-colony stimulating factor (GM-CSF). We have also cloned several immune-related transcription factors, including STAT4, STAT6, T-bet, and Fox-p3, which control the expression of some cytokine genes. To our knowledge, these are the first cytokine and cytokine-related genes cloned from any bat species.

1106

GENETIC VARIABILITY OF RVFV IN WEST AFRICA: IMPLICATIONS FOR VIRUS DISPERSAL AND DISTRIBUTION

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Rift valley fever (RVF) is a mosquito-borne viral disease infecting both human and animals. Human infection leads to a clinical picture ranging from mild to very severe syndromes including hepatitis, encephalitis, haemorrhages or blindness that can have a fatal outcome. Infection in animals is associated with high mortality among young animals and

massive abortions among pregnant females. RVF virus (RVFV) belongs to Bunyaviridae family, genus Phlebovirus. Its genome consists in 3 single stranded negative RNA and exhibits significant genetic variability. RVF is widespread in sub-Saharan Africa and spread recently to Yemen and Saudi Arabia and the ongoing outbreak in Indian Ocean (Comoros, Madagascar) emphasizes the need of surveillance and reinforce its impact as a major threat to human and animal health in relation with climate/environment changes and increasing animal and human traffic worldwide. In order to further understand factors leading to its emergence, a thorough study of its genetic variability in West Africa has been undertaken. Thus 40 strains isolated in epidemic or endemic context over X years from various hosts in West Africa have been sequence on the 3 segments and analyzed using phylogenetic methods. Such an analysis revealed that RVFV genetic diversity within West Africa ranges from 12.3 to 14 % at nucleotide level vs. 4.7 to 11.17 % at amino acid level. As expected the envelope glycoprotein G2 showed the highest genomic variability. Partial sequences of each of the segments revealed that isolates clustered in three distinct lineages. Clades within all three lineages contain combination of viral populations from different regions active migration within West Africa. In addition, co-circulation of 2 different lineages within the same areas was identified in 1998 and 2003 with emergence of reassortant mosquito-generated RVFV strains. It was also observed sequence homology between strains isolated several years apart suggesting re-emergences of RVF from endemic foci. Besides, distribution of RVFV lineages suggests RVFV in West Africa is linked to bioclimatic zones. Using data derived from that analysis, a model have been proposed and discussed.

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EARLY DETECTION OF HANTAVIRUS ACUTE INFECTION AND ECOLOGY STUDIES IN TONOSI, PANAMA. 2007-2008

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In Panama, 65.6% (60/93) of Hantavirus Cardiopulmonary Syndrome's cases have been detected in the province of Los Santos. Las Tablas 53% (32) and Tonosi 25% (15) were the most affected districts. With the objective of characterize hantavirus acute infections without acute distress respiratory syndrome, we conducted a hospital-based surveillance study in Tonosi and performed ecology investigations on rodents species. This region has a population of 10 223 inhabitants and a rural hospital with eight beds. Between January 2007 and February 2008, we included 23 patients that complied with the inclusion criteria such as: fever higher than 38.5°C, be a resident of the study area, be five or more years old, accept voluntarily to participate, and sign an inform consent. We excluded patients with rhinorrhea and chronic diseases. Eleven patients were identified with a mean of 31.6(SD+19.2) years old and a man:woman proportion of 1.2:1. Clinical symptoms were characterized by fever 11 (100%), fatigue 9 (82%), myalgia 9 (82%), nauseas 9 (82%) and headache 8 (73%). In addition, seven patients had cough (64%) and five patient presented dyspnea (45%). Three patients showed parahilar infiltrates and another one presented a discrete basal infiltrate. All of them had oxygen saturations higher than 95% and were positive for IgM antibodies against Hantavirus in both, Inmunoblot and ELISA tests. Between August 2007 and February 2008, ~33 600 Sherman tramps/night were put, and an increment of Oligoryzomys fulvescens specie was observed. During the same period, 6 of the 11 cases were reported. Preliminary results, revealed that the surveillance system can detect symptomatic cases on time and promote proper treatment administration. The increase in Oligoryzomys fulvescens population could suggest that Choclo virus is circulating in the area and affecting humans. The information generated, helped to alert the community and health authorities to adopt control and prevention measures for Hantavirus Cardiopulmonary Syndrome.

1108

VECTOR COMPETENCE OF *ANOPHELES GAMBIAE SENSU STRICTU* FOR O'NYONG-NYONG VIRUS

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O'nyong-nyong virus (ONNV; family, *Togaviridae*; genus *Alphavirus*) is a mosquito-borne virus transmitted in Sub-Saharan Africa that can cause severe arthralgia. The virus is unusual in that it is associated primarily with punctuated epidemics and is one of the few arboviruses transmitted by anopheline mosquitoes. This study explores the vector competence of *Anopheles gambiae sensu strictu* mosquitoes for ONNV in detail. Using a GFP-expressing recombinant ONNV, posterior midgut infections and an unusual tropism for the anterior midgut were common with this virus, however virus was never observed disseminated to hemocoelic tissues by examination for GFP fluorescence. This observation held with two different strains of *An. gambiae s.s.* Two different strains of wild type virus were then used in similar experiments. Wild type virus was able to escape from the alimentary canal, but we observed only low dissemination rates to *An. gambiae s.s.* salivary glands with these viruses, and a patchy infection pattern on the few salivary gland lobes that became infected. Quantification of viral RNA in mosquito saliva revealed that only minimal virus titres are shed in the saliva. We found that RNA interference influences these barriers to ONNV transmission, in particular, transient silencing of Argonaute 2 increased dissemination to the head and salivary glands, but these experimental treatments still did not change the low titres of virus in the saliva. In an examination of alternative transmission potential, we determined that infected *An. gambiae s.s.* could shed virus in prediuretic fluid during a second uninfected bloodmeal, but only at very low rates. In total, our vector competence data suggests that *An. gambiae s.s.* is a very poor and unlikely vector for ONNV transmission.

1109

DISEASE BURDEN DUE TO DENGUE AND INFLUENZA IN AN INDONESIAN FACTORY WORKER COHORT

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In Indonesia, more than 100,000 new cases of dengue infection are reported annually, with an attributable fatality rate of 1%, and influenza causes more than 20% of upper respiratory tract infections. Although both diseases are considered major public health problems, relatively little is known about their precise disease burdens and economic impacts. Here we analyze those features in the context of a factory-worker cohort study of dengue and influenza conducted since September 2006 in a textile factory in Bandung, Indonesia. Acute and convalescent blood specimens were collected from workers with suspected dengue infections. Nasal and throat swabs were taken from workers who experienced fever >37.7 C or had a history of acute fever with respiratory symptoms. Virus isolation, RT-PCR, serology test and clinical hematology tests were performed to identify dengue and influenza infections. Dengue caused 11.8 infections per 1000 workers per year, with over half resulting in hospitalization. Influenza caused 61.4 infections per 1000 workers per year; based upon

data from a larger study of influenza-like illness, we estimate more than 500 influenza cases per 1000 per year, the majority of which were mild illnesses. Absenteeism in dengue cases ranged from 1-14 days (mean 7 days), while in influenza cases ranged from 0-4 days (mean 1.45 days), resulting in 73 lost workdays/1000 employees annually due to dengue and 89 lost workdays/1000 due to influenza. Measured direct personal costs (absenteeism from work, payment for health care visits and hospitalization) attributable to dengue and influenza were US $1946 and US $520, respectively. These values likely underestimate the true direct costs of illness and do not consider indirect costs. In the context of the per capita GNP for Indonesia (US $1940) these two diseases are estimated to exact a substantial economic toll.

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NOVEL METHODS OF DETECTION AND CHARACTERIZATION OF RNA VIRUS PATHOGENS AND THEIR HOSTS IN THE KYRGYZ REPUBLIC

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RNA viruses belonging to several families are potential hazards for both human and animal populations in Central Asia. In the summer of 2007 we undertook a field trip to the Kyrgyz Republic where we harvested animals and ticks, to initiate establishment of a baseline for viral epizootiology and epidemiology studies. We collected tissues from 185 specimens of rodents and shrews from 4 collecting localities. Prior to this collecting effort, only 8 specimens of mammals from the Kyrgyz Republic were listed in the MaNIS database as archived in accredited museums in the United States. We have generated phylogenetic and phylogeographic results from the rodent genera *Alticola*, *Apodemus*, *Dryomys*, *Microtus*, *Myodes*, and *Rattus* as well as the soricid genus *Crocidura*. Results indicate that the mammalian fauna of the Kyrgyz Republic has complex biogeographic connections with East Asia, South Asia, and East Europe. Our data provide phylogeographic evidence of recent (< 20,000 ybp) colonization events by at least two genera (*Apodemus* and *Crocidura*) into the Kyrgyz Republic. We hypothesize that zoonotic viruses were introduced with colonization and have similarities to pathogens at the geographic origin of the colonizing mammals. In this study we examined the occurrence of viral RNA from hantaviruses, Crimean-Congo hemorrhagic fever virus and tick-borne encephalitis viruses (TBEV) in animals and ticks. Our data suggest that hantaviruses are associated with *Microtus*, *Apodemus*, *Rattus* and *Crocidura* species. We also detected TBEV in *Apodemus pallipes*. The occurrence of hantaviruses in *Apodemus pallipes* has, we believe, never been reported. Using a novel EIA for antibodies based on cloned viral antigens, we have been able to detect antibodies for all three studied viruses in a range of rodents and insectivores. We are currently isolating viral genomes for sequencing. We will correlate viral genomic information with serology and with the phylogeny of rodent and insectivore hosts.

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CHIKUNGUNYA VIRUS - MECHANISM OF ADAPTATION TO *AEDES ALBOPICTUS* MOSQUITO

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Chikungunya virus (CHIKV) is an emerging arbovirus associated with several recent large scale epidemics. In a recent study we showed that an E1-A226V mutation was directly responsible for a significant increase in CHIKV infectivity for *Aedes albopictus*, and led to more efficient viral dissemination into mosquito secondary organs and transmission to suckling mice, as reported previously. To test the hypothesis that increased CHIKV midgut infectivity of *Ae. albopictus* associated with E1-A226V mutation is responsible for increased dissemination and transmission of CHIKV to suckling mice we examined effect of the E1-A226V mutation on CHIKV fitness in orally and intrathoracically infected *Ae. albopictus* and *Ae. aegypti* mosquitoes. Data suggests that the E1-A226V mutation exerts its effect on *Ae. albopictus* vector competence mostly by modulating *Ae. albopictus* midgut cell infectivity. The effect of the E1-A226V mutation on cholesterol dependence of CHIKV was also analyzed, revealing an association between cholesterol dependence and increased fitness of CHIKV in *Ae. albopictus,* as reported previously. To further examine the relationship between CHIKV mosquito infectively and dependence on cholesterol, various amino acids substitutions were introduced into position E1-226 and the effects of these mutations on *Ae. albopictus* midgut infectively and cholesterol dependence was compared and analyzed. Additionally *in vitro* adaptation of CHIKV for growth in cholesterol depleted C6/36 cells was performed and mutations responsible for release of CHIKV cholesterol dependence were tested in *Ae. albopictus.* Data suggests that CHIKV dependence on cholesterol is not sufficient to cause CHIKV increased *Ae. albopictus* midgut infectively.

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DETERMINANTS AFFECTING OUTCOMES OF NATIONAL PROGRAMS TO ELIMINATE LYMPHATIC FILARIASIS (LF): DEFINING RESEARCHABLE PRIORITIES

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The Global Programme to Eliminate LF was launched in 2000. To understand why some of the National programs to eliminate LF have been more successful than others, a group of program-linked researchers reviewed the process and outcomes of program implementation in 8 countries - identifying factors responsible for increasing or decreasing the likelihood of program success and assessing the reasons for these outcomes. Success was defined as the rapid and sustained reduction in microfilaremia (mf) prevalence in communities to levels at or near zero. More than 40 different determinants affecting program outcome were identified and characterized as having either greater or lesser impact on the success of these MDA (mass drug administration)- based programs. The principal factors distinguishing programs achieving early success from other programs were : 1)initial level of endemicity; 2)vector capability; 3) drug regimen; 4) population compliance. Three categories of researchable issues were identified: - *Biologic researchable priorities: 1)* Quantifying differences in vector competence among vector species; 2) Identifying potential seasonal variations for LF transmission. - *Programmatic researchable priorities: 1)* Defining optimal drug distribution strategies for different settings; 2) Identifying programmatic implications of persistent antigenemia in mf-negative individuals and of low-level mf prevalence in communities after multiple treatments ; 3) Quantifying the operational factors essential for program success, including levels of population compliance, levels of mf- or antigen-positivity at which programs can safely be stopped, and number of rounds of MDA required for success in different epidemiologic settings. - *Community-focused' research priorities:* 1) Developing 'compliance profiles' in communities to identify those who are systematically non-compliant during MDAs and 2) Identifying the causes of non-compliance and approaches to overcoming it. Answers to each of these researchable issues should improve program guidelines and, ultimately, program outcome.

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EVALUATION OF DIAGNOSTIC TOOLS FOR BRUGIAN FILARIASIS ELIMINATION PROGRAMS

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The Global Programme to Eliminate Lymphatic Filariasis uses mass drug administration (MDA) to reduce infection rates to levels that cannot sustain transmission. More information is needed to define endpoints for MDA programs and to determine effective methods for post-MDA surveillance. The GAELF Diagnostics Study Group is testing various surveillance tools in 8 LF-endemic areas. We now report the results of diagnostic assays in *Brugia*-endemic areas in Sabah, Malaysia and Alor, Indonesia which had undergone 3 to 5 years of MDA. Blood was collected from 1,000 residents and 350 school children at each site and tested for microfilaremia (MF) by microscopy (60 µl thick smear) and by a qPCR test for *Brugia* DNA. Antibodies to recombinant filarial antigens were detected with two rapid tests (Brugia Rapid, BR and PanLF, PL) and by ELISA (Bm14). Filariasis rates were higher in Sabah (Mf 2.5% by smear and 1.4% with 95% CI 0.7-2.3% by PCR; antibody rates 18% BR, 18% PL, 60% Bm14) than in Alor (Mf 0.5% by smear and 0.28% CI 0.05-0.82 by PCR; antibodies 3% BR, 7% PL, and 30% Bm14). Using the BM14 assay, antibody rates in children were comparable to those seen in community surveys. Rapid tests were less sensitive than Bm14 for detecting low antibody levels indicative of exposure, but detected antibodies in most Mf carriers. We also estimated filarial DNA rates in mosquito pools by qPCR. *Anopheles* and *Mansonia* vectors in *Brugia*-endemic areas are difficult to catch, so we used gravid traps to collect non-vector *Culex* mosquitoes, as previous studies showed that filarial DNA persists in non-vector species. *Brugia* DNA rates in mosquitoes were 0.18% (CI 0.09-0.31%) in Alor and 0.08% (0.02-0.19) in Sabah. DNA rates could be higher in vector species, but *Culex* xenomonitoring efficiently detected *Brugia* parasites in both study areas. Results suggest that residual filariasis rates are higher in Sabah than in Alor. Additional study is needed to determine the optimal use and cost effectiveness of these tools for documenting interruption of LF transmission and for post-MDA surveillance.

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SPATIAL MODELING OF LYMPHATIC FILARIASIS RISK IN AMERICAN SAMOA BASED ON EPIDEMIOLOGICAL AND ENTOMOLOGICAL DATA

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Parasitological, serological and entomological surveys in three villages in American Samoa were undertaken to determine if residual transmission of *Wuchereria bancrofti* was occurring after 5 years of annual mass drug administration. Participants >4 years of age (n=597) were screened for circulating filarial antigen (ICT) and for antifilarial antibody (Bm14). ICT-positive persons were further screened for microfilariae (Mf) by thick smear. *Aedes polynesiensis* mosquitoes were collected from 10 sites within each village using BG-Sentinel™ mosquito traps and screened for the presence of *W. bancrofti* by dissection or polymerase chain reaction (PCR) assay. Antigen prevalence ranged from 3.7 to 4.6%; with only a single Mf-positive person found. Antibody prevalence was higher in all three villages, ranging from 12.5 to14.9%. The prevalence of *W. bancrofti* infection in *A. polynesiensis* by the dissection technique ranged from 0 to 0.23% (mean= 0.16%; 95% C.I. 0.05-0.46%) for all three villages, while the maximum likelihood estimate (MLE) of infection by PCR ranged from 0.52 to 0.9% (mean= 0.69%; 95% C.I. 0.34-1.2%). The overall point estimate of infection was significantly greater by PCR (0.69%) than by dissection (0.16%) ($p<0.05$). A Bayesian hierarchical generalized linear mixed model for data analysis found ICT- and Bm14-positivity were associated with age and with male gender. There was no spatial relationship between ICT or Bm-14-positivity and proximity to mosquito traps where *W. bancrofti*-infected mosquitoes were trapped. The presence of antibody positive children under the age of 10 in all three villages coupled with the detection of positive mosquito pools in all three villages indicates that residual transmission is still occurring in American Samoa. However, the lack of significant associations between infected mosquitoes and Mf positive individuals raises questions regarding the utility of xenomonitoring to target with precision the locations of infected individuals in areas with diurnal transmission, such as in Polynesia.

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COMPREHENSIVE MONITORING OF THE IMPACT OF A PILOT MASS DRUG ADMINISTRATION PROJECT FOR FILARIASIS IN PAPUA NEW GUINEA

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The purpose of this study was to assess the impact of mass drug administration (MDA) on bancroftian filariasis in Papua New Guinea and to gain experience with different monitoring methods. Three rounds of single dose diethylcarbamazine with albendazole were distributed in 2002-2004 in 12 villages in the Usino-Bundi district, near Madang. Sentinel villages were studied prior to and one year after each round of MDA. The mean MDA coverage rate was 72.9%. Coverage rates were low in children under 5 years of age. Three rounds of MDA decreased microfilaremia (MF, 1 ml night blood by filter) from 18.3% to 1.3% (93% reduction). MF clearance rates in infected persons were 71%, 90.6%, and 98.1% respectively, one year after 1, 2, or 3 rounds of treatment. Filarial antigenemia (a marker for adult worm infection, assessed by card test) rates decreased from 46.2% to 17.7% (62% reduction). Filarial antibody rates (IgG4 antibodies to Bm14, which indicate filarial infection or heavy exposure) decreased from 53.2% to 25.1% (53% reduction). MF, antigen and antibody prevalence rates decreased more rapidly in children under 11 years of age (by 100%, 79%, and 68%, respectively), perhaps reflecting their lighter infections and shorter durations of exposure/infection prior to MDA. Prevalence rates for parasite DNA in fed or gravid *An. punctulatus* mosquitoes (assessed by PCR/Poolscreen) decreased from 15.1% to 1.0 % (93% reduction). This study has provided useful information on the value of comprehensive monitoring for assessing effects of MDA. Each test provides a different type of useful information. MDA had dramatic effects on all filariasis parameters in the study area; reductions were similar to those reported after 3 rounds of MDA in Egypt. Residual infection rates in the Usino study area may be below levels needed for sustained transmission by the relatively inefficient *An. punctulatus* vector. A 4th round of MDA was distributed in early 2006. Follow-up studies will be needed to determine whether 4 annual rounds of MDA with moderate coverage rates were sufficient to permanently interrupt filariasis transmission in this area.

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IMPLEMENTATION AND MANAGEMENT OF LF CONTROL AND ELIMINATION PROGRAMMES: EIGHT YEARS OF EXPERIENCE FROM TANZANIA

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The Tanzania Lymphatic Filariasis Elimination Programme was launched in 2000 and is now functional in 34 districts in six regions. More than 9.2 million people have received the annual mass drug administration of ivermectin (Mectizan) and Albendazole. This paper describes the history and setting up of the present Lymphatic Filariasis Elimination Programme, its goals, principles as well as details of the implementation of the programme. It also address the key components of setting up the Tanzania programme which include social mobilization and advocacy, political will, training mechanisms, delivery mechanisms and strategies, funding as well as monitoring and evaluation. The paper will discuss the successes and challenges of the programme and experience that has been gained in the 8 years of the programmes existence. The discussion also includes the need to address financial sustainability and describes how Tanzania has managed with its meager resources to support programme implementation. As there are now districts that have received 6 rounds of treatment and there is need to expand the programme to cover a further 82 districts, the paper discusses strategies related to carrying out expansion without compromising the initial gains. Current approaches to implementing the programme in conjunction with other mass treatment efforts for infectious disease are also described in brief.

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PROGRESS TOWARD LYMPHATIC FILARIASIS (LF) ELIMINATION IN PLATEAU AND NASARAWA STATES, NIGERIA: SENTINEL VILLAGE EPIDEMIOLOGICAL AND ENTOMOLOGICAL EVALUATIONS AFTER SIX YEARS OF ANNUAL MASS DRUG ADMINISTRATION WITH IVERMECTIN AND ALBENDAZOLE

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Bancroftian LF is widespread in Plateau and Nasarawa States of central Nigeria, and mass drug administration (MDA) with ivermectin and albendazole has been provided to a treatment eligible population of 3.6 million since 2000. 2007 was the sixth MDA year with full geographic coverage (some areas have been treated for longer) and the reported treatment coverage has been >85% of eligibles all years (directly observed). According to the WHO, elimination of LF should be achieved after 5-7 years of MDA, if administered with good treatment coverage. In 2007 we conducted assessments in the two state area to determine if LF has been eliminated by the MDA program. We compared 2007 infection rates with baseline values gathered in 1999-2003 in 9 sentinel villages. *Wuchereria bancrofti* microfilariae in nocturnal thick smears decreased by 80%, from 9.8% in 2002-2003 to 0.7% in 2007 (the WHO threshold for elimination is for microfilaremia to be below 1%). The Filariasis Immunochromatographic Card Test (ICT) for LF antigenemia decreased by 83%, from 47% in 2000 to 8%. LF infection rate (L1-3) in anopheline mosquitoes decreased by 92%, from 5.2% in 2000 to 0.4%. These decreases were all statistically significant. A small cohort study of 174 permanent residents of the sentinel villages who were tested with ICT in 2004 were retested in 2007. We found 97 persons who were ICT negative in 2004 remained negative in 2007, indicating 0% incidence over a three year period (or 291 person-years). Among persons who were ICT positive in 2003 (n= 77), 19.5% became seronegative over that same period. While these findings are encouraging, disaggregated results show that there remain 'hot spots' of LF infection and that treatment should not be discontinued without further evaluations. The challenges of the 'end-game' for this LF MDA program will be discussed.

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INCREASING ADHERENCE TO MASS DRUG ADMINISTRATION FOR LYMPHATIC FILARIASIS - ORISSA STATE, INDIA

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India bears 40% of the world's burden of lymphatic filariasis (LF). Although mass drug administration (MDA) coverage with diethylcarbamazine (DEC) for LF has improved, India has not achieved the goal of 80% coverage of the at-risk population. Recently, a non-governmental organization (NGO A) in Orissa State implemented an educational campaign to increase adherence to MDA. A stratified, random cluster survey, with probability proportionate to size sampling, was performed between area A, where NGO A worked, and area B, where NGO A did not. Ten households (HH) were randomly selected in 30 villages in each area, using random walk methodology. All members of each HH were surveyed, and one member ≥ 15 yrs old was randomly selected to complete a knowledge, attitudes, and practices (KAP) survey. Replacement of those who were absent or declined to participate was not allowed. All results are adjusted for clustering and weighted for HH size when appropriate. LF MDA coverage for the entire population (Area A + Area B, n=3,449) was 56% (95% CI: 50.0-61.9). Although coverage in area A was 7.3% higher than in area B, this result was not statistically significant. The two most common reasons for not taking DEC in the KAP survey were fear of medication side effects (47.4%) and lack of recognition of one's risk for LF (15.8%). Side effects were reported by 12.6% of those who took DEC and were minor. Univariate predictors for taking DEC included knowing about the MDA in advance, knowing that DEC prevents LF, knowing that mosquitoes transmit LF, believing that water is involved in LF transmission, and knowing that anyone can acquire LF. Modifiable, statistically significant, multivariate predictors for taking DEC included: knowing that DEC prevents LF (aOR=2.62, 95% CI: 1.35-5.07), knowing that mosquitoes transmit LF (aOR=1.86, 95% CI: 1.08-3.21), and knowing both about the MDA in advance and that mosquitoes transmit LF (aOR=5.41, 95% CI: 2.80-10.44). India needs to increase LF MDA coverage to reach its goal of eliminating LF as a public health problem. These data suggest that promoting a simple public health message prior to MDA distribution specifying when the MDA will occur, that DEC prevents LF, and that mosquitoes transmit LF could effectively increase adherence to the LF MDA. In addition, these data demonstrate the need to improve education on the benefits of LF prevention and rarity of serious side effects to DEC.

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DELAYED *PLASMODIUM FALCIPARUM* PARASITE CLEARANCE FOLLOWING ARTESUNATE-MEFLOQUINE COMBINATION THERAPY IN THAILAND, 1997-2007

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The recently-reported, poor efficacy of artesunate (ATS)-mefloquine (MFQ) combination (ATS 12 mg/kg, maximum 600 mg, and MFQ 25 mg/kg, maximum 1,250 mg) against uncomplicated falciparum malaria on the Cambodia-Thailand border has raised much concern over the potential spread of resistance and the urgent need of an alternative therapy. ATS-MFQ combination has been the first line therapy for uncomplicated falciparum malaria in Thailand since 1995, initially only in selected areas known for high-level mefloquine resistance. We analyzed data obtained from the *in vivo* monitoring of ATS-MFQ therapeutic efficacy conducted according to WHO guidelines by the Thai National Malaria Control Program in 1,267 patients infected with *Plasmodium falciparum* from 1997-2007. Monitoring sites included were in 2 provinces bordering Cambodia and 5 provinces bordering Burma. Delayed parasite clearance as measured by the presence of parasitemia on Day 2 (D2, D0=day of treatment initiation) varied by region (Wald test, p=0.008): on the Thailand-Cambodia border, prevalence of D2 parasitemia increased from 0% in 1997 to 45.2% in 2007 while during the same time period on the Thailand-Burma border, it increased only from 5.5% to 13.3%. After controlling for age and initial parasitemia, the OR for each year for the Cambodian border was 1.40 (95% CI: 1.18, 1.66, p<0.0001) and was 0.98 (95% CI: 0.80, 1.19, p=0.83) for the Burmese border, thus the odds of having parasitemia on D2 is increasing, on average, 40% per year on the Cambodian border but not increasing on the Burmese border. D2 parasitemia was strongly associated with treatment failure at 28 days even after controlling for region, age, initial parasitemia and year (adjusted OR = 4.15, 95%CI: 2.35, 7.32, p<0.0001). Since MFQ is more slow-acting, ATS is the major determinant of parasite clearance on D2. These data suggest that parasite strains on the Cambodian border may be becoming less sensitive to ATS. Therefore increased malaria control efforts focusing on this multi-drug resistant hotspot is urgently needed. Surveillance for elevated parasite clearance times in the Greater Mekong Subregion, where artemisinin-based combination therapy is widely used, may be useful in tracking the sensitivity of *P. falciparum* to ATS.

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TREATMENT OF *PLASMODIUM FALCIPARUM* MALARIA WITH ARTESUNATE-MEFLOQUINE-PRIMAQUINE COMBINATION THERAPY IN TRAT PROVINCE, THAILAND

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Trat Province lies in the east of Thailand adjacent to the Cambodian border from where recent reports of treatment failures with artemisinin combination therapies (ACT) have begun to emerge. Despite small numbers of falciparum malaria cases and active public health measures, cure rates in Trat have shown a worrying decline in recent years, mirroring the situation directly across the border in western Cambodia. This is in stark contrast to adjacent border provinces of Thailand where cure rates with the same ACT regimens remain very high. The study is an on-going comparison of two short-course artesunate-mefloquine-primaquine regimens for the outpatient treatment of uncomplicated falciparum malaria, in which *in vivo, in vitro*, molecular and pharmacokinetic data are collected. A provisional review of the clinical data in the first 12 patients shows an overall 63-day cure rate of just 75% despite directly observed therapy and close follow-up. This figure is similar to the PCR-corrected 42-day cure rate of 81% that we described in 2005 at the same study site. To date 67% (8/12) subjects failed to clear malaria parasitemia by 72 hours, compared to 49% in 2005, and one subject still had a positive blood smear 120 hours after commencing treatment. However there were no early treatment failures and all subjects were aparasitemic by Day 6. The subjects who subsequently had recurrent parasitemia during follow-up did so between 14 and 28 days after initiation of therapy. Although the number of cases so far in this study is low, these data lend credence to an accumulating body of evidence that some malaria parasites from Trat Province are responding much more slowly to conventional ACT treatments than those from adjacent provinces in Thailand. There is a compelling need to establish the causes of declining ACT efficacy in Trat as well as western Cambodia and to characterize and contain these emerging parasite isolates.

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DURATION AND RECOVERY RATES OF ARTEMISININ INDUCED DORMANCY IN *PLASMODIUM FALCIPARUM IN VITRO*

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Parasite resistance to anti-malarials is widespread and clinical treatment of falciparum malaria presently relies on a limited number of drugs. Artemisinin and its derivatives are being used worldwide where multiple drug resistant falciparum malaria is prevalent. Despite the current success of artemisinin combination therapy the potential development of resistance to the artemisinin drugs is a growing concern and the threat of drug-resistant falciparum malaria remains severe. Despite the remarkable activity of artemisinin drugs, ~10% of patients fail treatment if artemisinins are given as a monotherapy to non-immune patients. The recrudescent parasites remain susceptible to artemisinin *in vitro*, suggesting a novel mechanism allowing parasites to tolerate drug treatment. The persistence of temporarily growth-arrested parasites (dormancy) following drug treatment such as described for bacteria and yeast provides a plausible explanation for this phenomenon. To investigate dormancy induced by artemisinin and its derivatives we determined the duration of growth-arrest and recovery rates in asexual stage *Plasmodium falciparum* parasites. Ring stage parasites were exposed to different concentrations of dihydroartemisinin (DHA) as a model drug and parasite growth was measured *in vitro* for *P. falciparum* lines from different genetic backgrounds. Our observations show that the parasite development is abruptly arrested for a period of up to 10 days post a single dose treatment before returning to normal growth in all cases. The majority of dormant parasites resume growth during the first week and the proportion of parasites recovering is dose dependant. Results from *P. falciparum* lines that have reduced susceptibility to artemisinin will also be discussed. Our results suggest that artemisinin-induced dormancy may be a key factor in treatment failure. Hence, a better understanding of drug-induced dormancy will provide valuable information for the effective use of artemisinin-combination therapies.

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EXAMINATION OF THE MOLECULAR BASIS OF RESISTANCE TO ARTEMISININ DRUGS IN *PLASMODIUM FALCIPARUM*

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Artemisinin (QHS) and its derivatives provide rapid relief from malaria symptoms and faster clearance of parasitemia than any other drugs. Because they represent the sole class of antimalarials for which clinical resistance has not been reported, it is critical to investigate potential resistance mechanisms. We have developed resistant parasites and are using these lines to determine the molecular basis of QHS resistance. Discontinuous exposure to artelinic acid (AL) or QHS *in vitro* produced AL and QHS resistant progeny of *Plasmodium falciparum* lines W2, D6, and TM91c235. Using this method, parasites were generated that tolerated 340ng/mL of QHS (D6) and 160ng/mL of AL (TM91c235). We are currently adapting these parasites to higher levels of pressure as well. Prior microarray studies performed on early drug selected progeny of W2 (W2.QHS40 and W2.AL80) identified >40 probesets that were significant in terms of differential expression (p<0.001). The most pronounced expression changes were observed for *pfmdr1* with ~4-fold increase in W2.AL80 and ~2 fold increase in W2.QHS40 when compared to W2. A subset of putative ABC transporters was under-expressed in the resistant progeny (ex. *pfmdr2*). Of the differentially expressed genes, the most significant was PFE1050w, which encodes a S-adenosyl-L-homocysteine hydrolase. Targeted analysis of 42 other transporters found six that were highly significant (p<0.05), with PF11_0466 being the most significant. We hypothesized that the differential expression of genes may be due to novel SNPs. Therefore, we sequenced *pfmdr2*, PFE1050w, and PF11_0466 in W2 and W2.QHS200, and compared these results to known SNPs in PlasmoDB. Full sequence analysis of *pfmdr2* and PFE1050w did not identify any nucleotide differences between W2 and W2.QHS200. Analysis of PF11_0466 was inconclusive. We used a real-time QPCR assay to assess copy number of *pfmdr1* and *pfmdr2* in parental and resistant lines. A RT-QPCR assay was used to assess expression of *pfmdr1* in W2 and its resistant progeny. Copy number of *pfmdr1* was elevated in W2.QHS200, TM91c235, and TM91.AL80, but it was not increased in D6.QHS300. Copy number of *pfmdr2* was 1 for the same parasites. Transcription of *pfmdr1* was increased in the resistant lines of W2. Future research will focus on whole genome sequencing of the above parental and resistant parasites to elucidate mechanisms of resistance to artemisinin drugs.

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ADAPTIVE COPY NUMBER EVOLUTION OF A KEY GENE IN THE FOLATE PATHWAY OF MALARIA PARASITES

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The first gene in the *Plasmodium* folate biosynthesis pathway - GTP-cyclohydrolase I (*gch1*) - shows extensive copy number variation (CNV). We provide compelling evidence that CNV in this gene is an adaptive consequence of selection by antifolate drugs, which target enzymes downstream in this pathway. (1) We compared *gch1* amplification in parasites from Thailand (strong historical antifolate selection) with those from neighboring Laos (weak antifolate selection). We observed <2% of chromosomes with copy number amplification in Laos, while 71% carried multiple (2-10) copies in Thailand. This dramatic geographical differentiation (F_{ST} = 0.7) is maintained despite abundant gene flow, suggesting strong local adaptation. (2) Genetic variation flanking *gch1* was reduced in Thailand consistent with hitchhiking resulting from rapid recent spread of chromosomes carrying multiple copies of this gene. (3) We found that parasites bearing *dhfr*-164L, which causes high level resistance to antifolate drugs, carry significantly (p<0.0001) higher copy numbers of *gch1* than parasites bearing 164I, indicating functional linkage between genes on different chromosomes. (4) We examined the span and breakpoints of amplified chromosomal regions using real-time PCR and sequencing. We found 5 different amplicon types containing 1 to 6 genes, consistent with parallel evolution and strong selection for this gene amplification. *Gch1* was the only gene occurring in all amplicons suggesting that this locus is the target of selection. (5) We found a strong positive correlation between gene expression and copy number demonstrating that increased gene dosage has the potential to increase flux through the folate pathway. These data provide a striking example of copy number variation in *P. falciparum* involved in adaptation, and suggest that investigation of structural variation may provide a fast-track to locating genes underlying important traits in this and other pathogens.

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INTERMITTENT PRESUMPTIVE TREATMENT FOR MALARIA DURING PREGNANCY: REDUCED EFFICACY AND SELECTION FOR RESISTANCE

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WHO recommends that African women receive intermittent presumptive treatment during pregnancy (IPTp) with sulfadoxine-pyrimethamine (SP) to prevent poor outcomes, but resistance to SP has spread and may limit efficacy. We analyzed the effects of IPTp use on pregnancy outcomes as well as selection for resistant parasites in a cohort of 872 women in Muheza, Tanzania, where malaria transmission is intense. 87% of women delivering at the Muheza Designated District Hospital reported using IPTp during pregnancy, and plasma assays for sulfa confirmed women's verbal reports (99.2% concordance of positive results). Use of IPTp was not associated with a significant reduction in the odds of placental malaria (PM), low birth weight (LBW), or severe maternal anemia (SMA) within any parity group or within the subset of women who received two or more doses of IPTp. In a preliminary study, isolates from the area were predominantly wild-type at codons DHFR 50 and 164, and DHPS 536 and 613, but were resistant at codons DHFR 108 and DHPS 437. Using pyrosequencing, we quantified the proportion of parasites within individual placental isolates that carried resistance markers at four additional positions. Isolates contained on average 92.2% parasites with a resistance marker at codon DHFR 51, 95.5% at DHFR 59, and 89.6% at codon DHPS 540, which may explain lack of IPTp efficacy. At codon DHPS 581, women's placental infections were 14.1% drug resistant in women without IPTp exposure, as compared to 39.7% resistant in those with exposure (p=0.035), suggesting selection for resistant variants by IPTp. The mutant form of DHPS 581 is associated with high levels of resistance *in vitro*. In this area of widespread drug resistance, IPTp does not improve pregnancy outcomes and may further select for drug resistant parasites. New drug combinations for IPTp are urgently needed.

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ARTEMETHER-LUMEFANTRINE VERSUS DIHYDROARTEMISININ-PIPERAQUINE FOR TREATMENT OF UNCOMPLICATED FALCIPARUM MALARIA: A RANDOMIZED TRIAL TO GUIDE NATIONAL POLICY IN UGANDA

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Artemisinin-based combination therapies (ACTs) have been strongly advocated for use in Africa but obstacles to their widespread use remain. Uganda recently adopted artemether-lumefantrine (AL) as the recommended first-line treatment for uncomplicated malaria. However, AL has several limitations, including a twice-daily dosing regimen, recommendation for administration with fatty food, and a high risk of reinfection soon after therapy in high transmission areas. We compared the efficacy and safety of AL with DP in Kanungu, an area of moderate malaria transmission in Uganda. Patients aged 6 months to 10 years with uncomplicated falciparum malaria were randomized to therapy and followed for 42 days. Genotyping was used to distinguish recrudescence from new infection. Of 414 patients enrolled, 408 completed follow-up. Compared to patients treated with AL, patients treated with DP had a significantly lower risk of recurrent parasitaemia (33.2% vs. 12.2%; risk difference = 20.9%, 95% CI 13.0-28.8%) but no statistically significant difference in the risk of treatment failure due to recrudescence (5.8% vs. 2.0%; risk difference = 3.8%, 95% CI -0.2-7.8%). The prevalence of fever was similar over the first 3 days of follow-up in the two treatment groups. Both treatments produced rapid clearance of parasitemia with no parasites detected by Day 3. Patients treated with DP also had a lower risk of developing gametocytaemia after therapy (4.2% vs. 10.6%, p=0.01). Both drugs were safe and well tolerated. DP is highly efficacious, and operationally preferable to AL because of a less intensive dosing schedule and requirements.

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CYTOKINE EXPRESSION IN A HAMSTER MODEL OF HANTAVIRUS PULMONARY SYNDROME

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Andes virus (ANDV) is a major cause of hantavirus pulmonary syndrome (HPS) in South America. Mononuclear leukocytes and various cytokines appear to play a central role in the pathogenesis of the interstitial pneumonitis and, ultimately, the life-threatening pulmonary edema in HPS caused by ANDV and certain other hantaviruses. The ANDV in the Syrian golden hamster can cause a highly lethal disease that is pathologically remarkably similar to HPS. The purpose of this study was to assess whether the cytokine pattern in the lungs of ANDV-infected hamsters proximal to death is similar to the cytokine pattern in the lungs of fatal HPS cases. Adult Syrian golden hamsters were inoculated with 100 median infectious doses of ANDV by transthoracic injection in the left thorax. Subsequently, 4 animals were sacrificed each day through day 8 post-inoculation (PI). The level of ANDV RNA and levels of TNF-α mRNA and the mRNA of certain other cytokines were measured in the right lung of each animal. Microscopic examination of samples of right lung revealed interstitial pneumonitis as early as day 3 PI and alveolar edema as early as day 5 PI. The most severe pulmonary pathology was observed in the animals that were sacrificed or died on day 8 PI. High levels of viral RNA were detected as early as day 1 PI. The levels of ANDV RNA peaked on day 4 PI and then remained high through day 8 PI. Levels of IFN-γ, IL-2, IL-12, TNF-α and TGF-β mRNA increased through day 2 PI. Levels of TNF-α and TGF-β mRNA remained elevated through day 8 PI whereas the levels of IFN-γ, IL-2, and IL-12 mRNA dramatically decreased on day 3 PI by as much as 10-fold below the baseline levels, remained below baseline levels through day 7 PI, and then increased above the baseline levels on day 8 PI. Previous studies on samples of lung from fatal HPS cases revealed an increase in the number of cells positive for IFN-γ, IL-2 and TNF-α. The elevated levels of TNF-α mRNA in the lungs of the ANDV-infected hamsters sacrificed on day 8 PI relative to the sham-inoculated control animals are further evidence that the pathogenesis of the HPS-like disease in ANDV-infected hamsters is similar to the pathogenesis of HPS. The elevated levels of TNF-α and IFN-γ in the lungs of the hamsters sacrificed on days 1 and 2 PI suggests that the cytokine response in the lungs of fatal HPS cases begins long before the abrupt transition from febrile prodrome into severe pulmonary edema.

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CLINICAL COURSE OF HANTAVIRUS CARDIOPULMONARY SYNDROME IN CHILEAN PATIENTS

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Hantavirus cardiopulmonary syndrome (HCPS) is a pan-American zoonosis. Andes virus is the etiologic agent in Chile, where 567 HCPS cases and 208 deaths (37%) have been reported through May 2008. We analyzed clinical data from 104 Andes virus HCPS cases enrolled in natural history and treatment protocols. Cases were classified as mild (no mechanical ventilation, no shock), moderate (required mechanical ventilation) and severe (mechanical ventilation and shock). Among 147 subjects enrolled in these studies, hantavirus infection was confirmed by IgM anti-hantavirus ELISA in 104 (median age 37 y/o, 78% male). Most cases were seen in summer (49%) with a second wave in winter (15%). Mean time from first symptoms to hospitalization was 5.8 days, and 45% sought medical care during the prodrome. The clinical syndrome was characterized by fever (97%), myalgia (88%), headache (82), dyspnea (79%), nausea-vomiting (53%), abdominal pain (49%), arthralgia (34%), conjunctivitis (32%), diarrhea (31%), bleeding signs (27%), petechiae (23%), and blurred vision (11%). Laboratory evaluation at admission showed increased LDH (98%), platelet count <150.000/mm3 (95%), elevated liver enzymes (93.5%), plasma Na < 135 (68%), leukocyte count > 15.000/mm3 (42%), elevated creatinine (44%), hematocrit >48% (46%), hematuria (20%) and increased amylase (14%). 44 (42%) were classified as mild, 11 (11%) as moderate and 49 (47%) as severe. 83 (79%) required admission to ICU (mean stay 6.5 days), and most severe cases developed DIC. Among the 22 deaths (21%), 18 (81%) died within 48 hrs of admission. Case fatality rate was higher for women (29.2 versus 18.8) (p=0.20). Variables associated with higher risk of death were: shock (21/49, 43%) versus no shock (1/55, 1.5%) (p<0.0001), hematocrit >48% (64 versus 29%, p=0.004), leukocyte count > 15.000 (73 versus 33% p=0.001), elevated creatinine (65 versus 38%, p=0.09) and platelet count (44,770 versus 68,389/ml p=0.037). In conclusion, HCPS has a bimodal seasonality in Chile. The clinical course of HCPS in Chile is similar to that of the cases in North America, but renal involvement and clinical bleeding are more common in Chile. Shock, hemoconcentration and renal involvement predicted increased risk of death, and there was a trend toward higher mortality in women.

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GUAROA VIRUS: AN EMERGENT PATHOGEN AMONG HUMANS IN PERU

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Guaroa virus, a member of the Family Bunyanviridae, was first isolated in Guaroa, Colombia in 1959 from apparently healthy individuals. Since this first report, additional isolations of the virus were made from human patients and mosquitoes in Brazil, Colombia and Panama, and serosurvey studies revealed the presence of antibodies to Guaroa virus in residents from several areas in Colombia, Brazil, Argentina, Peru and Guatemala. However, despite these previous serological studies from Peru, it remained a question whether the virus was associated with human febrile illness and whether the virus circulating in this country was genetically similar to strains isolated from other countries in South and Central America. As part of a febrile illness study initiated primarily in Iquitos, Peru in 1993, a total of 12 Guaroa virus strains were obtained from patients with mild febrile illness and 4 additional human cases were confirmed by IgM seroconversion. Eleven out of the sixteen Guaroa confirmed cases were reported in 2007 (between January through November) suggesting the re-emergence of Guaroa virus as a human pathogen. These data also showed that the virus circulated throughout the year and epidemiological studies conducted in 1997 revealed an overall seroprevalence of 31% to Guaroa viral infection among residents in Iquitos and surrounding communities of the Amazon region of Peru. Thus, these data have documented more precisely that Guaroa virus is an emergent pathogen responsible for human illness in Peru. Genetic characterization of the Guaroa virus strains from Peru and other strains isolated from South and Central America is currently underway to identify the factors involved in the emergence of this virus as a human pathogen in Peru.

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HTLV INFECTION IN AMAZONIAN COMMUNITIES IN PERU

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HTLV is endemic in South American indigenous groups. This study examines the prevalence and distribution of HTLV infection in indigenous communities living along the Peruvian Amazon basin. As part of a randomized household-based survey, adults aged 18-29 from 27 indigenous communities completed a questionnaire regarding demographics and sexual behavior and provided a blood sample. Study participants represented 12 different ethnic groups in the Peruvian Amazon. Sera were screened for HTLV-1 and HTLV-2 using ELISA (Vironostika, Biomereux, Netherlands) with Western blot confirmation (INNOLIA, Innogenetics, Belgium). Sera were obtained from 638 (99%) of the 644 participants, 282 males and 356 females. The overall prevalence for Western blot-confirmed infections was 1.9% (12/638) for HTLV-2 and 0.9% (6/638) for HTLV-2. All but one of the infections were detected in people from Shipibo-Conibo (S-C) communities, with prevalences of 4.7% (11/232) and 2.3% (6/232) for HTLV-1 and 2 respectively. HTLV-2 infection was also detected in one S-C woman enrolled in a mostly mestizo (mixed blood) community; all other HTLV-2 infections occurred in communities where HTLV-1 infection was also detected. One additional HTLV-1 infection was detected in a female resident of a S-C community who self-identified as "mestiza." Risk of HTLV infection did not differ significantly by gender, age, or sexual experience. In conclusion, this study is the largest study of HTLV infection in indigenous communities in Peru. We detected HTLV-1 and 2 infections that were surprisingly restricted to the Shipibo-Conibo ethnic group or residents from their communities. The geographic proximity of S-C communities to urban cities may explain the recent detection of HTLV-2 infection in non-indigenous sexually active adults living in cities adjacent to the Amazon river. Future studies of HTLV genotypes are planned to may compare HTLV strains from different indigenous to urban populations.

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KNOWLEDGE, ATTITUDES, AND PRACTICES REGARDING LASSA FEVER IN POST-CIVIL WAR SIERRA LEONE

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Lassa fever (LF) is a severe hemorrhagic illness caused by Lassa virus (LASV). LASV is transmitted to humans by contact with the excreta or blood of rodents of the genus *Mastomys*. LF is endemic in West Africa, with an estimated 300,000 infections and 5,000 deaths annually. Kenema District, Sierra Leone, is thought to have the highest incidence of LF in the world. Massive population displacement and destruction of health infrastructure occurred during the 11-year civil war in Sierra Leone. Knowledge, attitudes and practices (KAP) regarding LF in the resettled population are unknown. We are therefore conducting a cross-sectional design KAP study to inform future community-based interventions for LF. A 2004 national census was used to select 40 clusters of geographic sampling units with probability-proportional to size. Within each cluster, ten households were selected and a survey administered to the head of household. Data presented here are based on 149 surveys. Virtually all participants (97%) had heard of LF, with the most common sources being radio (38%), a person who had LF (34%), health care workers (21%), the District LF Outreach Team (17%), a colleague (17%), and family members (8%). However, 63% could not name a sign or symptom of the disease. Rats were the most frequently identified source of transmission of LASV (57%), while 39% did not know how LASV was transmitted. The majority of respondents (52%) did not know how to prevent LF. Poisoning was the most common method for rodent control (62%), followed by owning cats (28%), trapping (22%), no action (7%), and keeping a clean house (2%). Of particular concern was the finding that nearly 40% of those surveyed reported consumption of rodents, which has been associated with increased risk of LF. The World Health Organization currently recommends storing food in rodent-proof containers, disposing of garbage far from homes, maintaining clean houses and keeping cats, although no rodent control interventions for prevention of LF have ever been empirically evaluated. Carefully designed studies to elucidate and communicate the most effective control methods are needed.

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UNDERSTANDING BATS ACCESS TO DATE PALM SAP: IDENTIFYING PREVENTATIVE TECHNIQUES FOR NIPAH VIRUS TRANSMISSION

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Nipah virus outbreaks coincide with the date palm sap harvesting season in Bangladesh. Drinking raw date palm sap was identified as a risk factor for Nipah virus transmission in two outbreaks. Bats (*Pteropus spp.*)

frequently visit date palm trees and occasionally shed Nipah virus through saliva and urine. Understanding how bats contaminate date palm sap is a key to develop preventative techniques. Method: We asked sap collectors to identify 11 date palm trees that bats visited regularly and observed them for 11 nights. Per their usual routine, sap collectors shaved the bark of the date palm trees (*Phoenix sylvestris*) just below the base of branches, and placed a bamboo tap to collect sap in to a clay pot. We mounted two infrared cameras that silently capture images upon detection of motion at each tree from 5:00 PM to 6:00 AM. Out of 22 infrared camera nights of observations, 16 captured 184 visits of bats around the tree (mean: 9.7), 151 in and around the shaved part (mean: 10.8), four at the stream (mean: 0.5), and no bats at the tap or on the collection pots. Bats accessed the shaved part of the date palm tree in three ways by either directly landing on the shaved part (n=82); or landing directly to the left or right of the shaved part (n=63); landing on tree branches above the shaved part (n=6). Bats visited the trees most commonly from 9:00 PM to twelve midnight (37%) and from 2:00 AM to five AM (45%). Bats contaminated the shaved part and the sap stream 157 times, mostly by landing and licking (n=145) and few by only landing (n=12); duration of contamination varied (mean: 111 seconds, range: 1-1093). In conclusion, bats commonly visited date palm trees and contaminated the sap collected for human consumption. This is further evidence that date palm sap is an important link between Nipah in bats and Nipah in humans. Efforts that prevent bat access to the shaved part and the sap stream of the tree could reduce Nipah spillovers to the human population.

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RIFT VALLEY FEVER VIRUS INFECTION IN AFRICAN BUFFALO (*SYNCERUS CAFFER*) HERDS IN RURAL SOUTH AFRICA--EVIDENCE OF INTER-EPIZOOTIC TRANSMISSION

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Rift Valley fever virus (RVFV) is an emerging biodefense pathogen that poses significant health threats to human and livestock communities in Africa and the Mideast. To date, the inter-epizootic/epidemic reservoirs of RVFV are not well defined. In a prospective random subsets survey of infectious diseases among African buffalo during the 5½ year period between November 2000 and July 2006, 550 individual buffalo were tested for anti-RVFV antibodies in 820 capture events in 302 georeferenced locations in Kruger National Park (KNP) area on the eastern border of South Africa. Overall, 115 individual buffaloes (21%) were seropositive by hemagglutinin-inhibition assay. Prevalence of anti-RVFV seropositive animals was highest (32%) in the first year of the study (2001), immediately following a period of anomalous heavy rainfall during 1999-2000. Prevalence then dropped progressively in subsequent years (30%, 18%, 19%, and 14% in 2002, 2003, 2004 and 2005, respectively). However, despite a return to normal rainfall patterns in 2001, nine of 126 resampled, initially seronegative (at-risk) animals converted their anti-RVFV serology status to positive (7 % incidence) during the period of observation. Anti-RVFV seroconversions were detected in significant temporal clusters between mid-2001 and the first half of 2003 (six of 9, upper central region of KNP), and between March and October of 2004 (2 of 9, upper and lower central region). The observed seroconversions occurred during time periods outside any reported RVFV outbreaks in South Africa or neighboring Mozambique and Zimbabwe. These findings highlight the potential importance of focal, inter-epizootic RVFV transmission among wildlife in perpetuating the regional risk for Rift Valley fever virus transmission.

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CHARACTERIZATION OF THE CELL DEATH MACHINERY IN *AEDES AEGYPTI*

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Detailed knowledge of the pathways that regulate apoptosis in mosquitoes will be important for designing strategies that utilize apoptosis as a means to interrupt pathogen transmission. At this point, little is known about apoptosis regulation in mosquitoes, but the sequencing of mosquito genomes has allowed for the identification of genes that are predicted to play a role in apoptosis, based on their known function in *Drosophila*. Our laboratory has undertaken a systematic approach to identifying and studying the function of a number of these conserved apoptotic regulatory genes in *Aedes aegypti*. As an initial method to study the function of these genes, we have used RNA interference (RNAi) to silence a number of genes in the *Ae. aegypti* cell line Aag2 and examine their roles in regulating apoptosis. In *Drosophila*, the core apoptotic pathway includes the master regulator DIAP1, which inhibits the accumulation and activity of the caspases Dronc and DrICE, while the IAP antagonists Reaper, Hid and Grim negatively regulate DIAP1. Continuous synthesis of DIAP1 is required to prevent spontaneous apoptosis in *Drosophila* cells, while the caspase-activating protein Ark, the initiator caspase Dronc and the effector caspase Drice, along with at least one of the IAP antagonists, are required for apoptosis. Silencing of the *Aeiap1* gene resulted in spontaneous apoptosis in Aag2 cells, indicating that AeIAP1 plays a similar role in *Ae. aegypti*. Silencing *AeArk* or *AeDronc* inhibited apoptosis triggered by several different apoptotic stimuli. There has been expansion of effector caspases in *Ae. aegypti* compared to *Drosophila*, and our results indicate that there has been specialization in the function of specific effector caspases in *Ae. aegypti*. These initial results suggest that the pathways that regulate apoptosis in *Ae. aegypti* are similar to those in *Drosophila*, but there are subtle differences in how these pathways are regulated in the yellow fever mosquito.

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A ROLE FOR *AEDES AEGYPTI DNR1* IN REGULATING APOPTOSIS

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Although the genetic pathways that regulate apoptosis have been studied extensively in *Drosophila*, little is known about the genes that regulate apoptosis in other insects. It is especially important to study regulation of apoptosis in mosquitoes because of the role of apoptosis in innate immunity, and because of the importance of mosquitoes as vectors for many human and animal pathogens. *Dnr1* (*defense repressor 1*) is known to play roles in innate immunity and apoptosis in *Drosophila*, and a single copy of *Dnr1* exists in the genome of the yellow fever mosquito, *Aedes aegypti*. The goal of this study was to examine the function of Dnr1 in regulating apoptosis in *Ae. aegypti* cells. RNAi-mediated depletion of Dnr1 from *Drosophila* S2 cells results in increased protein levels of the initiator caspase Dronc and, although it does not induce apoptosis, makes S2 cells more sensitive to apoptotic stimuli. Silencing of *Dnr1* by RNAi in the *Ae. aegypti* cell line Aag2 resulted in spontaneous apoptosis, accompanied by caspase activation. This suggests that Dnr1 plays a role in *Ae. aegypti* apoptosis, and that Dnr1 perhaps may be even more critical in regulating apoptosis in *Ae. aegypti* than in *Drosophila*. We also found that silencing *AeDnr1* reduced transcript levels for certain other known apoptotic regulatory genes. Some of the apoptotic genes downstream of *AeDnr1* were also identified, providing information about the position of *AeDnr1* in the apoptotic pathway. Our results suggest that Dnr1 may play a dynamic regulatory role in the apoptotic pathway in mosquitoes.

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THE ROLE OF KEY PTEN SPLICE VARIANTS ON REPRODUCTION AND LIFESPAN IN THE MOSQUITO *AEDES AEGYPTI*

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The insulin/insulin growth factor I signaling (IIS) cascade regulates key physiological processes, such as life span and reproduction, in a wide range of organisms including mosquitoes. PTEN is one of the key IIS inhibitors that reset the IIS cascade to its resting state. In the yellow fever mosquito, *Aedes aegypti*, six splice variants of AaegPTEN have been identified. Previous studies on the expression of the *AaegPTEN* transcripts indicated that *AaegPTEN6* is expressed predominantly in the fat body and ovary, key reproductive tissues in insects, while *AaegPTEN3* is expressed predominantly in the head and midgut. We generated polyclonal antibodies specific for AaegPTEN3 and 6. Consistent with the transcript data AaegPTEN3 protein is predominantly expressed in the head with a small amount of protein detected in the midgut. In contrast, the AaegPTEN6 protein is highly expressed in all four tissues. We hypothesize that AaegPTEN6, not AaegPTEN3, is the key regulator of reproduction. To determine the affects of AaegPTEN on reproductive success we used RNA interference to suppress expression of all the AaegPTEN splice variants or AaegPTEN3 and 6 specifically. Double stranded RNA against the conserved phosphatase region of all AaegPTEN splice variants was injected into newly emerged female mosquitoes resulting in a greater number of more developed follicles compared to DsRed controls. To further elucidate the roles of AaegPTEN3 and 6 in the mosquito, we have recently generated dsRNA against the unique 3' ends of both AaegPTEN3 and 6. The affect of AaegPTEN6 on the number of developed follicles is compared to AaegPTEN3 affects. In addition to knocking down AaegPTEN expression we examined what happened when AaegPTEN was overexpressed in specific tissues. We engineered a transgenic line of mosquitoes that overexpresses AaegPTEN6 in the fat body following a blood meal. The transgenic line has been repeatedly outcrossed with the colony wild types to achieve maximum genetic diversity and tested for expression and its effects on lifespan and reproduction.

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INSIGHT INTO METABOLIC PATHWAYS INVOLVED IN AMMONIA FIXATION, ASSIMILATION, AND EXCRETION IN *AEDES AEGYPTI* MOSQUITOES

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During the process of blood feeding, female mosquitoes can transmit etiological agents that cause millions of deaths annually. Attempts to control mosquito populations using biorational approaches depend on a thorough understanding of mosquito biology. An important by-product of amino acid metabolism is ammonia, which is highly toxic to animal tissues. It is not entirely clear how female mosquitoes are able to survive the massive deamination that takes place during the catabolism of the amino acids derived from blood meal proteins. In order to elucidate the metabolic pathways that blood-fed mosquitoes use to avoid ammonia toxicity, we have studied the kinetics of incorporation of 15N from labeled ammonia chloride into several nitrogen compounds in *Aedes aegypti* females by mass spectrometry techniques. We previously reported that the labeled nitrogen of 15NH4Cl is initially fixed and assimilated in *Ae. aegypti* into [5-15N]-Glutamine by a glutamine synthetase/glutamate synthase pathway, followed by the production of [15N]-Glutamate which is converted to [15N]-Proline, as reported previously. More recently, we have shown that mosquitoes can use the 15N from the amide group of two [5-15N]-Glutamine molecules to produce one molecule of uric acid labeled at two nitrogen positions. This uric acid can either be excreted, or further metabolized via an amphibian-like uricolytic pathway that utilizes urate oxidase, allantoinase, and allantoicase to produce glyoxylic acid and two molecules of urea labeled at one position, as reported previously. Taken together, our results demonstrate that urea synthesis in *Ae. aegypti* mosquitoes is not limited to arginine cleavage by arginase as these mosquitoes have an alternate metabolic pathway for urea synthesis. Current studies are aimed at elucidating the relative contributions of the uricolytic pathway and arginase in nitrogen excretion in blood fed mosquitoes. The discovery of unexpected nitrogen metabolizing pathways and regulatory mechanisms for ammonia metabolism in *Ae. aegypti* females could lead to the identification of novel targets that could be exploited for vector control.

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MOLECULAR ANALYSIS OF LIGHT PULSE STIMULATED BLOOD FEEDING INHIBITION IN *ANOPHELES GAMBIAE*

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Circadian clocks regulate rhythmic phenomena in a variety of organisms and control different biochemical, behavioral and physiological processes. The most important clock entrainment cues are light, heat and food. We have initiated a systematic dissection of light regulated circadian clock of the mosquito *Anopheles gambiae* and how it controls the host seeking and blood feeding behavior to understand the vectorial capacity of the mosquitoes. The *A. gambiae* mosquitoes exhibit an endophilic, nocturnal blood feeding behavior; light playing a major role in the transmission of malaria. We have shown that mosquito blood feeding behavior is under circadian control and can be modulated by light cues, both in a clock dependent and independent manner. Short light pulses in the dark period can inhibit the feeding propensity temporarily, however longer periods of light stimulation induces a phase advance phenomenon in mosquitoes. The temporary feeding inhibition after short light pulses reflects a masking effect of light; an unknown mechanism which superimposes on the circadian rhythm. The shorter light pulses, however, differentially regulates a variety of genes including those implicated in circadian control; suggesting that light induced masking effect implicates clock components. Other regulated genes were related to feeding as well as various physiological processes like metabolism, transport, immunity and protease digestions. RNAi-mediated gene silencing assays of the light pulse regulated circadian factors *timeless*, *cryptochrome* and three *takeout* homologues significantly up-regulated the mosquito's blood-feeding propensity. In contrast, gene silencing of light pulse regulated olfactory factors down-regulated the mosquito's propensity to feed on blood, suggesting that the observed feeding inhibition is mediated by the light pulse induced regulation of chemosensory factors that are important for host seeking.

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RNA INTERFERENCE (RNAI) OF RIBOSOMAL PROTEIN S3A (RPS3A) SUGGESTS A LINK BETWEEN THIS GENE AND ARRESTED OVARIAN DEVELOPMENT DURING ADULT DIAPAUSE IN *CULEX PIPIENS*

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Arrested ovarian development is one of main characteristics of adult diapause in Culex pipiens. Ribosomal protein S3a, a small ribosomal subunit, is a highly conserved protein that contributes to numerous physiological functions, including ovarian development in several species including Drosophila melanogaster and Anopheles gambiae. In mosquitoes that do not enter diapause, rpS3a is consistently expressed, but in mosquitoes programmed for diapause, expression of rpS3a is intermitted (high on day 6, 12, 15, but low on day 8 and 10) during early diapause, but is then consistently elevated in females 1 month and older. RNA interference was performed to evaluate a possible function for rpS3a

related to the arrested ovarian development during diapause. dsRNA injected into non-diapausing females suppressed ovarian development. When primary follicle sizes were measured 2 and 4 days after injection, the follicles were significantly smaller than those from controls of the same age and were similar to those of diapausing mosquitoes. Follicle sizes of non-diapausing females again increased 10 days after dsRNA injection, presumably a result of degeneration of the injected dsRNA. Decreased expression of rpS3a following dsRNA injection was confirmed by Northern blot hybridization. Topical application of juvenile hormone III, an endocrine trigger for diapause termination in this species, was used to stimulate ovarian development in the dsRNA injected non-diapause mosquitoes. Application of JHIII 4 days after dsRNA injection yielded an almost complete recovery from the RNAi effect. We propose that rpS3a is involved in the shut down of ovarian development that characters the adult diapause of Cx. pipiens.

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TRANSCRIPTIONAL EFFECTS OF LONG-TERM BACTERIAL CHALLENGES DURING LARVAL DEVELOPMENT IN MOSQUITO VECTORS OF HUMAN DISEASE

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Mosquitoes are able to mount an effective innate immune reaction in response to invasion by pathogens or parasites. Exposure of adult mosquitoes to a bacterial challenge results in profound transcriptional changes, leading to the activation of immune pathways and the production of anti-microbial factors. In several instances, the immune factors produced in response to bacterial infection are similar to those produced in response to infection with mosquito-borne agents of disease, suggesting that exposure to bacteria could influence vector competence in mosquitoes by stimulating their immune system. Immune reactions during larval development are less well understood. Preliminary data obtained in our laboratory shows that the exposure of mosquito larvae to high bacterial concentrations in the rearing water triggers a significant increase in the expression of immune-related transcripts. It is not known, however, whether these transcriptional changes would be lost during the pupal stage, or would be maintained in the gene expression profile of newly emerged adults. Interestingly, it has been recently demonstrated that the vector competence of adult mosquitoes is influenced by, among other factors, the environmental conditions prevailing during the insect's larval development. This has led us to hypothesize that it is possible to alter the expression profile of immune-related transcripts in newly emerged adult mosquitoes by manipulating the bacterial load of the larval rearing environment. To test this hypothesis, *Aedes aegypti* and *Anopheles gambiae* larvae were reared under semi-sterile (control) and high-bacterial load (either *Escherichia coli* or *Micrococcus luteus*) conditions. Total RNA was extracted from newly emerged adult females, and used to perform whole-transcriptome microarray analysis of gene expression. Significant differences in the gene expression profiles of the experimental and control groups are discussed.

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UNDERSTANDING TRANSMISSION OF CRYPTOSPORIDIOSIS IN THE UNITED STATES, 2007: MOLECULAR ANALYSIS OF SPORADIC *CRYPTOSPORIDIUM* ISOLATES WITH A CASE REPORT OF A HUMAN INFECTION WITH *CRYPTOSPORIDIUM* HORSE GENOTYPE

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At least 21 outbreaks of cryptosporidiosis were documented across the United States in the summer of 2007. To understand the transmission of non-outbreak-associated cryptosporidiosis in the U.S., 6, 10, 11, and 31, *Cryptosporidium*-positive specimens were collected in late 2007 from sporadic human cases in New Mexico, Iowa, Colorado and Idaho, respectively. These specimens were characterized at the species and subtype levels by PCR-RFLP analysis of the small subunit rRNA gene and DNA sequence analysis of the 60 kDa glycoprotein gene. The majority of infections (88%) were caused by *C. hominis*, with *C. parvum* being found in only six cases. Additionally one patient was infected with a new *Cryptosporidium*, the *Cryptosporidium* horse genotype, which has so far only been detected in one horse. The patient infected with the horse genotype had diarrhea for two weeks, visited an emergency room, and received nitazoxanide treatment. The patient cared for dogs, cats, rodents and rabbits in a pet store, but had no contact with horses. There was also no other known exposures to cryptosporidiosis. Altogether, five subtypes were found in the 51 *C. hominis*-positive specimens, with one of the subtypes, IaA28R4, being found in all study states and responsible for 40/51 (78%) of *C. hominis* cases. Although this subtype was only sporadically detected in the United States previously, it was responsible for two recreational water-associated outbreaks of cryptosporidiosis in 2007. This may indicate that these "sporadic" case-reports were actually part of larger multi-state outbreaks or that widespread secondary transmission occurred. To better understand the transmission of cryptosporidiosis in the United States, a national system to broadly characterize sporadic and outbreak-related *Cryptosporidium* isolates from humans and animals is needed.

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TEMPOROSPATIAL DETERMINANTS OF CRYPTOSPORIDIOSIS IN UGANDAN CHILDREN

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Transmission of cryptosporidiosis is believed to occur through two major pathways, one exclusively involving humans *(Cryptosporidium hominis*) and one involving humans and animals (*C. parvum).* Species identity is often used in epidemiological studies as a proxy for route of exposure. However, the need for molecular methods to differentiate between species often dictates that studies in resource poor areas be located in urban referral hospitals, where rural populations are underrepresented. Accordingly, we hypothesized that *C. hominis* and *C. parvum* may display unique temporal and spatial patterns, which are not revealed when data

is aggregated at the hospital level. GIS and time series techniques were applied to a large database of children attending Mulago Hospital in Kampala, Uganda (n = 2778, n with cryptosporidiosis = 476). We explored whether the prevalence of cryptosporidiosis and the distribution of each species was related to various factors including population density, land use activities and heavy rainfall. While *C. hominis* was the dominant species in all regions studied, considerable heterogeneity existed in the prevalence of cryptosporidiosis and in *Cryptosporidium* species by area of residence. Preliminary findings revealed that children living in the more rural, sparsely populated District of Luwero were 3.7 times more likely to be infected with *C. parvum* compared to children residing in the urban Kampala District (p=0.039). *C. hominis* and *C. parvum* did not display a high degree of correlation in time, suggesting that the environmental drivers for transmission may be asynchronous. This study highlights how molecular and environmental data can be combined to gain a more complete knowledge of cryptosporidiosis transmission in specific populations.

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SOURCES OF *TOXOPLASMA GONDII* INFECTION IN THE UNITED STATES

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Toxoplasmosis can cause severe ocular and neurological disease. We conducted a case-control study to determine risk factors and attributable risks for *T. gondii* infection in the United States. We administered a questionnaire from August 2002 through May 2007 to recently infected adults ≥18 years old selected from the Palo Alto Medical Foundation *Toxoplasma* Reference Laboratory case logs (*T. gondii* IgM and IgG antibody positive, low avidity), and to controls randomly selected from seronegative persons. Risk factors for recent *T. gondii* infection were determined using logistic regression analysis. Of 148 case-patients with recent *T. gondii* infection, 126 (85%) were female and 76 (51%) were pregnant; of the 413 controls, 412 provided gender, and of these, 394 (96%) were female and 301 (73%) pregnant. Elevated risk for recent *T. gondii* infection was associated with the following factors: eating undercooked or rare meat (OR 1.92, 95% confidence limits [CL] 1.16, 3.18; attributable risk [AR] 25%), eating raw meat (OR 2.12, 95% CL 1.06, 4.23; AR 13%), working with meat (OR 3.37, 95% CL 1.20, 9.49; AR 5%), eating raw oysters, clams, or mussels (OR 1.97, 95% CL 1.01, 3.86; AR 15%); drinking untreated water from a stream, lake, river, or pond (OR 3.44, 95% CL 1.21, 9.78; AR 8%); and having 3 or more kittens (OR 22.87, 95% CL 4.87, 107.39; AR 10%). Among pregnant women, drinking unpasteurized goat's milk elevated risk (OR 5.49, 95% CL 1.30, 23.25), and washing hands after handling raw meat was protective (always versus sometimes/never, OR 0.28, 95% CL 0.10, 0.81). Exposure to certain raw or undercooked foods, untreated water, and kittens were important risk factors for *T. gondii* infection. Pregnant women should be made aware of healthy food preparation practices, be advised against drinking untreated water, wash their hands thoroughly after potential contact with cat/kitten feces, and if possible, avoid handling litter boxes.

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URBAN FERAL PIGEONS (*COLUMBIA LIVIA*) AS A SOURCE FOR AIR-AND-WATERBORNE CONTAMINATION WITH *ENTEROCYTOZOON BIENEUSI* SPORES

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Enterocytozoon bieneusi-associated microsporidiosis, a severe disease for immunosuppressed people, can be due to environmental transmission of spores of zoonotic origin. Testing of water, excrements, and disturbed condition air samples from a long-lasting and weather-sustained aggregation site of urban feral pigeons (*Columbia livia*) for human-virulent microsporidia by the multiplexed fluorescence *in situ* hybridization (FISH) and PCR resulted in identification of *E. bieneusi*. The overall concentration of *E. bieneusi* spores in water and pigeon guano samples was 3.8 x 10^4/L, and 3.6 x 10^3/g, respectively, and the concentration of airborne spores tested while the site was swept for 30 min varied from 0.9 x 10^4 to 1.8 x 10^4/m^3. Pigeon guano contained the highest fraction of potentially viable spores (i.e., 85%), followed by water samples (i.e., 65%) and air samples, i.e., from 25 to 30%. The present study demonstrated that a person with 30 minutes of occupational or non-occupational exposure to pigeons, such as cleaning surfaces with pigeon excrement could inhale approximately 3.5 x 10^3 of *E. bieneusi* spores, and 1.3 x 10^3 of spores could be inhaled by a nearby person. Workers with potential occupational exposure to feral pigeons should be informed about zoonotic microsporidiosis risks and advised of appropriate ways of protection. Individuals conducting home renovations, demolitions, or community clean-up activities should also be targeted for education and risk management. Immunocompromised people who are at much higher risk of acquiring microsporidiosis from feral urban pigeons should limit or avoid occupational or casual contacts with these birds.

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REDUCTION OF CEREBRAL INFECTION AND MORTALITY, AND EFFECTS ON TRANSPLACENTAL TRANSMISSION OF *NEOSPORA CANINUM*, UPON IMMUNIZATION OF MICE WITH RECOMBINANT NCROP2 ANTIGEN-BASED VACCINES

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Rhoptry antigens are involved in a variety of cellular functions related to host cell invasion, formation of the parasitophorous vacuole and parasite-host cell interplay. The cDNA sequence of one of these antigens, NcROP2 was identified from *Neospora caninum* ESTs, amplified by RT-PCR, expressed in Escherichia coli as a (His)6-tagged recombinant protein (recNcROP2) and purified over Ni2+-affinity chromatography. Both, recNcROP2 and antibodies directed against recNcROP2 had a negative impact on *N. caninum* tachyzoite host cell invasion *in vitro*, indicating that this protein participates in the host cell entry process. Subsequently, the protective efficacy of NcROP2 as a potential vaccine candidate was evaluated in a C57BL/6 mouse cerebral disease model. Mice were vaccinated three times with 2 weeks intervals with recNcROP2 emulsified either in Freunds incomplete adjuvants (FIA) or saponin, and control groups were treated with adjuvants alone (adjuvants control) or PBS (infection control). Subsequently, mice were challenged with 2 x 106 *N. caninum* tachyzoites. Nine mice, all belonging to the infection control or adjuvants control groups, exhibited clinical signs of cerebral neosporosis and succumbed to infection, while no clinical signs were noted for recNcROP2-vaccinated mice. For all other animals, the experiment was terminated 35 days post infection. Cerebral parasite burdens were assessed by real-time PCR in all mice, and were revealed to be significantly

reduced in the recNcROP2 vaccinated mice. ELISA of sera revealed IgG1 to be elevated in recNcROP2-saponin vaccinated mice, while IgG2a was higher in recNcROP2-FIA vaccinated animals. This shows that, depending on the adjuvants used, vaccination with NcROP2 induces a protective Th-1- or Th-2-biased immune response against experimental *N. caninum* infection. Currently, NcROP2, is being evaluated in a fetal infection mouse model, in conjunction with other antigens such as MIC1 and MIC3, and results show that NcROP2-based vaccines significantly reduce the parasite burden and development of neosporosis in the offspring, demonstrating that vaccination can diminish fetal infection in mice.

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THIOUREIDES OF 2-(PHENOXYMETHYL) BENZOIC ACID 4-R SUBSTITUTED: A NOVEL CLASS OF ANTI-MICROBIAL AND ANTI-PARASITIC AND ANTIMICROBIAL COMPOUNDS

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Fifty members of a novel class of antimicrobial compounds, 2-(4-R-phenoxymethyl)benzoic acid thioureides, were synthesized and characterized with respect to their activities against a selection of gram-positive and gram-negative bacteria, the yeast *Candida albicans*, the extracellular and intracellular protozoan parasites *Giardia lamblia* and *Toxoplasma gondii*, respectively, and the larval (metacestode) stage of the tapeworm *Echinococcus multilocularis*. To determine the selective toxicity of these compounds,the human colon cancer cell line Caco2 and primary cultures of human foreskin fibroblasts (HFF) were also investigated. The new thioureides were obtained in three steps. In step 1, 2-phenoxymethylbenzoic acid derivatives 4-R substituted with a fluoro-, chloro- or methoxy-group were prepared by reacting phtalide with para- substituted potassium phenoxide. In the second step, 2-(4-R-phenoxymethyl)benzoic acid chloride was synthesized. Finally in step 3 this acid chloride was refluxed with ammonium thiocyanate, and the resulting 2-(4-R-phenoxymethyl)benzoyl isothiocyanate was treated with primary aromatic amines to obtain the new compounds, subsequently characterized by their physical constants (melting point, solubility). The chemical structures were elucidated by 1H-NMR, 13C-NMR, IR spectral methods and elemental analysis. The analyses confirmed the final and intermediate compounds structures. A number of thioureids exhibited moderate anti-microbial activities against methicillin-resistant *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Salmonella enteriditis* and *Candida albicans*. All thioureides, except two compounds with a nitro group, were totally ineffective against *Giardia lamblia*. The structural integrity of *E. multilocularis* metacestodes was significantly affected by 22 compounds, and 23 compounds inhibited the proliferation of *T. gondii*, three of them with an IC_{50} of approximately 1 µM. In contrast, HFF were not susceptible to any of these thioureides, while Caco2 cells were significantly affected by 17 compounds, two of them inhibiting proliferation with an IC_{50} in the micromolar range. Thioureides may thus present a promising class of anti-infective and anti-cancer agents.

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EVALUATION OF THE CYTOTOXICITY OF MULTIPLE AMPHIPATHIC ANTI-MICROBIAL PEPTIDE COMBINATIONS TO POTENTIAL BACTERIAL HOSTS AND *TRYPANOSOMA CRUZI*

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The "Paratransgenic" model for control of disease transmission has three distinct characteristics; a parasitic organism that causes disease via delivery to the human by an insect vector, the vector that harbors the parasite within its gut in close proximity to symbiotic or commensal bacteria able to express foreign gene products upon transformation, and gene products that are capable of eliminating the parasite or its transmission while exhibiting minimal effects on the transformed bacteria or insect vector. The parasite *Trypanasoma cruzi* is responsible for Chagas disease and its chief triatomine vector, *Rhodnius prolixus*, has a symbiotic relationship with the soil bacterium, *Rhodococcus rhodnii. R. rhodnii* that was genetically engineered previously to produce the anti-microbial peptide (AMP), Cecropin A was co-infected with *T. cruzi* into *R. prolixus* resulting in complete clearance of the infectious *T. cruzi* in 65% of the vectors. Similar AMP molecules have been isolated elsewhere and were studied in experiments reported here for differential toxicity against *T. cruzi* and *R. rhodnii.* Of the six AMPs tested initially, four of them; Apidaecin, Magainin, Melittin, and Cecropin A had toxicity profiles applicable to the Chagas paratransgenic system, killing *T. cruzi* at lower than 10 µM and requiring greater than 100 uM to show any effect on *R. rhodnii.* Subsequent treatments of *T. cruzi* with these peptides in pair-wise combinations resulted in improved killing efficiency, indicating that improvement of the 65% clearance seen in previous experiments may be possible utilizing multiple populations of transformed bacteria expressing different AMP species.

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LANDSCAPE GENETICS REVEALS FOCAL TRANSMISSION OF *ASCARIS LUMBRICOIDES*

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We combine molecular epidemiological and landscape genetic approaches to examine the transmission dynamics of the human parasitic roundworm *Ascaris lumbricoides* in Jiri, Nepal. Our goal is to determine if there are focal points of transmission and if so, what are the epidemiological factors that are correlated with these foci? We genotyped 1094 adult nematodes with 23 microsatellite markers. The worms were collected from 165 households (320 individual human hosts) in an area less than 20 square km. Bayesian genetic cluster methods make it possible to examine if there are distinct genetic groups of parasites within a host population (i.e., focal points of transmission). Landscape genetic analyses can then be used to test for correlations with epidemiological factors that may affect the distribution of genetic variation within and among these genetic clusters. Our analyses indicate that there is significant genetic structuring of parasite genotypes within this small sampling area. Several variables were examined for significant correlations with the observed genetic structure of the parasite. These variables included hosts, household, host age, host gender, host density, altitude, infection intensity, parasite gender, geographic distance, and time. Household explained over 60% of the variance in the distribution of *Ascaris* genetic variation within Jiri. Furthermore, households that were sampled 3 years apart showed no difference in the genetic composition of the parasites. These results highlight that a significant amount of transmission is concentrated around households and that this transmission is stable over time. We discuss the implications of these results for modeling dynamics and potential control strategies. This study illustrates how molecular analyses complement epidemiological information in providing a better understanding of parasite population biology.

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FACTORS AFFECTING THE FECUNDITY OF *ASCARIS LUMBRICOIDES* AND THEIR IMPACT ON PATTERNS OF DENSITY DEPENDENCE

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Density-dependent fecundity is a ubiquitous feature of intestinal nematode infections and has important implications for diagnosis, host-parasite system stability, transmission dynamics of the parasite population, and rates of reinfection after chemotherapy. Density dependence is usually incorporated into transmission models of *Ascaris lumbricoides*, by considering that female fecundity rate is a decreasing function of the number of worms per host (worm burden). A number of other factors, however, may influence worm fecundity and thus alter patterns and strength of density dependence with implications for helminth epidemiology and control. In this work we analyze data from the largest ever *A. lumbricoides* chemo-expulsion study, carried out between 1988 and 1989 in Dhaka, Bangladesh by Hall and co-workers. Pyrantel pamoate was administered to an initial cohort of 1,765 individuals and the expelled worms were collected, sexed, and weighed. The cohort was followed up and the procedure repeated after two six-month periods of re-infection. Statistical models show that both the mean weight of female worms and the age of the host harboring the worm population are significant determinants of worm fecundity. Furthermore, female weight declines with increasing worm burden (negative density dependence), the rate of which depends on host age. The interplay between these density-dependent constraints and host age accentuates the severity of density-dependent fecundity compared with results obtained by assuming that fecundity is a function of worm burden alone. These findings contribute to further our current understanding of *A. lumbricoides* population biology and have implications for the implementation, monitoring, and evaluation of control programs based on chemotherapy and targeted against soil-transmitted intestinal nematodes.

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TEMPORAL DYNAMICS OF THE SEX RATIO OF *ASCARIS LUMBRICOIDES* AND ITS IMPLICATIONS FOR TRANSMISSION

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The sex ratio of the worm population is an important determinant of the mating probability (the probability that female worms inhabiting a host will be mated) in transmission models of dioecious (separate sexes) macroparasites. Female-biased sex ratios in *Ascaris lumbricoides* infections of humans have been described in the helminth epidemiology literature, but few studies have determined whether the sex ratio is static or dynamic, or dependent on the structure of the parasite population (e.g. density dependent). To explore these questions we analyzed data obtained from the largest *A. lumbricoides* chemo-expulsion study, carried out by Hall and co-workers between 1988 and 1989 in Dhaka, Bangladesh. An initial cohort of 1,765 individuals was treated with pyrantel pamoate and the expelled worms were counted and sexed. The cohort was followed up and the procedure repeated after two six-month periods of re-infection. Statistical models were used to investigate the adult worm sex ratio across stratified samples of the collected worm populations. Our results suggest that the sex ratio in this *Ascaris* population can be described as temporally dynamic, and female-biased. The dynamics were investigated using a simple deterministic mathematical model and found to be adequately explained by a strong female bias in the sex ratio of establishing, incoming worms and a male longevity bias. The impact of these dynamics on the female mating probability is explored under a range of assumptions regarding the mating system (from complete monogamy to complete polygamy of male worms) and is compared with the mating probability given the ubiquitously assumed 1:1 sex ratio and complete polygamy of male worms. The findings of this work are discussed both in terms of the transmission dynamics and biology of *A. lumbricoides*. We propose to conduct similar analyses with other chemo-expulsion datasets in order to ascertain any geographical heterogeneity in sex ratio dynamics within the distributional range of *Ascaris*.

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EFFECT OF DEWORMING AND INTESTINAL HELMINTH (RE)INFECTIONS ON ATOPY AND ATOPIC DISEASE: LONGITUDINAL ANTHELMINTHIC TREATMENT STUDIES IN CUBAN SCHOOLCHILDREN

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Although helminth infections have been suggested to protect from atopy and atopic diseases, there is still no consensus on their relationship. We investigated the effect of deworming and intestinal helminth (re)infections on atopy, asthma, allergic rhinoconjunctivitis and atopic dermatitis. We examined 440 4-13 year-old Cuban schoolchildren in six-monthly intervals for 24 months. Intestinal helminth infections were diagnosed by stool examination. Atopic diseases were diagnosed by ISAAC (International Study of Asthma and Allergies in Childhood) questionnaire, asthma additionally by spirometry, and atopy by skin prick testing (SPT). After deworming the frequency of current wheeze ($p<0.001$) and allergic rhinoconjunctivitis ($p=0.015$) significantly decreased. The percentage of SPT positives temporarily increased from 9.7% (95% CI: 5.5 - 16.6%) to 32.7% (95% CI: 24.7 - 42.9%) ($p<0.001$) and subsequently returned to baseline values (11.9%, 95% CI: 6.9-19.6%). (Re)infection with *Ascaris lumbricoides* and *Trichiura trichiura* was positively and hookworm negatively associated with the development or retention of these atopic diseases, while for atopy an opposite trend was seen. In conclusion, our data indicate that atopic diseases improve after anthelminthic treatment. Atopy on the other hand increases after deworming. As this increase appears only temporarily, deworming of schoolchildren does not seem to be a risk factor for the development of atopy, nor for atopic disease. Effects of helminth (re)infections on atopy and atopic diseases appear to be species-specific.

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THE INTERPLAY BETWEEN HUMAN B CELLS, EOSINOPHILS AND HELMINTHS: A NOVEL ASPECT OF THE HYGIENE HYPOTHESIS

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The hygiene hypothesis is based on the premise that lack of exposure to helminths predisposes certain individuals to immune-mediated disease, such as inflammatory bowel disease (IBD). This hypothesis is supported by epidemiological data that shows developed countries with a low prevalence of helminth infections have higher incidence of allergic and inflammatory diseases. Helminths modulate the host immune response in a manner that dampens the exaggerated response to innocuous antigens, such as commensal bacteria. Suppression of inflammation via helminths has been linked to T regulatory cells and IL-10, although the mechanisms

remain poorly understood. In this communication, we provide another potential mechanism by which helminths modulate the gastrointestinal environment. IBD has its origins in a malfunctioning epithelial barrier where the host interfaces with commensal bacteria. We have found that B cells from IBD patients express high levels of surface Toll-like receptor 2 (TLR2) in both the blood and mucosal lesions and display a chronically activated phenotype. TLR2 expression is directly correlated with disease severity suggesting that these cells arise within the inflammatory milieu of active disease. Commensal microbes are a rich source of TLR2 ligands in the gut. TLR2-activation of IBD B cells induces production of chemo-attractants for neutrophils (IL-8) and eosinophils (eotaxin). We predict that neutrophils are recruited to clear any potential bacteria that have breeched the epithelial barrier. However, the role of eosinophils appeared enigmatic. Upon further investigation, we found that TLR2+ B cells express TLR4 in response to IL-4. Chemokine secretion was suppressed upon culture with helminth TLR4 ligands. In contrast, TLR4+ B cells did not respond to *E. coli* LPS, suggesting a unique host-parasite interaction between helminths and TLR4+ human B cells. We predict that eosinophils are recruited by B cells for a source of preformed IL-4 to aid in the upregulation of TLR4. Helminths provide immunosuppressive TLR4 ligands to help curb inflammatory B cells. Overall, these results suggest that gut inflammation is regulated in part through B cells, worms, and eosinophils.

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NEUTROPHIL RECRUITMENT TO SOLUBLE EXTRACT FROM *STRONGYLOIDES STERCORALIS* IS IL-17 INDEPENDENT

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Neutrophil recruitment via CXCR2 is required for innate and adaptive protective immunity in mice to the larvae of *Strongyloides stercoralis*. CXCR2 is the receptor for the murine orthologs of IL-8, MIP-2 and KC, and CXCR2 has also been shown to respond to parasite antigens. The goal of the present study was to determine whether CXCR2-mediated neutrophil recruitment to S. stercoralis was dependent on stimulation with host chemokines or parasite products. IL-17A and IL-17F have been well characterized as important upstream mediators of MIP-2 and KC production. When IL-17R KO mice were infected with *S. stercoralis* larvae there was no difference, as compared to wild type mice, in larval survival, neutrophil recruitment, or production of MIP-2 and KC. The possibility that larvae directly induce neutrophil recruitment was investigated in a series of *in vitro* assays. *S. stercoralis* soluble extract caused a significant neutrophil recruitment response, which was not diminished by Polymyxin B treatment to eliminate LPS. Zigmond-Hirsch checkerboard analysis found that both chemokinesis and chemotaxis were involved in the neutrophil recruitment to the extract. Characterization of the signaling pathway determined that it was a G protein coupled response involving tyrosine kinase and PI3K. Furthermore, inhibition of CXCR2 blocked the recruitment response, while inhibition of CXCR4 did not. Finally, it was demonstrated that stimulation of neutrophils with *S. stercoralis* soluble extract caused significantly increased production of the neutrophil recruiting chemokines MIP-2 and KC. Therefore, extract of *S. stercoralis* is capable of directly recruiting neutrophils through CXCR2. In addition the extract also induced neutrophils to release MIP-2 and KC which further enhanced the recruitment of neutrophils.

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DIFFERENTIAL GENE EXPRESSION BETWEEN INFECTIVE AND NON-INFECTIVE STAGE *STRONGYLOIDES STERCORALIS* LARVAE REVEALED BY MICROARRAY

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The biological differences between non-infective first stage (L1) and infective third stage larvae (L3i) of the parasitic nematode *Strongyloides stercoralis* (Ss) at a molecular level remain to be elucidated. Thus, microarrays were developed and utilized to explore the transcriptional differences between these two important *Strongyloides* stages. Three iterations of Ss microarrays were developed based on 3571 clusters identified from the 11335 expressed sequence tags (ESTs) obtained as part the Nematode EST project. Differentially labelled RNA obtained from Ss L3i and L1 was used to hybridize to multiple and optimized final versions (V3) of these microarrays. Average adjusted signals across multiple probes on the array were calculated using a mixed model ANOVA to adjust for differences between slides, arrays and amount of labeled material hybridized. Genes that were more highly expressed in either stage (based on a conservative cutoff of 0.3 $\log_2$ signal ratio L3/L1) were examined for differences in gene function. In a preliminary analysis, 879/3571 (25%) genes were more highly expressed in the L1 stage with 642/3571 (18%) genes in the L3i stage. When compared to previously analyzed EST data, inconsistencies were found between expression assessed by microarrays and that inferred from EST abundance data. Nevertheless, there was a concordance of the two methods in 449/879 L1-specific and 170/642 L3i-specific genes. Although 64% of L1- specific and 40% of L3i-specific genes were of unknown function, significant differences in gene expression were found between the two stages with higher numbers of genes involved in signal transduction ($P = 0.003$, Page-Z score test) and metabolism ($P = 0.005$, Page-Z score test) being found in the L1 stage. Some of the most abundantly expressed genes in the L1 encoded for NADH dehydrogenase and cytochrome B in contrast to the L3i where the most highly expressed included genes of unknown function. These data suggest that the L1 is more transcriptionally active than the L3i and the Ss microarray will be a powerful method to not only dissect molecular differences among different parasite stages but also to identify new (and abundant) targets for chemotherapy and vaccine development.

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ONE STEP FORWARD, TWO STEPS BACK? ASSESSING THE IMPACT OF A MISSED MDA CYCLE IN HAITI

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Lymphatic filariasis (LF) elimination programs face challenges in monitoring the impact of multiple rounds of mass drug administration (MDA) on transmission, particularly in settings where the regularity or quality of MDA may be affected by resource constraints. Annual mass treatment with diethylcarbamazine and albendazole began in Leogane, Haiti, in 2000 and has continued yearly, with the exception of 2006. In September 2007, as part of a multi-country research project designed to standardize and compare available diagnostic tools, we conducted surveys in two sentinel sites, Centre Ville and Masson Mathieu, in the Leogane area. Samples from each participant were examined for the presence of

microfilaremia, antigenemia, and antifilarial antibody (Bm14). Since 2000, there has been a significant decrease of antigenemia in both sites as measured by immunochromatographic card test (ICT). From 2000 to 2005, ICT prevalence had dropped from 48.6% to 23.2% (p<0.001) in Centre Ville and from 36.8% to 8.2% (p<0.001) in Masson Mathieu. Because of an interruption of funds in 2006, there was no MDA in Leogane. In 2007, approximately two years after the most recent MDA, ICT prevalence increased to 31.5% in Centre Ville and 14.1% in Masson Mathieu, representing a significant recrudescence of infection in both areas. Furthermore, a total of 18 of 102 (17.6%) children <6 years old were found to be ICT positive, suggesting recent LF transmission. The potential for ongoing transmission was supported by finding both Bm14 and microfilaria-positive children. These data suggest that missed MDA cycles can be damaging to LF elimination programs, and that five or more rounds of MDA may not be enough to successfully interrupt transmission in highly endemic settings.

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RATES OF MICROFILARIAL PRODUCTION BY *ONCHOCERCA VOLVULUS* ARE NOT CUMULATIVELY REDUCED BY MULTIPLE IVERMECTIN TREATMENTS

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Regular distribution of ivermectin reduces onchocerciasis transmission and morbidity by killing microfilariae (microfilaricidal effect). In addition, ivermectin exerts an embryostatic effect, by which microfilarial production by adult female worms becomes suppressed during a number of weeks after treatment. To assess the overall effect of ivermectin on onchocerciasis transmission and evaluate the likelihood of local elimination of the infection it is important to estimate the magnitude of the anti-fertility effect over the course of a treatment program. We estimated the effect of repeated drug treatments on the production of microfilariae by *Onchocerca volvulus* by developing a mathematical model that was fitted to data collected from three hyperendemic communities of the central onchocerciasis focus in Guatemala. Eligible residents had received ivermectin twice per year for two and a half years. The data consist of microfilarial load measurements in the skin, collected just before each six-monthly treatment during the program. The model that is developed describes the dynamics of an individual host's expected microfilarial load over the 30-month study period. We adopt a Bayesian hierarchical approach and use Markov chain Monte Carlo techniques to fit the model to the data. Combining estimates from the three villages, average microfilarial production in the first six months post-treatment was reduced by approximately 64% of its pre-treatment level, regardless of values chosen for the pre-ivermectin fertility rate within plausible ranges. Increased adult worm death rate after treatment (to mimic removal of macrofilariae via nodulectomy during the program) resulted in a smaller estimated magnitude of the embryostatic effect (rate of microfilarial production was reduced by 58% of pre-ivermectin value). After subsequent treatments, the rate of microfilarial production appeared to be similarly decreased. The data and analyses therefore do not support the hypothesis of a cumulative effect of multiple ivermectin treatments on microfilarial production by female worms.

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DYNAMICS OF *ONCHOCERCA VOLVULUS* MICROFILARIAL LOADS OF CAMEROONIAN PATIENTS SUBMITTED TO REPEATED (5 - 23) IVERMECTIN TREATMENTS OVER 14 YEARS (1994 - 2007)

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The main effects of ivermectin (IVM) on *Onchocerca volvulus* are a microfilaricidal effect, leading to a rapid decrease in the microfilarial loads, and a temporary blockage of the release of microfilariae (mf) by the adult worms. The mf loads re-increase slowly from 3 months after treatment. While reports from Ghana suggest that this re-increase is more rapid in patients who have received many IVM doses, the long term impact of repeated IVM treatments on the dynamics of mf loads is still unclear. To evaluate this, mf loads have been monitored in 1994, 1997 and 2007 in a cohort of Cameroonian subjects. They all had received either 4 annual or 13 3-monthly IVM doses between 1994 and 1997 (during a closely monitored trial), and were proposed the drug during the subsequent years as part of annual community-directed treatments with IVM (CDTI). In 2007, information was collected from each individual about the date of their last dose. Mf loads measured in 1994 (before the 1st dose), 1997 (one year after the 4th dose), and 2007 (10 months after the last round of CDTI) in a group of 32 patients treated annually between 1994 and 1997 and who had actually taken IVM in 2006, were 137.7, 15.2 and 15.4 mf/mg, respectively. Thirty-eight other individuals had been treated either annually or three-monthly between 1994 and 1997, and had not participated in the CDTIs organised in 2006; in these patients, the mf loads measured in 1994 and 2007 were 99.5 and 47.3 mf/mg, respectively. When extending the non compliance period to more than 3 years, the mean mf load in 2007 was 109.2 mf/mg (14 subjects). 105 skin-snip positive individuals from the 1994 cohort were retreated in 2007 after the parasitological examination, and then followed up at 15, 80 and 180 days after treatment. Mf loads recorded on D15 showed that the microfilaricidal effect of IVM was similar to that classically reported. However, the mf prevalence observed at D80 (41%) and D180 (77%) and the significant mf loads observed at D180 (13% of individuals present with >10mf/mg) reflect the persisting reproductive capacities of some adult worms. These observations suggest that despite 13 years of intervening treatments halting IVM treatment could result in a rapid return to the initial hyperendemic state. Embryograms and genotyping of parasites collected as part of this study are underway to assess whether the repopulation rates of skin by mf observed in some individuals reflects an emerging resistance of *O. volvulus* to IVM.

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PROGRESS TOWARD LYMPHATIC FILARIASIS (LF) ELIMINATION IN PLATEAU AND NASARAWA STATES, NIGERIA: INTEGRATED POPULATION-BASED PREVALENCE SURVEYS AFTER SIX YEARS MASS DRUG ADMINISTRATION

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Mass drug administration (MDA) with ivermectin and albendazole was initiated in the year 2000 to eliminate LF from Plateau and Nasarawa States, Nigeria. The program scaled up by 2002 and all 30 local government areas (LGAs) in the two states were receiving MDA. The national LF program has supported sentinel village surveillance to monitor the impact of MDA. Sentinel monitoring, while useful, is limited in the ability to generalize to a larger implementation unit such as an entire LGA. In an effort to integrate monitoring and evaluation of neglected tropical disease programs, we conducted a population-based prevalence survey of *Wuchereria bancrofti* antigen along with needed baseline surveys for blinding trachoma in 14 LGAs. A systematic sample of 20 census enumeration areas (EA) per LGA served as the primary sampling units. Households were randomly selected with equal probability of selection within the EA. All persons aged 2 years and older were eligible for examination of LF antigen using the NOW® Filariasis Immunochromatographic Card Test (ICT). Household residents of all ages were examined for clinical signs of trachoma using the WHO Simplified Grading System. A total of 18,502 persons were examined for LF. Overall antigen prevalence among the LGAs ranged from 0.106% to 14.01%. Antigen prevalence of all age groups was below 2.0% in 8/14 LGAs. Out of 3,365 children aged 2-6 years, 18 (0. 48%), had a positive ICT result (95%CI 0.23-0.73). No young children had positive ICT results in 4 LGAs. LGA estimates of prevalence of trachomatous inflammation follicular (WHO grade TF) among children aged 1-9 years ranged from 1.81% to 5.62%. 11581158115811LF antigen prevalence is below the threshold set by the Pacific Program to Eliminate LF (PacELF) for stopping MDA in 8 of the 14 evaluated LGAs. The results of this population-based survey are consistent with those of sentinel sites: both suggest potential interruption of LF transmission in some areas and ongoing transmission in focal villages of some LGAs. This raises questions concerning the applicability of MDA-stopping rules in Africa. District level surveys of LF antigen and trachoma can be conducted simultaneously.

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LONG TERM REDUCTION OF *WUCHERERIA BANCROFTI* TRANSMISSION IN PAPUA NEW GUINEA AFTER CESSATION OF MASS DRUG ADMINISTRATION

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Four annual cycles of Mass anti-filarial Drug Administration (MDA) given in 1994-1997 dramatically reduced human infection rates and nearly interrupted mosquito-borne transmission of *Wuchereria bancrofti* in an endemic area of Papua New Guinea , as described previously. We conducted entomological studies in 2003, 2004 and 2007 to determine whether there had been a resurgence in transmission by the Anopheles punctulatus complex, the major vector of *W. bancrofti*. Pre-MDA (1993-4) annual vector infection, i.e. any filarial stage, and infective rates, i.e. infective third-stage larvae, determined by dissection of individual mosquitoes were 7.5% and 1.8%, respectively. Infection and infective rates decreased significantly to 1.9% and 0.5% following the first two annual rounds of MDA. Surveys conducted in 2003-4 in a village where transmission was almost interrupted did not produce any mosquitoes harboring infective larvae among 764 specimens that were individually dissected. One infected mosquito was observed. PCR pool assays that enable detection of low numbers of infected mosquitoes without regard to their developmental stage were performed for 563 *Anopheles punctulatus* collected in 2004 during high transmission months. We observed a minimum infection rate of 0.7% in the PCR pool assay compared to an infection rate of 0.8% determined by dissection for the same months in 1996. In 2007, 10 years after MDA was stopped, we initiated collection of mosquitoes again. Aggregate infection and infective rates were 1.2% and 0.3%, similar to those observed during MDA and one year after treatment ceased. We conclude that MDA alone can interrupt transmission of *An. punctulatus*-transmitted filariasis in this area of Papua New Guinea, and that fewer than 5 annual rounds of MDA may be sufficient to achieve this.

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PROGRESS TOWARDS ELIMINATION OF ONCHOCERCIASIS AS PUBLIC HEALTH PROBLEM IN PROBLEMATIC AREAS IN WEST AFRICA

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Although the Onchocerciasis Control Program (1975-2002) has successfully eliminated onchocerciasis as a public health problem in large parts of West Africa, the situation remained problematic in four river basins. These basins required continued control measures beyond 2002 and they were designated as special intervention zones (SIZs). To improve our understanding of the epidemiology of onchocerciasis and of the barriers to successful control, we analyzed the trends in epidemiological and entomological indicators of onchocerciasis transmission in the SIZs. The SIZs include the Oti basin in Togo/Benin, the Pru basin in Ghana, and the Upper Niger/Mafou and Tinkisso basins in Guinea-Conakry. We examined the trends in epidemiological parameters (microfilariae (mf) prevalence, community microfilarial load), entomological parameters (annual biting rate, annual transmission potential) and treatment coverage, in each of these basins from the start of OCP until present. Control activities started in 1977 in the Oti basin, while they started only in the late eighties in the other SIZs. The 4 SIZs were meso- to hyperendemic before the start of control, with village mf prevalence levels of 60-80%. All SIZs still had significant ongoing transmission at the closure of OCP in 2002. The situation was most problematic in the Oti basin, where mf prevalence levels >40% were still observed in 2002. The situation had strongly improved by 2007. Prevalence levels were far below 10% in the Guinean basins and levels >20% were no longer observed in the Pru and Oti basins. In cobclusion, unfavorable transmission conditions, incomplete vector control, poor treatment coverage, human migration, and invasion of flies from uncontrolled areas may all have hampered the control of onchocerciasis in the SIZs. Other control programs, like the African Program for Onchocerciasis Control and the Global Program for Elimination of Lymphatic Filariasis, should anticipate that some areas require more intensive or longer continuation of control than others.

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ONCHOCERCIASIS ELIMINATION IN AFRICA: THE POSSIBILITY OF SUCCESS IN AN ISOLATED FOCUS IN SUDAN

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Sudan is a country that has a long history of onchocerciasis, and despite many efforts to control the disease over the past fifteen years areas of severe disease remain. There are a number of identifiable endemic foci in both the south and north of the country, some large and with cross border characteristics. Despite the difficulty of implementing Mectizan® (ivermectin) disease control programmes in many of these foci due to geographic and civil disturbance issues, one focus in the north of the country, Abu Hamad, is possibly now approaching *Onchocerca volvulus* free status. It is an isolated focus on the Nile River in northern Sudan where some 100,000 people live in villages along the sides for around 150 kilometers of the river. They have received symptomatic treatment for onchocerciasis with anti-filarial drugs for over 30 years and then ivermectin use on a community wide distribution basis for the past ten years. The levels of skin positivity in the population have changed dramatically since the last detailed recorded study in 1982 when as high as 37.5% of the population was positive for microfilariae in skin snips. In an investigation made in mid 2007, three villages in the same vicinity as those studied in the original study were surveyed and were all found to free of skin snip positivity. The number of nodules present in residents was also very much lower than that seen in previous years; however, cases of acute oncho-dermatitis (3-5% of the population) were identified suggesting that low levels of microfilaria are still present in a small proportion of the population. Of clinical significance was the definition of distinct dermal reactions to the *Simulium* vector present in this area - this dermal condition can be confused by local personnel as being due to the parasite, and thus presents a challenge to the elimination efforts and ultimate perceptions of programmatic success. If elimination is achieved in Abu Hamad in the near future this will provide considerable impetus to the African program for onchocerciasis and its final goals.

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VISUALIZATION OF MONKEYPOX VIRUS PATHOGENESIS BY *IN VIVO* IMAGING

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Monkeypox viruses cause human monkeypox, a zoonotic smallpox-like disease with significant public health importance. It is also a potential bioweapon (Category A) that can be transmitted by the airborne route. The virus circulates enzootically in the rainforests of Central and Western Africa and is occasionally transmitted to humans. In 2003 monkeypox emerged for the first time in the Western Hemisphere and caused an outbreak in the Midwestern states affecting 37 people exposed to ill prairie dogs purchased from pet stores. The virus entered the US upon the importation of exotic rodents from Ghana (West Africa). It is now recognized the existence of two distinct genetic clades that exhibit different virulence characteristics and slightly different genome. We have constructed recombinant viruses expressing the luciferase gene that can be use to monitor viral infection *in vivo* using biophotonic *in vivo* imaging. Using these viruses, we have characterized MPXV infection in several animal models including laboratory mice and prairie dogs. Our studies revealed that Balb/C mice became infected with MPXV but did not succumb to the disease and cleared the infection within 10 days post-infection (PI). However, severe combined immune deficient (SCID) Balb/c mice were highly susceptible to MPXV, with infection resulting in 100% lethality. In vivo imaging studies using the Luc+-expressing virus in SCID Balb/c mice showed that luminescence, indicative of MPXV infection, was visible in the abdominal region as early as 24 hours post IP injection and subsequently spread to other parts of the body. Surprisingly, the highest viral titers (~10e5 PFU) were found in the ovaries and this was also evident in the imaging studies. Immunohistochemical staining of organs, revealed the presence of MPXV antigen in ovaries, intestines and skin. For black-tailed prairie dogs (C. ludovicianus), following intranasal (IN) infection luminescence signal indicative of viral replication was visualized in the nares as early as day 1 PI in infected animals. Viral infection was then detected in the lymph nodes a few days later, and then progressed to the skin where pox lesions ultimately developed. Sentinel animals showed evidence of infection by day 9 PI. These studies provide a better understanding of the virulence of MPXV strains, their pathogenesis in mice and prairie dogs, an index of their transmission potential to humans and other animals.

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IDENTIFICATION AND RELATIVE ABUNDANCE OF SMALL RNAS IN ALPHAVIRUS INFECTED MOSQUITOES

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RNA interference (RNAi) is a mechanism triggered by dsRNA resulting in a sequence specific silencing of gene expression. In cells, dsRNA is recognized and cleaved into 21-28 nucleotide small dsRNA products. Small RNAs found in somatic cells include microRNAs (miRNAs) and small interfering RNAs (siRNAs). miRNAs are derived from the cleavage of endogenous dsRNA and regulate host gene expression. siRNAs are derived from the cleavage of exogenous dsRNA, such as viral dsRNA intermediates, and in invertebrates function as the sequence-specific mediators of an antiviral RNAi pathway. While there is evidence to support a functional antiviral RNAi response in mosquitoes, it remains unclear if siRNAs affect the ability of mosquitoes to vector human diseases caused by arthropod-borne viruses (arboviruses). To begin to address the question of how intensely arboviruses are targeted by the RNAi pathway, we used high-throughput sequencing to obtain the identity and relative abundance of small RNAs in alphavirus infected mosquitoes. In brief, mosquitoes were injected with an alphavirus. After an incubation period, total RNA was size fractionated and small RNAs (18-30nts) were isolated. RNA adapters of known sequence were ligated to the 5' and 3'ends of the small RNAs. This was followed by RT-PCR amplification. Reverse transcribed and amplified products were then sequenced on an Illumina genome analyzer. Bioinformatic analysis provided the relative abundance and strand specific genome-wide distribution of viral siRNAs. Results indicate that the RNAi response targets the entire viral genome; however, some regions are more highly targeted than others.

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ALPHAVIRUS DERIVED SMALL RNAS MODULATE PATHOGENESIS IN DISEASE VECTOR MOSQUITOES

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Mosquito-borne viruses cause significant levels of morbidity and mortality in humans and domesticated animals. Maintenance of mosquito-borne viruses in nature requires a biological transmission cycle that involves alternating virus replication in a susceptible vertebrate and mosquito host. While the vertebrate infection is acute and often associated with disease, continual transmission of these viruses in nature depends on the establishment of a persistent, non-pathogenic infection in the mosquito vector. An antiviral RNAi response has been shown to limit the replication of RNA viruses in flies. However, the importance of the RNAi pathway as an antiviral defense in mammals is unclear. Differences in the immune responses of mammals and mosquitoes may explain why these viruses are not generally associated with pathology in the invertebrate host.

siRNA profiling of *Aedes aegypti* infected with a replicating alphavirus, an important group of mosquito-borne pathogens, indicated that > 11% of the total siRNAs present were derived from double stranded RNA (dsRNA) of viral origin. To investigate whether the inhibitory effects of the mosquito's antiviral RNAi response on the replication of RNA viruses is sufficient to control the pathogenic potential of an arbovirus in this host, we engineered two members of the alphavirus genus to express suppressors of RNA silencing (SRS). Recombinant alphaviruses expressing each SRS effectively suppressed a dsRNA triggered siRNA response and became highly pathogenic to the mosquito host. Our results suggest that the siRNA pathway is essential to the survival of mosquitoes infected with alphaviruses, and thus the maintenance of these viruses in nature.

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TEMPORAL PATTERNS OF ROTAVIRUS GENOTYPE VARIATION IN RURAL, NORTHERN ECUADOR

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Rotavirus is the most common cause of acute gastroenteritis among infants and young children throughout the world, but developing countries account for nearly all of the ~600,000 annual deaths attributable to rotavirus. We studied the epidemiology of rotavirus in 22 rural communities in northern coastal Ecuador over a five year period using a serial case control design. Each community was visited approximately every eight months for a 15 day period, for a total study population of ~5000. In five years, 839 stool samples were collected from symptomatic residents and 2308 stool samples were collected from healthy controls. Rotavirus antigen was detected in 22% of the cases and 3% of the controls. Although the prevalence of asymptomatic rotavirus was relatively constant across age groups, in the 15-20 year old age group, we detected significantly higher prevalence among women than men [8.6% vs. 1.1%, odds ratio = 8.3, 95% C.I. = 1.0-398.0, p = 0.043], suggestive of child-care associated transmission. The same trend was observed in other age groups as well. For genotyping of the VP4 and VP7 genes, RNA was purified from all 250 rotavirus positive stool samples. In 2005, the prevalence of genotype G9 spiked dramatically, constituting 75% of the samples, but in later years declined to less than 10%. Curiously, the decline of G9 rotavirus was concomitant with a dramatic rise in the prevalence of untypeable samples. 61% of the rotavirus positive samples could not be genotyped for VP4 or VP7. The possibility of sample degradation was considered but discounted after an experimental examination of rotavirus stability and the visualization by electron microscopy of rotavirus-like particles in several untypeable samples. Finally, a novel strain, which had been initially untypeable, was characterized for the VP4, VP6, and VP7 gene segments. Sequence analysis showed that primer sequence mismatch was the cause of genotyping failure. The diversity of rotavirus genotypes has important implications for current and future genotyping schemes and the vaccine programs that they inform.

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MOLECULAR EVOLUTION OF CHIKUNGUNYA VIRUS IN WEST AFRICA AND EPIDEMIOLOGICAL IMPLICATIONS

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Chikungunya fever (CHIKF) is an arboviral disease transmitted by *Aedes* mosquitoes. Infection in humans leads to intenses arthralgia that can last for several months. Chikungunya virus (CHIKV) is a member of the togaviridae family, genus alphavirus. Over the last 3 years, Chikungunya have been responsible of major outbreaks in Asia and Africa primarily in Indian Ocean namely Reunion Island. In Senegal, Chikungunya, has been isolated in 1962and since then has reemerged in 1966, 1982, 1996-97, and more recently 2004-2006. Although, CHIKV is endemic in West Africa, very limited data are available for its genetic varability. Therefore, in order to further understand its dispersal in Africa and within countries, we analyzed sequences of complete E1 coding region of 41 isolates collected over 45 years from various hosts species and west African countries with special emphasis on Senegal. Results obtained showed that 2 distinct lineages of CHIKV circulate in West Africa: one which is specific of the region and a second "cosmopolite" which includes isolates from East Africa. Such an observation led to hypothesize and discusses exchange between east and west Africa. In addition sequences of E1 revealed that mutation of Alanine at 226 of E1 to Valine - which is believed to be a critical factor of emergence of CHIKV in Reunion Island - is not present in isolates collected in West Africa. Altogether, the results showed that although CHIKV isolates from East and West Africa are related, the epidemiology of the virus in the 2 areas appears to be different. Such differences are analyzed to gain further insights on the emergence factors of CHIKV.

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DOUBLE INTRODUCTION OF HIGHLY PATHOGENIC AVIAN INFLUENZA H5N1 IN GHANA IN 2007

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In 2006, the first highly pathogenic avian influenza (HPAI) subtype H5N1 in Africa was detected in Nigeria. The reported cases were followed by several outbreaks in other countries later during the same year. In Nigeria, there have been multiple introductions of more than one lineage. Here, we report a similar scenario for multiple introductions of HPAI H5N1 in Ghana. Twelve cloacal swab samples were collected in April and May 2007, from domestic chicken and migratory birds from Accra, the capital and from Tema, a port city 35km east of Accra. Samples were tested at the Noguchi Memorial Institute for Medical Research for HPAI H5N1 by real time PCR using gene specific primer/probe sets. Samples that were H5N1 positive were shipped under IATA regulations to NAMRU-3, a WHO Reference Center for Avian influenza (AI) in Cairo, Egypt for confirmation of results and genetic characterization. Sequencing of the HA and NA genes was performed and phylogenetic analysis was conducted. All twelve samples were confirmed positive for AI H5N1 using real time PCR assays. Sequence analysis of the HA and NA genes from these samples indicated characteristic highly pathogenic avian influenza (HPAI) with the multibasic amino acid motif at the HA cleavage site. HPAI H5N1 from Ghana belongs to the Clade 2.2, Qinghai-like viruses and formed two distinctive clusters. Strains collected from Tema clustered with viruses first characterized in Nigeria (similar to GenBank accession #AM503002), while the strains collected from Accra clustered with H5N1 strains initially described in Cote d'Ivoire (similar to GenBank accession #CY020693). The Accra cluster HA sequence data shows specific genetic signature which is commonly seen in low pathogenic H5 avian influenza from migratory bird strains. In conclusion, phylogenetic analysis of sequence data of these H5N1viruses from Ghana indicate a nearly simultaneous introduction of two HPAI H5N1 strains described in Nigeria and Cote d'Ivoire. Whether the origin of these parent strains was from migratory birds or bird trade is unknown. We hypothesize that the existence of variable strains in the same geographic region potentially provides an ideal environment for virus reassortment.

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CASE FATALITY OF SEVERE ACUTE RESPIRATORY SYNDROME (SARS) IN MAINLAND CHINA AND ASSOCIATED RISK FACTORS

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This study was undertaken to analyse the case fatality ratio (CFR) and its risk factors for Severe Acute Respiratory Syndrome (SARS) in mainland China by using a comprehensive dataset of all probable cases. The data of all probable SARS cases was derived from the Infectious Disease Reporting System of the Center of Diseases Control and Hospital Information Systems, during the 2003 epidemic in mainland China. The definition of probable SARS case was consistent with the definition for clinically confirmed SARS issued by the Ministry of Health of the People's Republic of China. We performed univariate and multivariate logistic regression analysis to determine the association of CFR with age, sex, residence location, occupation, the period of the epidemic, and the duration from symptom onset to admission into hospital. he overall CFR was 6.4% among 5327 probable SARS cases in mainland China. Old age, being a patient during the early period of a local outbreak, and being from Tianjin led to a relatively higher CFR compared to young age, late stage of a local outbreak and cases from Beijing. Guangdong province resulted in an even lower CFR compared to Beijing. In conclusion, the deteriorated health status and apparent complications of SARS patients with relatively old age (> 60 years) has caused a much higher risk of dying than for younger patients. In the early stage of local outbreaks, lack of experience in patient care and perhaps treatment has also lead to a relatively higher CFR. The Tianjin SARS outbreak happened mainly within a hospital, leading to a high impact of co-morbidity. The relatively young age of the cases partly explains the low CFR in mainland China compared to other countries and areas affected by SARS.

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HIGH HYBRIDIZATION RATE BETWEEN *ANOPHELES GAMBIAE* MOLECULAR FORMS AT THE WESTERN EXTREME OF THEIR RANGE HIGHLIGHTS POSSIBLE GENE-FLOW IN THE X-CHROMOSOME "SPECIATION ISLAND"

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Anopheles gambiae M and S molecular forms are considered to be incipient species: in fact, only 6 M/S putative hybrids (i.e. showing a M/S rDNA-IGS-RFLP pattern) have been reported so far out of almost 7,000 *A. gambiae* s.s. from north-west Africa and none from central-west Africa (N>10,000). High genetic differentiation between M and S have been shown to be restricted to three genomic "speciation islands". One of these is the pericentromeric region of the X-chromosome, where reduced recombination and natural selection have been suggested to have contributed to the accumulation of alleles of genes involved in reproductive isolation between the two forms. We here report an unusually high degree of hybridisation between M and S forms at the western extreme of their distribution range, where no previous data were available. In fact, we recorded 35 M/S hybrid specimens out of almost 2.000 *A. gambiae* s.s. collected in The Gambia (M/S freq=0.6%-7%, in sympatric sites, with N>100) and 37/179 (20.7%) in Bissau city (Guinea Bissau, about 200 km southwards). Quite intriguingly, we also have evidence of possible gene-flow within the X-pericentromeric region between the two molecular forms in the study area. This is shown by the preliminary analysis of M/S hybrids by a novel approach based on the study of a Short Interspersed Transposable Element (SINE200) at a single locus, which we found to be consistently specific of the M-form in other geographic areas. This SINE200 locus maps about 1 Mb from the IGS-rDNA region on which the M and S-form identification is based. Unexpectedly, both IGS-RFLP and SINE200 approaches provided only partially consistent results, possibly suggesting a higher-than-expected recombination between the two markers in the X-pericentromeric area. Does selection play a role in determining the observed pattern? Are we observing a phenomenon peculiar of the study area, where the two forms hybridise more frequently? These issues will be discussed also in relation to the further analysis of laboratory offspring originated from crosses between M/S specimens.

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ECOLOGICAL DIVERGENCE AND REPRODUCTIVE ISOLATION ALONG AN URBANIZATION GRADIENT: HABITAT SEGREGATION OF *ANOPHELES GAMBIAE* MOLECULAR FORMS IN A FOREST AREA OF CAMEROON

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Consequences of human activity on the biosphere include loss of biodiversity, alteration of ecological communities, and biological invasions. However, an overlooked effect of the impact of humans on the environment is the creation of new species. Anthropogenic habitat disturbance of primeval landscapes generates contrasting ecological settings where divergent selection for alternative eco-phenotypes can promote the evolution of reproductive isolation and speciation. We will present an example of how urbanization in the Central African rain forest is driving a process of ecological segregation and incipient speciation between two cryptic taxa of the major Afro-tropical malaria vector *Anopheles gambiae*. The molecular forms M and S of this mosquito, distinguishable by fixed nucleotide sequence differences in the inter-genic spacer of ribosomal DNA, sharply segregate along an urbanization cline at a geographical scale of a few kilometres. The molecular form M occurs exclusively in the most human-disturbed habitats where it can breed in polluted sites associated to waste waters, whereas the S form is found in rural settings, where it breeds in rain-dependent water puddles on bare soil. In the metropolitan area of Yaoundé, populations of the two forms come in contact along a narrow peripheral zone. Hybrids have never been found in strictly sympatric natural populations. An index of urbanization based on remotely-sensed data was used as a predictor of the probability of occurrence of the M form in 306 randomly-chosen georeferenced localities (57 positive, 249 negative) across the forest of Cameroon. The binary logistic regression model was statistically significant (P<0.001, area under the ROC curve=0.72), and correctly predicted M occurrence in about 83% of the sampled localities. With a threshold of 50%, the model was highly specific (2% false positives), but moderately sensitive (21% true positives), indicating that either the model did not capture a significant portion of the underlying explanatory variables, and/or the M form can occur at lower densities in less suitable habitat. The ongoing adaptation of the M form to a new ecological niche is of epidemiological

significance, as it may lead in the future to increased malaria transmission in urban settings, where levels of immunological premunition by the human population are currently in equilibrium with lower rates of parasite exposure.

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A TEP1 MEDIATED RESPONSE IS REQUIRED BUT NOT SUFFICIENT FOR MELANIZATION OF *PLASMODIUM FALCIPARUM* IN THE *ANOPHELES GAMBIAE* MIDGUT

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It is known that the survival of the *Plasmodium* parasite in its mosquito vector is in part determined by the innate immune response of the mosquito. A powerful tool to identify mosquito genes that mediate killing of Plasmodium are mosquito strains refractory to the parasite. An *Anopheles gambiae* laboratory strain (L3-5), selected from an African mosquito line to be refractory to *P. cynomolgi*, was used to study the nature of the innate immune response of the mosquito against *P. falciparum*. The L3-5 strain was found to be susceptible to African *P. falciparum* but it was highly refractory to a *P. falciparum* strain from Brazil, melanizing 98% of the parasites in the mosquito midgut. Using dsRNA mediated gene knockdown was found that *P. falciparum* killing and melanization in *An. gambiae* L3-5 requires TEP1. In order to test whether activation of the TEP1 mediated pathway is sufficient to kill different *P. falciparum* strains, a coinfection of *P. falciparum* strains was done in the L3-5 mosquito. Coinfection of *P. falciparum* from Africa and from Brazil gave rise to a mixed phenotype with live oocysts and melanized parasites in each of the mosquito midguts analyzed. There was no indication of any of the two parasite phenotypes predominating over the other, indicating that even when the mosquito immune system is activated and is able to melanize parasites through TEP1, this is not sufficient to kill different invading Plasmodium lines. This suggests that besides activation of the immune response of the mosquito, there are Plasmodium factors that determine whether the parasite is susceptible or not to the mosquito defenses.

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LARVAL ANOPHELINE MOSQUITO RECTA EXHIBIT A DRAMATIC CHANGE IN ION TRANSPORT PROTEINS IN RESPONSE TO SHIFTING SALINITY

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Mosquito larvae live in dynamic aqueous environments which can fluctuate drastically in salinity due to ecological events such as rainfall and evaporation. Larval survival depends on the ability of tissues such as the rectum to regulate hemolymph osmolarity by absorbing and excreting ions. The recta of several culicine genera (including Aedes and Culex) have been studied in detail, but very little is known about the recta of anopheline larvae. Here we report several lines of evidence which suggest anopheline larvae differ from culicine larvae both in rectal structure and regulation of protein expression. Whereas obligate fresh-water and saline-tolerant culicines have structurally distinct recta, immunolocalization patterns of carbonic anhydrase and Na+K+-ATPase reveal that all anophelines examined (regardless of saline tolerance) have structurally similar recta composed of distinct DAR (dorsal anterior rectal) cells and non-DAR cells. In larvae reared in fresh water, carbonic anhydrase localizes to the cytoplasm of DAR cells and Na+K+-ATPase localizes to the basal membrane of non-DAR cells. Additionally, saline-tolerant anopheline larvae undergo a dramatic shift in rectal Na+K+-ATPase protein localization from the non-DAR cells to DAR cells when reared in saline water compared to those reared in fresh water. A similar shift in protein localization is not seen in any culicine larvae examined. We also report preliminary physiological data obtained using a self referencing potassium selective electrode which suggests a change in potassium flux in the non-DAR cells of Anopheles albimanus larvae reared in fresh versus saline water. From these data we suggest that saline-tolerant anopheline larvae adapt to saline water in a distinctive way by shifting ion regulatory proteins such as Na+K+-ATPase to alter the primary function of specific rectal cells. This likely changes the overall regulatory functionality of the tissue from adsorption to secretion of specific solutes.

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FUNCTIONAL CHARACTERIZATION OF A PLATELET AGGREGATION INHIBITOR FROM THE SALIVARY GLANDS OF *AEDES AEGYPTI*

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A complex repertoire of pharmacologically active molecules in blood feeding arthropod saliva is responsible for modulating host hemostasis, immune defenses, pain/itch, and wound healing, which facilitates blood feeding and pathogen transmission. Genomic strategies yielded previously unobtainable insights into the nature and diversity of salivary gland molecules. Understanding the function(s) of these molecules is vital to development of novel vector and disease transmission control strategies. Salivary glands of *Aedes aegypti* contain a Kazal serine protease inhibitor (AeKSPI) that inhibits fibrinogen mediated platelet aggregation. AeKSPI is a 7kDa protein containing a single Kazal domain with 6 cysteine residues forming three disulfide bonds, and shares high sequence homology to a potent thrombin inhibitor from the triatomine bug, *Dipetalogaster maximus*. Recombinant AeKSPI (rAeKSPI) specifically inhibits trypsin; is less inhibitory of α-thrombin; and, lacks activity against γ-thrombin. Mass spectrometric analysis of the protein bands obtained from a quartz crystal microbalance capture strategy and His-tag pull down experiments revealed that recombinant AeKSPI binds to the γ domain of human fibrinogen. Fibrinogen γ domain recognizes sequences for the platelet receptor GPIIa-IIIb, which mediates aggregation. We observed dose dependant inhibition of fibrinogen mediated platelet aggregation and adhesion by rAeKSPI. Far-Western blots revealed the mechanism of inhibition as the binding of rAeKSPI to fibrinogen regions that recognize GPIIa-IIIb. Results suggest that AeKSPI is a platelet aggregation inhibitory protein contributing to successful blood feeding by *Ae. aegypti*.

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SURVIVAL AND REPLICATION OF *WOLBACHIA PIPIENTIS* IN *ANOPHELES GAMBIAE*

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Cytoplasmic incompatibility (CI), induced by Wolbachia endosymbionts is of extreme interest to control vector insects for disease control. The mosquito Anopheles gambiae is the primary vector of Plasmodium parasites in Africa. No naturally occurring Wolbachia infections have ever been identified in Anopheles mosquitoes. Artificial horizontal transfer of Wolbachia not yet succeeded in Anopheles mosquitoes, although Wolbachia can infect cultured Anopheles cells in vitro. To assess the ability of Wolbachia to colonize Anopheles cells in vivo, we purified

the virulent Wolbachia strain wMelPop from infected cell cultures and injected purified symbionts into female adult Anopheles gambiae. Semi-quantitative PCR indicated that Wolbachia are able to replicate in injected mosquitoes, suggesting that injected symbionts survive and are able to infect mosquito tissues. Tissue distribution of Wolbachia in injected mosquitoes was investigated using fluorescence in situ hybridization and immunofluorescent microscopy. The virulent effect of wMelPop was investigated using life-table analysis.

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THE ROLE OF SERPINS IN MELANIZATION AND TOLL IMMUNE PATHWAY IN THE MOSQUITO, *AEDES AEGYPTI*

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The melanization reaction and the Toll immune pathway are insect defense reactions employed against microbial and parasite infections. One common character in both immune responses is that signaling is mediated by a clip-domain serine protease (SP) cascade. A group of proteins, serine protease inhibitors (serpins), are required to regulate and restrict SP function. Knock-down (KD) of Serpin-2 caused massive melanotic tumors; however, it had no effect against the avian malaria parasite, Plasmodium gallinaceum. Furthermore, we found that CLIP-B5, a mosquito orthologue of Drosophila Easter, activates the Toll immune response in the mosquito, *Aedes aegypti*. In *Drosophila*, Spn27A is a counterpart of Drosophila Easter in the SP cascade of Toll embryonic pathway. However, Serpin-2, an *Aedes* orthologue of Drosophila Spn27A and Manduca Serpin-3, interacts with ClipB29 but not with Easter. We found that the Serpin-2/CLIPB29 cassette is involved in a minor activation of Toll immune pathway. But, double KDs of Serpin-2 and CLIPB29 were not able to dismiss the melanotic tumors resulting from Serpin-2 KD. This suggests the role of other mosquito serpins in the melanization reaction and the Toll immune pathway. As a first step to figure out the roles of Serpins, we tested the expression profiles of immune-inducible Serpins. We will compare the KD phenotypes of all immune Serpins in female mosquitoes. We will address this further to elucidate the specific SPs interacting with the serpins and the immune function of each SP-Serpin cassette involved against parasitic and microbial infections.

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CLINICAL DEVELOPMENT OF THE *NA*-ASP-2 HOOKWORM VACCINE IN PREVIOUSLY-INFECTED BRAZILIAN ADULTS

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Necator americanus secretory protein-2 (*Na*-ASP-2) is thought to play a role in the development of hookworm larvae in the host. Vaccination of laboratory animals with *Na*-ASP-2 induces partial protection against challenge with hookworm larvae, and anti-ASP-2 antibodies in naturally-infected humans are associated with reduced infection intensity. Recombinant *Na*-ASP-2 formulated with Alhydrogel did not result in significant vaccine-related adverse events in a Phase 1 study in hookworm unexposed adults living in the US and induced significant levels of antigen-specific IgG1 but minimal IgG4 and IgE. However, upon initiation of a Phase 1 study in previously infected healthy Brazilian adults in an area of high *Necator* transmission, generalized urticarial reactions were observed within 2 hours of injection with 10 µg *Na*-ASP-2 in 3 of 7 volunteers. No further significant vaccine-related adverse events were observed beyond mild-to-moderate injection site pain and swelling in the 7 volunteers after 290 days of follow-up. Anti-*Na*-ASP-2 IgE and IgG subclasses were quantified by ELISA on sera collected at baseline and at post-vaccination time-points. Individuals who developed urticaria had markedly elevated pre-vaccination levels of *Na*-ASP-2-specific IgE and IgG4 compared to those who didn't develop urticaria. Fourteen days after immunization, anti-ASP-2 IgG responses increased significantly compared to controls vaccinated with the hepatitis B vaccine ($p = .04$). Subsequent seropidemiological studies in the same *Necator* endemic area have shown that hookworm infected individuals have high levels of IgE and IgG4 to *Na*-ASP-2 but low levels of IgG1. The data suggest that natural infection induces IgE and IgG4 to *Na*-ASP-2 that can result in immediate-type hypersensitivity reactions upon vaccination with this antigen, whereas vaccination of naive individuals induces a different antibody profile consisting primarily of anti-ASP-2 IgG1. Our data have important implications for future helminth vaccine research and highlight the important differences between the immune response induced by natural infection compared to vaccination. Further development of this vaccine is awaiting the results of skin testing and anti-ASP-2 antibody assessments of young children living in hookworm-endemic areas to detect sensitization to *Na*-ASP-2 and thus determine if this age group could be safely vaccinated with this antigen.

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DEVELOPMENT OF A REAL-TIME PCR ASSAY FOR SPECIFIC DETECTION OF *ANGIOSTRONGYLUS CANTONENSIS* IN CLINICAL AND ENVIRONMENTAL SAMPLES

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Angiostrongylus cantonensis is the most common cause of human eosinophilic meningitis. Due to the absence of reliable laboratory diagnostic tests, most cases of infection are currently diagnosed based on clinical symptoms together with a history of exposure. The aim of this study was to develop a real-time PCR assay for the identification of *A. cantonensis* in clinical as well as environmental samples. Third stage larvae extracted from naturally and experimentally infected mollusks were used to establish the sequence variability of the PCR target gene, the ITS1 region, in *A. cantonensis* and its close relative *A. costaricensis*. A TaqMan assay specific for *A. cantonensis* was then designed and its sensitivity was evaluated using DNA extracted from 39 samples: 21 CSF and 7 brain tissue samples from laboratory rats infected with *A. cantonensis*; 2 CSF specimens from two cases of suspected angiostrongyliasis; and 9 mollusks collected from endemic regions in the U.S, of which three were positive for *A. cantonensis* by a conventional PCR assay. The real-time PCR assay detected *A. cantonensis* in 17 of the samples from the infected rats and in one of the human CSF specimens. The real-time PCR returned positive results for the three previously positive plus four additional mollusks, suggesting potential greater sensitivity. The specificity of the real-time PCR assay was further evaluated with DNA extracted from 1 CSF and 1 brain tissue sample from one non-infected rat, 7 human CSF and brain tissue samples from patients with other CNS infections and larvae from two strains of *A. costaricensis* and two mollusks containing a non-identified nematode species. No false-positive results were obtained with the negative controls. In summary, the real-time PCR assay showed 63% sensitivity (it detected 21 of 33 positives) and 100% specificity for the detection of *A. cantonensis* in the samples tested. The identification *of A. cantonensis* DNA in one of the human CSFs indicates a promising potential of this assay as a diagnostic test for human angiostrongyliasis.

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CRYSTAL PROTEINS AS A NEW CLASS OF ANTHELMINTICS

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Crystal proteins made by the soil bacterium *Bacillus thuringiensis* (Bt) have been used for decades as a biological control for insects that eat crops and

vector human diseases. These proteins are toxic to insects but non-toxic to vertebrates. As such they are the main source of biological control agent used around the world and are approved for expression in transgenic food crops. Their mode of action is to bind to intestinal cells of target invertebrates and form a pore. We have been studying nematicidal Crystal proteins, in particular Cry5B. We have studied Cry5B intoxication pathways in the nematode *Caenorhabditis elegans*. We have shown that Cry5B is toxic to a wide variety of nematodes and can effect a near complete cure of an Ancyolostoma ceylanicum infection in hamsters, as reporte previously. Currently we are extending this study to a different model of infection, the natural mouse parasite *Heligmosomoides polygyrus*. We will discuss our results on using Cry5B and other crystal proteins to cure *H. polygyrus* infections *in vivo*. Given the repeated and urgent cries for the development of anthelmintics with novel mechanisms of action, our goal is to develop the full potential of crystal proteins as cures for soil-transmitted helminth infections in humans.

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STUDIES OF TRIBENDIMIDINE MECHANISM OF ACTION AND RESISTANCE IN *CAENORHABDITIS ELEGANS*

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Tribendimidine is a derivative of amidantel and has a broad spectrum of activities against soil-transmitted helminthes. It has been used recently in clinics in China to treat human hookworm infections and is the only new human anthelmintic to be developed and used in the last 25 years, as reported previously. However, little is known about its toxicity and resistance pathways in nematodes. The free-living nematode *Caenorhabditis elegans* has been used to study and discover the mechanism of action of almost all of the anthelmintics currently in use. Here, we apply *C. elegans* to study tribendimidine. We found that the larva of *C. elegans* exposed to tribendimidine exhibit stunted growth, loss of integrity of internal structures, decreased fertility, and death. These effects can be quantitated by means of LC50, developmental delay, and progeny production assays. We performed a forward genetic screen using mutagenesis to isolate mutants resistant to tribendimidine. In three different screens, we isolated 10 tribendimdine resistant mutants. Complementation analyses indicate that these mutants fall four complementation groups. We have characterized these mutants and identified the genes associated with them. We will show our results as well as discuss the implications towards understanding the mechanism of action and possibility for resistance with tribendimdine.

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IMPACT OF INTESTINAL PARASITIC INFECTIONS ON VITAMIN A STATUS AMONG ABORIGINAL SCHOOLCHILDREN IN RURAL PENINSULAR MALAYSIA

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The impact of intestinal parasitic infections on vitamin A status was investigated through out a cross-sectional study on 241 aboriginal schoolchildren aged 7-12 years in Pos Betau, Pahang, Malaysia. All children underwent physical examination including anthropometric measurements and screened for *Ascaris lumbricoides*, *Trichuris trichiuara*, and hookworm by using Kato-Katz and Harada Mori techniques. Trichrome staining technique was used to detect *Giardia duodenalis* trophozotes and/or cysts. About 3-4 mls of venous blood samples was collected and vitamin A status was assessed using the level of serum retinol. Socioeconomic data were collected using pre-tested questionnaires. The overall prevalence of ascariasis, trichuriasis, hookworm infections and giardiasis were 67.8%, 95.5%, 13.4% and 17.8% respectively. Moreover, 66 (27.4%) children had low serum retinol (<0.70 µmol/L) and considered to have vitamin A deficiency (VAD). The results showed that giardiasis and severe ascariasis were associated significantly with low serum retinol levels with *P*-values of 0.004 and 0.018, respectively. The output of logistic regression analysis confirmed that giardiasis (OR=2.7; 95%CI=1.3, 5.5) was a strong predictor of low serum retinol among these children. In conclusion, the intestinal parasitic infections and VAD are still public health problems among aboriginal schoolchildren. The findings also showed the importance of intestinal parasitic infections in developing VAD. Hence, deworming and vitamin A supplementation programmes should be implemented periodically to these children in order to improve their health and nutritional status.

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HOW DO MASS CAMPAIGNS AFFECT DISTRICT HEALTH SERVICES? THE CASE OF A NATIONAL CAMPAIGN FOR NEGLECTED TROPICAL DISEASES IN MALI

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In 2007 an integrated drug administration platform addressing seven neglected tropical diseases (three Soil-transmitted helminthiases, Onchocerciasis, Schistosomiasis, Lymphatic Filariasis, Trachoma) was launched in 5 African countries, including Mali. The main strategy is community-based preventive chemotherapy. There is only a scarce body of knowledge about interactions between mass campaigns and health systems. During the first national campaign in Mali against Neglected Tropical Diseases (NTDs) in May 2007, we conducted a qualitative field study with the aim to assess interactions between this campaign and the district health services. The study revealed a significant input of district health systems in the management and implementation of the NTDs mass campaign, leading to destabilizing effects (interferences, imbalances and duplications), especially in the weaker health centers: 1) *Interferences with health centre functioning*: health centre staff, responsible for training and supervising community volunteers, coordinating community mobilization, and collecting, analyzing and reporting campaign- related information, was found repeatedly absent from the health centre, disturbing or interrupting routine activities; 2) *Imbalances in resource allocation*: financial and non-financial incentives for campaign-related activities, as well as high availability of drugs during the campaign, established a distinction with regular health service activities, likely to affect health staff behavior and local priority setting in health care provision; and 3) *Duplication of activities*: the creation of campaign specific systems for drug supply and health information, administratively dissociated from the existing national systems, contributed to increased workload and district costs. This study suggests that the current multiplication of mass campaigns has destabilizing effects on already weakened health care delivery systems. Yet, strengthened health care services will be necessary to consolidate campaign results in the long term, to implement complementary disease control activities, and to respond to communities' felt health needs.

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PREVALENCE OF SOIL-TRANSMITTED HELMINTHS IN 50 RICE FARMING VILLAGES OF THE SAMAR PROVINCE OF THE PHILIPPINES

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Soil-Transmitted Helminths (STH) are the most common neglected tropical diseases and the most prevalent infections worldwide. The aim of this study is to estimate the prevalence of *Ascaris lumbricoides, Trichura trichiura*, and hookworm, in the Samar province (Philippines). A cross-sectional study was conducted in 50 villages of Western Samar province between August 2003 and November 2004. In each village, a maximum of 35 households with at least five members and one farmer who worked in a rice farm were selected. 5624 participants provided at least one stool sample to determine infection with *A. lubricoides*, *T. trichiura*, and hookworm. Two-slides per stool sample were examined using the Kato-Katz method. Demographic information was collected through interviews. The data were analysed with a random-effect logistic regression. The overall prevalence proportions of *A. lumbricoides*, *T. trichiura*, and hookworm infection are 43.3%, 66.1%, and 31.4%, respectively. Prevalences varied extensively from village to village ranging from 4.6% to 76.6%, 19.1% to 95.6%, and 11.1% to 57.8% for *A. lumbricoides*, *T. trichiura*, and hookworm, respectively. There is a correlation between *A. lumbricoides* and *T. trichiura* prevalences at the village level. Adjusting for other two STHs, female gender was associated with higher risk of *A. lumbricoides* (adjusted Prevalence Odds Ratio (aPOR) =1.38; 95% Confidence Intervals (CI): 1.13, 1.69) and lower risk of hookworm (aPOR=0.28; 95% CI: 0.23, 0.34). Being over 16 years was associated with lower prevalence of *T. trichiura* and Hookworm, whereas for *A. lumbricoides*, the prevalence was lower among those aged over 40 years (reference: 0-10 years). The prevalence was lower among non-farmers only for hookworm infection (aPOR=0.55; 95% CI: 0.38, 0.78; ref: rice-farming). In conclusion, high overall prevalence of these STHs demonstrates their endemicity in Samar province, Philippines. Lower risk of infection in older age groups agrees with the findings from other studies. Lower risk of hookworm infection among non-farm workers and females may be explained by its transmission through skin penetration. Correlation between *A. lumbricoides* and *T. trichiura* infection prevalence may be explained by their similar route of transmission. The observed village-to-village variation in prevalence was remarkable, and confirms the importance of taking clustering into account in studying STH.

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NITRIC OXIDE DEPLETION AND ENDOTHELIAL DYSFUNCTION IN CHILDREN WITH MALARIA AND MARKED ANEMIA

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Intravascular hemolysis produces nitric oxide (NO) depletion and endothelial dysfunction in conditions as diverse as sickle cell disease, thalassemia, paroxysmal nocturnal hemoglobinuria and transfusion reactions. In the studies reported here, we tested for similar mechanisms in 52 children 1-5 years of age with *Plasmodium falciparum* malaria and marked anemia (Hb < 7 gms per dL) relative to 31 healthy controls. Evidence for intravascular hemolysis in these children included increased indirect bilirubin and lactate dehydrogenase (LDH) levels relative to healthy controls (1.1 *vs.* 0.4 mg/dL, 659 *vs.* 416 IU/mL, $p<0.001$), consistent with increased plasma Hb levels. During acute hemolysis, free plasma Hb irreversibly and rapidly scavenges NO, a regulator of endothelial and vascular function. Arginase 1, released from the erythrocyte compartment during hemolysis and measureable in plasma, further reduces NO bioavailability by diverting its precursor, arginine. NO consumption assays confirmed the reduction of NO. Preliminary evidence for endothelial dysfunction included increased plasma levels of sVCAM-1 in cases vs. controls. As with other hemolytic disorders characterized by low NO bioavailability, endothelial dysfunction and aberrant vasoconstriction caused clinical sequeale and hemodynamic changes including elevated pulmonary pressures and elevated NT-proBNP levels (2,010 *vs.* 128 pg/mL, $p<0.001$). These results suggest that the consequences of intravascular hemolysis with *P. falciparum* malaria and marked anemia are fundamentally similar to those in other diseases with intravascular hemolysis: initial release of intraerythrocytic arginase 1, LDH and Hb into plasma, secondary reduction of NO levels *in vivo,* and subsequent endothelial dysfunction as a result of NO depletion. Consistent with this conclusion, there was a negative correlation between Hb and NT-proBNP at the time of admission for cases, but not controls (ρ=-0.46, -0.06; $p<0.001$, p=0.76).

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ANGIOPOIETIN-2, AN AUTOCRINE MEDIATOR OF ENDOTHELIAL ACTIVATION IS ASSOCIATED WITH PARASITE BIOMASS, ENDOTHELIAL DYSFUNCTION AND MORTALITY IN SEVERE FALCIPARUM MALARIA

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Adherence of parasitized red cells to activated endothelium causes microvascular obstruction and pathology in severe falciparum malaria, however mechanisms of endothelial activation remain unclear. The angiogenic factor, angiopoietin-2 (Ang-2), is an autocrine mediator of endothelial activation stored in endothelial Weibel-Palade bodies (WPBs). Endothelial bioavailability of nitric oxide (NO), the major inhibitor of WPB exocytosis, is low in severe malaria and may contribute to Ang-2 release and endothelial activation. Exocytosis of WPBs occurs during subclinical experimental malarial infection, but the role of Ang-2 in malaria pathogenesis is unknown. Serial venous samples were obtained from adults with and without severe malaria, in parallel with longitudinal measures of endothelial function using reactive hyperemia-peripheral arterial tonometry (RH-PAT, a measure of endothelial NO bioavailability). Concentrations of Ang-2 were associated with malaria disease severity, biomarkers of perfusion, endothelial activation and parasite biomass. The longitudinal relationship between Ang-2 and endothelial function was assessed using a mixed-effects model. Ang-2 was elevated in SM and associated with increased venous lactate, plasma intercellular adhesion molecule-1 concentrations, parasite biomass (but not parasitemia) and mortality. In contrast, concentrations of the related angiopoietic factor, vascular endothelial growth factor (VEGF) were reduced in severe malaria. Recovery of endothelial function was associated with falling concentrations of Ang-2. In conclusion, Ang-2 release from endothelial cells with reduced NO bioavailability likely contributes to endothelial activation, impaired perfusion, sequestered parasite biomass and poor outcome in severe falciparum malaria. Agents which improve endothelial NO and/or reduce WPB exocytosis may have therapeutic roles in severe malaria.

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ANGIOPOEITIN-1 AND -2 AS NOVEL BIOMARKERS OF CEREBRAL MALARIA

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Well-validated biomarkers do not currently exist to accurately identify patients infected with malaria who are at risk of developing serious complications such as cerebral malaria. Based on the hypothesis that widespread endothelial cell activation is central to the pathogenesis of severe malaria we examined the endothelial activation markers, angiopoeitin-1 (ANG-1) and angiopoeitin-2 (ANG-2), in serum samples from healthy controls and *Plasmodium falciparum*-infected patients with uncomplicated or cerebral disease, from two diverse populations - Thai adults and Ugandan children. ANG-1 and ANG-2 levels were compared to tumour necrosis factor-α (TNF), which has been previously shown to correlate with severe and complicated malaria. In both populations, ANG-1 levels were significantly decreased in cerebral malaria versus uncomplicated malaria and healthy controls, and significantly decreased in uncomplicated malaria patients versus healthy controls (Kruskal-Wallis (K-W) test, $p<0.001$). Additionally, ANG-2 levels were significantly increased in cerebral malaria versus uncomplicated malaria and healthy controls (K-W test, $p<0.001$) but not in uncomplicated malaria versus healthy controls. The standard marker of severe disease, TNF, was elevated in malaria-infected individuals compared to healthy controls (K-W test, $p<0.001$) but was only significantly elevated in cerebral compared to uncomplicated cases in the Thai adult population ($p<0.001$). Receiver operating curves were used to compare how well each biomarker identified cerebral malaria patients from uncomplicated malaria patients. ANG-1 and the ratio of ANG-2:ANG-1 were found to be highly accurate tests to discriminate cerebral malaria patients in both populations (Area under the receiver operating curve (AUROC) for Thai / Ugandan population: ANG-1 - 1.0/0.795; ANG-2:ANG-1 - 1.0/0.782). TNF was only an accurate test for cerebral malaria in the Thai population (AUROC: 0.834) but did not discriminate well between cerebral and uncomplicated malaria patients in the Ugandan samples (AUROC: 0.544). ANG-1 and the ANG-2/1 balance are promising biomarkers for cerebral disease. Further work should address their usefulness as prognostic biomarkers and as potential therapeutic targets in malaria infection, as well as their utility in other infectious disease states that disrupt endothelial integrity.

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ADAMTS13 DEFICIENCY WITH ELEVATED LEVELS OF ULTRA-LARGE AND ACTIVE VON WILLEBRAND FACTOR IN MALARIA

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Malaria shares clinical characteristics with the rare disease thrombotic thrombocytopenic purpura (TTP), most notably intravascular platelet aggregation, thrombocytopenia and organ dysfunction. A severe deficiency in ADAMTS13, the enzyme responsible for proteolysis of ultra-large and hyperactive von Willebrand factor (VWF) multimers, is the underlying pathogenic mechanism in TTP. We therefore studied VWF release and proteolysis in patients with a symptomatic *Plasmodium falciparum* (n=26) or *P. vivax* (n=16) malarial infection and in asymptomatic controls without microscopically detectable parasitemia (n=32) on the Indonesian island Sumba. Compared with controls, patients with either falciparum or vivax malaria had significantly lower median platelet numbers (269 vs. 122 vs. $117x10^9$/L; $p<0.001$), higher median VWF levels (10.9 vs. 28.2 vs. 19.7 μg/mL; $p<0.001$) and higher VWF activation factors (1.4 vs. 2.6 vs. 1.9; $p<0.001$). The latter indicates that a higher amount of the circulating VWF in malaria patients was in a platelet binding conformation, which was also reflected by a significant inverse correlation between VWF activation factors and platelet number (Spearman's R -0.453; $p=0.004$). All malaria patients and a substantial proportion (66%) of controls had reduced ADAMTS13 activity and antigen levels. Severe (<10% activity) ADAMTS13 deficiency was found in 30.7% and 37.5% of *P. falciparum* and *P. vivax* patients, respectively, and in 22% of controls and it was associated with the presence of ultra-large VWF multimers. The pathogenic mechanisms responsible for the low ADAMTS13 remain to be elucidated, but ADAMTS13 autoantibodies or functionally important mutations in the *ADAMTS13* gene were not found. In conclusion, we show that ADAMTS13 deficiency is highly prevalent in malaria endemic regions. The combination of endothelial cell perturbation with increased release of active and ultra-large VWF and reduced VWF inactivation by ADAMTS13 may contribute to the malaria-associated thrombocytopenia and to microcirculatory disturbances and organ dysfunction.

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SINGLE MOLECULAR FORCE SPECTROSCOPY STUDY OF *PLASMODIUM FALCIPARUM*-INFECTED ERYTHROCYTE CYTOADHERENCE TO ENDOTHELIAL RECEPTORS

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Cytoadherence or sequestration is essential for the pathogenesis of the most virulent human malaria species, *Plasmodium falciparum*. In this paper, we applied single molecular force spectroscopy technique to quantify the molecular interaction forces and the binding kinetics between *P. falciparum* infected red blood cell (IRBC) and two endothelial receptors: thrombospondin (TSP) and CD36. Temperature was found to play an important role in affecting the dissociation rates as well as free energy barrier profile of different receptors binding to *P. falciparum* IRBCs. Results from the comparison of CD36 with TSP at physiological temperature showed that CD36 mediated interaction was much more stable than that mediated by TSP, although TSP-IRBC interaction was stronger than CD36-IRBC interaction in the high pulling rate regime. This suggests that TSP may initiate cell adhesion by catching the fast flowing IRBCs whereas CD36 functions as the 'holder' for providing stable binding. Our study should provide valuable information on the structure-function relationship and the biophysical and pathological functions of host receptors and parasite ligands, which would help to identify therapeutic targets and to develop novel drug candidates to reduce the morbidity and mortality burden caused by malaria.

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C5A POTENTIATES DYSREGULATED INFLAMMATORY AND ANGIOGENIC RESPONSES IN PREGNANCY-ASSOCIATED MALARIA

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Pregnancy-associated malaria (PAM) is a leading cause of maternal and infant mortality. The accumulation of parasitized erythrocytes

(PEs) and monocytes within the placenta is thought to contribute to the pathophysiology of PAM. However, there are critical gaps in our understanding of the molecular basis of PAM and the downstream mediators of placental and fetal injury. Based on the hypothesis that excessive complement activation, in particular generation of the potent inflammatory peptide C5a, may contribute to PAM, we investigated the role of C5a in the pathogenesis of PAM *in vitro* and *in vivo*. Using human monocytes we assessed the interaction between C5a and malaria *in vitro*. In the presence of the malaria toxin pfGPI, monocytes generate C5a and induce expression of the C5a receptor (CD88). Co-cultivation of monocytes with C5a and *pf*GPI resulted in the synergistic production of cytokines (IL-6, TNF, IL-1 β, and IL-10), chemokines (IL-8, MCP-1, MIP1 α, MIP1 β) and the anti-angiogenic factor sFlt-1, in a time and dose-dependent manner. This enhanced inflammatory response was abrogated by C5a receptor blockade. We assessed the role of C5a in PAM *in vivo* by examining C5a plasma levels in pregnant malaria-exposed women. Compared to pregnant women without malaria, C5a levels were significantly elevated in women with PAM, with increases most pronounced in women with higher placental parasite burdens. In conclusion, these data indicate that C5a contributes to a dysregulated inflammatory and angiogenic response to malaria and implicate complement activation in the pathogenesis of PAM. Further studies will be required to assess the clinical utility of C5a as a biomarker of PAM pathogenesis and a potential target for therapeutic interventions.

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DIFFERENTIAL IMMUNOPATHOGENIC OUTCOMES OF *PLASMODIUM CHABAUDI* AS INFECTION DURING PREGNANCY IN A/J AND B6 MICE

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The immunopathogenesis of malaria during pregnancy remains poorly understood. However, excessive placental production of Th1-type cytokines has been linked with poor pregnancy outcomes. Because successful pregnancy occurs in a Th2-cytokine dominant environment, Th2-biased A/J mice were expected to experience better pregnancy outcomes despite increased malaria susceptibility when compared to resistant, Th1-biased B6 mice. A/J and B6 mice were infected with 10^3 *Plasmodium chabaudi* AS-infected red blood cells on day 0 of pregnancy, followed clinically, and sacrificed on days 9-11. Plasma cytokines were detected by ELISA. Infected pregnant (IP) A/J mice experienced higher parasite burdens than B6 mice (peak parasitemia: 45% vs 32%, respectively; p= 0.0008). Contrary to expectation, A/J mice, like B6 mice, aborted at mid-gestation, concurrent with peak parasitemia and high levels of plasma pro-inflammatory cytokines. A/J mice had significantly higher levels of tumor necrosis factor (TNF)-α (A/J: 180 ± 42 pg/mL vs B6: 82 ± 27 pg/mL; p=0.004), whereas B6 mice exhibited significantly higher levels of interferon-γ (A/J: 575 ± 179 pg/mL vs B6: 1792 ± 405 pg/mL; p=0.001) and soluble TNF receptor II (A/J: 16,589 ± 3,456 pg/mL vs B6: 42,040 ± 821 pg/mL; p=0.001). Histological analysis of infected A/J placenta revealed a significant inflammatory infiltrate whereas placental sections from IP B6 mice were free of inflammation but had evidence of significant hemorrhage and thrombosis. In summary, both A/J and B6 mice infected with *P. chabaudi* AS suffer acute pregnancy loss in association with heavy parasite burden and pro-inflammatory cytokines. B6 abortion appears to additionally rely on placental damage together with thrombosis while A/J abortion is associated with placentitis. Since these phenomena are all associated with the pathogenesis of human placental malaria, these two models will serve to expand our understanding of the mechanisms leading to these pathogical outcomes and their respective roles in poor pregnancy outcomes associated with malaria.

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CLINICAL SYNDROMES OF *PLASMODIUM FALCIPARUM* MALARIA INFECTION IN KAMPALA, UGANDA: INITIAL RESULTS FROM THE CYTOADHERENCE IN PEDIATRIC MALARIA (CPM) CASE-CONTROL STUDY

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Plasmodium falciparum pathogenesis and severity may result from binding of PfEMP-1 on infected red cells to host cellular ligands. The Cytoadherence in Pediatric Malaria (CPM) Study, an observational case-control study, was launched in October 2007 at Mulago Hospital, Uganda's national referral hospital, intending to enrol 2000 children (6 mos - 12 yr, without concomitant HIV). *Cases* have severe disease (WHO criteria ± death), and *controls* have mild malaria. The primary aim is to explore the association between clinical outcomes and host expression of cytoadhesive PfEMP-1 ligands, including ABO blood group, ICAM1 (CD54), and platelet glycoprotein IV (CD36). Two full-time research physicians prospectively collect clinical data according to protocol. We present preliminary results from this cohort. Over the study's 1st 6 mo, 658 patients were screened, 405 enrolled, and 26 excluded, for a total of 365 analyzed (168 cases; 197 controls). Exclusions were due to 23 malaria false positives and 3 HIV cases. Patients, 58% male: 42% female, were unwell for 3.7±2.0 days before presenting to hospital. Cases were younger (2.5±2.1 y, 92% < 5 y) than controls (3.7±2.8 y, 74% <5 y), $p < 2x10^{-6}$. Compared with controls (median Hb 9.3 g/dL), cases were severely anemic (median Hb 4.7 g/dL), and 129 (35%) were transfused. Platelet counts were slightly lower among cases than controls (114,000/μL [IQR 67,000-174,000] vs 133,000/μL [IQR 84,000-212,000], p=0.01). Of the 14 (4%) patients who died, the median time to demise was 1 day, despite use of guideline-based antimalarials. Among cases, severe malarial anemia (SMA) was the most common syndrome (n=110, 65%), followed by hyperlactatemia (HL, n=73, 43%), cerebral malaria (CM, n=36, 21%), severe thrombocytopenia (ST, platelets <50,000/μL, n=32, 19%), hyperparasitemia (HP, >250,000/μL, n=31, 18%), and hypoxia (HO, n=11, 7%). Many cases suffered syndrome overlaps, with 53% exhibiting at least 2 of either SMA, HL, CM, ST, HP, or HO. Fatalities rose with increasing composite syndrome count: 10% for 2, 22% for 3, 40% for 4, and 50% for 5. In conclusion, pediatric malaria in Kampala can be characterized by 6 distinct syndromes distinguished by precise definitions. Composite clinical scoring may prove suitable for mortality risk assessment and for endpoint analysis of this large case-control study examining host cytoadherence ligands in malaria outcomes.