domains of *cg2* gene was analysed by nested PCR. *Pfcrt*-K76T mutation showed significant and complete association with *in vitro* CQ susceptibility status with 100% sensitivity and specificity. *Pfmdr1*-N86Y mutation also showed strong association with *in vitro* CQ resistance with 99.05% sensitivity and 100% specificity. Length polymorphism in *cg2*-kappa and omega repeat domains showed significant but incomplete association with CQ resistance with lower sensitivity (88.57% and 67.61% respectively) and specificity (65.22% and 60.87 respectively). Furthermore, strong linkage disequilibrium was observed between alleles of codon 76 of *pfcrt* and alleles of codon 86 of *pfmdr1* gene. The results show that *Pfcrt*-K76T and *pfmdr1*-N86Y mutations are good diagnostic molecular markers of CQ resistance in NE India while length polymorphism in kappa and omega repeat domains of *cg2* gene are not good markers. Thus, *Pfcrt*-K76T and *pfmdr1*-N86Y mutations either alone or in combination can be used as epidemiological tools for surveillance of CQ resistance in India, especially in areas like NE Indian states where falciparum malaria is highly endemic and CQ resistance is high.

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RESPONSE OF FALCIPARUM MALARIAL PARASITE TO STANDARD TEST DOSE OF CHLOROQUINE IN PUNJAB, PAKISTAN

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Present study represents the results of a 28-day follow-up of 404 patients treated with chloroquine for uncomplicated falciparum malaria in five districts of Punjab, Pakistan. Chloroquine resistance checked in the subjects harboring *Plasmodium falciparum*, using *in vivo* techniques was 35.4%, with 31.2% RI and 4.2% RII and nil RIII. During the non-transmission seasons for the years 1999-2005, among the rural populations Among five districts maximum *P. falciparum* (%) noted in Muzaffargarh (29.2) and minimum in Jhang (20.8). Among the five districts, maximum RI (35.2%) and RII (5.4%) were noted in Multan. Maximum resistance was noted in 5-15 year age group, but with a low RII (3%) value. Two patient characteristics were found to be independent and important predictors of resistance in a Cox proportional hazards model i.e. 5-15 years age group and parasitemia count >6000/µl. The weekly risk of resistance (%) was 18.7, 35.4, 19.0 and 25.0 during 1st, 2nd, 3rd and 4th week respectively, with lowest in 1st and highest in 2nd week. During the 28-day test 5 cases (1.2%) did not complete follow-up. From seven districts of the Punjab, 604 cases of *P. falciparum* satisfied the entry criteria for *in vitro* test. Out of 604 subjects with uncomplicated malaria 228 were treated with chloroquine, showed overall 38.6% resistance, with higher values in males (79.5%) than females (20.4%).192 subjects tested with basoquine in six districts of Punjab, showed 34.8% overall resistance with higher values in males (72%) than in females (28.35%). Another 192 subjects tested with sulphadoxine / pyrimethamine in six districts of Punjab, showed 5.7% resistance with higher values in males (63.6%) than females (36.4%) ($p<0.005$). Difference between the resistances was highly significant ($p<0.000$) between sulphadoxine / pyrimethamine and chloroquine or basoquine and non significant between basoquine and chloroquine ($p<0.177$). So it is suggested that among these drugs sulphadoxine / pyrimethamine could safely replace the present first line drugs (chloroquine and basoquine) in Pakistan.

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RAPID DETECTION OF DIHYDROFOLATE REDUCTASE RESISTANCE ALLELES IN *PLASMODIUM FALCIPARUM* USING REAL-TIME PCR WITH LOCKED NUCLEIC ACID TAQMAN PROBES

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Drug resistance in *Plasmodium falciparum (Pf)* is a major problem in malaria endemic areas and constant and continuous monitoring is vital for effective implementation of control effort. Molecular markers, *in vitro* and *in vivo* tests have been developed for the purpose of monitoring antimalaria drug resistance. Real-time PCR offers a fast and reliable method for rapid detection and measurement of molecular markers for resistant allele. *Pf* Dihydrofolate reductase (*dhfr*) and Dihydropteroate synthase (*dhps*) genes single nucleotide polymorphisms have been shown to modulate the resistance of the parasite to Sulfadoxine pyrimethamine (SP), an antifolate class of drug. In this study, an assay using real-time PCR and sequence specific Locked Nucleic Acid (LNA) probes that detect the important mutations on *Pfdhfr* is described. Using DNA from laboratory *Pf* parasites and one hundred and thirty three clinical samples, real-time PCR was able to rapidly, simultaneously and correctly distinguish the important *Pfdhfr* mutations at codon 51, 59 and 108. Moreover, mixed infections caused by *Pf* clones with wild-type or mutant alleles could be efficiently distinguished as confirmed by genomic sequencing. The prevalence of the mutant I51, R59 and S108 in the clinical isolates was 85%, 95% and 98.5% respectively. None of the samples had the T108 mutation. The high level of the triple *dhfr* mutation in *P. falciparum* isolates in the study area is an indication that the use of SP either in combination with artemisinin or non artemisinin drugs might not be suitable for effective control. The lowest detection limit of the assay was about $1.5x10^{-3}$ng/µl of DNA below 40 cycles, that is, parasite density as low as 2 parasites/200 WBCs could be detected. In addition to its sensitivity and specificity, the new assay has a low per test cost, fast, easily automated and well-suited for large scale epidemiological studies.

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CLEARANCE OF AMODIAQUINE-RESISTANT *PLASMODIUM FALCIPARUM* IN NIGERIAN CHILDREN BY IMMUNOGLOBULIN G ANTIBODIES TO THE 19-KDA C-TERMINAL REGION OF MEROZOITE SURFACE PROTEIN 1 ($MSP\text{-}1_{19}$)

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In this study, we investigated the role of humoral immune responses (IgG, IgG1 and IgG3) against the 19-kDa C-terminal region of merozoite surface protein 1 ($MSP\text{-}1_{19}$) on clearance of amodiaquine (AQ) resistant parasites (harboring mutant alleles of pfcrt and pfmdr1 genes) and recovery in Nigerian children under the age of 12 years. 120 children with acute uncomplicated falciparum malaria were treated with AQ and followed-up for 28 days. Mutations in pfcrt and pfmdr1 associated with *in vivo* resistance to AQ were determined in all patients prior to treatment and after treatment in patients who failed treatment with the drug. Sera collected from 120 children at enrollment and during follow-up were assayed by ELISA for total IgG, IgG1 and IgG3 to recombinant $MSP\text{-}1_{19}$. Recovery was defined as the absence of severe malaria at any time and absence of fever and parasitemia 72 hours post-treatment. IgG3 sub-class titers to $MSP\text{-}1_{19}$ was significantly higher than total IgG ($p=0.033$; 95%CI= -0.144 to -1.049) and IgG1 ($p=0.000$; 96%CI=-1.05 to -0.889) in patients at enrollment. In addition, IgG3 titers to $MSP\text{-}1_{19}$ were also higher in plasma samples taken from children who carried resistant parasites with resistant markers (pfcrtT76+pfmdr1Y86) but cleared these parasites.

These results not only validates the importance of MSP-1_{19} as an antigen associated with protection from malaria, but also point to the importance of IgG3 antibodies in clearing amodiaquine resistant parasites.

(ACMCIP Abstract)

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IDENTIFICATION OF SINGLE NUCLEOTIDE POLYMORPHISMS (SNPS) IN *PLASMODIUM FALCIPARUM* DHFR, PFDHPS AND PFCRT GENES USING A MICROSPHERE-BASED MINISEQUENCING ASSAY

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The rapid spread of antimalarial drug resistance over the past few decades has necessitated increased monitoring for resistance. Constant vigilance is required to ensure early detection of the ever-changing patterns of resistance so that national treatment policies can be revised. We designed a mini-sequencing procedure using FlexMapTM technology to facilitate the identification of known SNPs associated with antimalarial drug resistance in *Plasmodium falciparum*. Using *P. falciparum* genomic DNA, we prepared standard PCR reactions to amplify Pfdhfr, Pfdhps and Pfcrt. SNP specific primers containing a 5' capture sequence were designed so that the terminal dNTP of the primer anneals directly over the SNP. Two primers were designed for each SNP; one primer terminates with the wild type and one terminates with the mutant nucleotide. Fluorescently labeled allele-specific extension products (ASEP) were generated using the PCR amplified gene, SNP primers and a standard PCR reaction mixture containing biotin-labeled dCTPs and unlabeled dATPs, dGTPs and dTTPs. Following the extension step, the tagged ASEPs were captured using fluorescent microsphere containing DNA complimentary to the tags. Following incubation with streptavidin-R-phycoerythrin the reactions were analyzed using a BioplexTM suspension array system. The procedure correctly identified fifteen different polymorphisms in three multiplex reactions using a total of 32 different allele specific primers and 32 FlexMAP® microspheres. The procedure is robust and qualitative and can easily be transferred between laboratories and due to the cost savings associated with this procedure, it could replace traditional DNA sequencing as the gold standard for molecular markers determination in *P. falciparum*.

(ACMCIP Abstract)

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MUTATIONAL ANALYSIS OF THE *DIHYDROFOLATE REDUCTASE* AND *DIHYDROPTEROATE SYNTHASE* GENES FROM *PLASMODIUM VIVAX* IN ISOLATES FROM IQUITOS, PERU

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First line treatment of *Plasmodium falciparum* with Sulfadoxine-Pyrimethamine (SP) drug in Peruvian Amazon Region was changed in 2001. During this time, primary infections of *P. vivax* were not treated with SP, but co-infections could have exposed the species to high levels of drug inducing mutations in orthologs shown previously in *P. falciparum* to be linked to drug resistance. Double or triple mutations at codons 57, 58 and 117 in *Pvdhfr* have been shown to be associated with a decrease in *in vitro* susceptibility to SP. We identified mutations in *Pvdhfr* and *Pvdhps* in samples from Iquitos collected in 2006 and from 1998-1999 when SP was the first line therapy. Using a limited number of isolates, we observed an increase in the number of mutations in *Pvdhfr* from isolates collected in 2006 compared to 1998-99 samples. We found that the prevalence of double and triple mutations were greater in isolates from 2006 (76% and 20%) than in 1998-99 samples (67% and 17%). In addition, we found a new genotype in isolates (13%) from 2006, which includes a novel insertion joined to 57L/58R. For *Pvdhps*, 24% of samples had just a single mutation that has been implicated in sulfadoxine resistance in *P. falciparum*. The high prevalence of double and triple mutants could signify a baseline level of circulating resistant phenotypes to pyrimethamine in Peru. However we did not find a correlation between *Pvdhfr* mutations and SP use, signifying that mutational rate in these genes are independent of drug pressure.

(ACMCIP Abstract)

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SELECTION OF *PLASMODIUM FALCIPARUM* MULTIDRUG RESISTANCE GENE 1 ALLELES BY ARTEMETHER-LUMEFANTRINE IN NIGERIAN CHILDREN WITH ACUTE UNCOMPLICATED MALARIA

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We assessed *Plasmodium falciparum mdr1* (pf*mdr1*) polymorphisms and copy numbers as well as *P. falciparum* Ca^{2+}ATPase (*pfATPase6*) gene polymorphisms in 96 Nigerian children presenting with uncomplicated falciparum malaria and enrolled in an artemether-lumefantrine (AL) efficacy study. The nested PCR-RFLP and the quantitative real-time PCR methodologies were used to determine alleles of *pfmdr1* and copy numbers respectively in samples collected from all patients prior to treatment and in all recurrent parasites during a 42 day follow-up. The *pfmdr1* 86N (p= P=0.01; z=-2.53 Wilcoxon-signed rank test) and 184F (P=0.003; z=-2.97; Wilcoxon-signed rank test) alleles increased significantly and were strongly selected among post-treatment samples obtained from patients with newly acquired or recrudescing infections and gametocytes after treatment with AL. All pre- and post-treatment samples as well as gametocytes harbored a single copy of the *pfmdr1* gene and the wild-type allele *pfATPase6*. These findings suggest that polymorphisms in *Plasmodium falciparum pfmdr1* gene are under directional AL selection. *Pfmdr1* polymorphisms may result in reducing the therapeutics efficacy of this newly adopted combination for uncomplicated falciparum malaria in sub-Saharan countries of Africa.

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CHARACTERIZATION OF FOUR MICROSATELLITES AROUND *PLASMODIUM VIVAX* DIHYDROFOLATE REDUCATSE *(PVDHFR)* GENE ASSOCIATED WITH PYRIMETHAMINE RESISTANCE

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The antifolate primethamine competitively inhibits dihydrofolate reductase (DHFR) enzyme of both *Plasmodium falciparum* and *P. vivax*. Although primethamine in combination with sulfadoxine (SP) is commonly used against *P. falciparum* malaria, it also causes mutations in the *P. vivax dhfr* gene. Thus, there is a need to monitor the status of *P. vivax* DHFR mutations in the field isolates and to identify genetic markers to study the origin and spread of pyrimethamine associated mutations in different populations as being studied in the case of *P. falciparum dhfr*. Further, limited numbers of genetic markers are available to study the population

genetic structure of *P. vivax*. Therefore, in a comprehensive study, we analyzed DHFR mutations among 177 *P. vivax* isolates from India. In addition, we also identified and characterized four microsatellite loci flanking *pvdhfr* gene (two nearest loci at -38.83kb and +6.15kb, and two farthest loci at -230.54kb and +283.28kb) among 110 of these *P. vivax* isolates. Around 43% of the isolates had wild-type PvDHFR ($F_{57}S_{58}T_{61}S_{93}S_{117}$) alleles whereas 57% showed mutant PvDHFR alleles. Among mutant type, 1.5 % isolates had single PvDHFR mutation ($F_{57}S_{58}T_{61}S_{93}N_{117}$) whereas ~ 2.2 % and ~ 44 % had double PvDHFR mutations with $L_{57}R_{58}T_{61}S_{93}S_{117}$ and $F_{57}R_{58}T_{61}S_{93}N_{117}$ alleles respectively. We also found quadruple PvDHFR mutations ($L_{57}R_{58}M_{61}S_{93}T_{117}$) in 9% of the isolates. We found all four microsatellite loci as highly polymorphic with number of alleles ranging from 4 to 10. The expected heterozygosity (*He*) at these loci ranged from 0.50 to 0.82. However, no associations were observed between PvDHFR alleles and the microsatellite genotypes. In conclusion (i) the sulfadoxine-pyrimethamine should be used with caution to minimize the development of cross-species resistance in the field. (ii) Although no association was found between PvDHFR alleles and the flanking microsatellite genotypes, these loci in *P. vivax* were showing extensive heterozygosity and rate of variation were comparable with the variations observed at *P. falciparum* microsatellite loci.

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CONSUMER PERCEPTIONS AND CARE-SEEKING FOR FEBRILE ILLNESS ASSOCIATED WITH THE AVAILABILITY OF ARTEMISININ-CONTAINING ANTIMALARIAL COMBINATION THERAPY IN RUFIJI DISTRICT TANZANIA, 2003 TO 2006

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Artemisinin-combination therapy (ACT) has been adopted in many malaria endemic countries. Introduction of a highly efficacious treatment may be associated with shifts in care-seeking behavior if consumers perceive its advantages over other treatments. Such changes may also be affected by the relative availability of ACT at health facilities and treatments from other sources in the community. ACT with sulfadoxine/ pyrimethamine plus artesunate was introduced for routine management of uncomplicated malaria at all health facilities in Tanzania's Rufiji District beginning in 2003 as part of a multi-year evaluation. Availability of ACT was assessed through routine audits and prescribing data. We conducted household surveys of care-seeking behavior at baseline and annually from 2004 to 2006 (1500 to 2000 households annually). Qualitative data on community perspectives of ACT and alternative treatments were collected in individual and group interviews throughout the same period. The introduction of ACT was associated with a 78% increase in reported use of health facilities for recent febrile illness in 2004. Despite this, a consistent proportion (23%) of households continued to rely on drug retail outlets. Health facility utilization grew steadily until 2006, when the household survey coincided with a period of stockouts affecting ACT and essential drugs in general. Consumer perceptions of ACT in qualitative interviews were mildly positive when it was first introduced and became substantially more so during the stock out period. In conclusion, highly efficacious ACTs may be perceived as advantageous by consumers and increase demand and utilization of health facilities where they are easily obtainable. Maintaining improvements in health facility use and ACT uptake were highly dependent on maintaining regular stocks. Expansion of ACT in other African countries should anticipate increased demand and must be accompanied by improvements in delivery and maintenance of adequate supplies.

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EXPANDED SURVEILLANCE TO CONFIRM THE DISAPPEARANCE OF CHLOROQUINE RESISTANT MALARIA FOLLOWING CHLOROQUINE WITHDRAWAL IN MALAWI

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Following chloroquine's withdrawal from Malawi in 1993 the prevalence of the chloroquine-resistant PfCRT K76T genotype in Blantyre decreased from 85% to 13% by 2000. The K76T mutation has not been detected in Blantyre since 2001. To learn how far chloroquine sensitive falciparum malaria extends beyond Blantyre, we expanded surveillance to districts nearer to Zambia and Mozambique, where chloroquine resistance has remained highly prevalent. Filter paper blood samples were collected in drug efficacy studies conducted in Lilongwe, closer to Malawi's western border with Zambia and Mozambique; Machinga, on Malawi's eastern border with Mozambique; and Nkhotakota, along Malawi's central lakeshore. Five hundred and seventeen pretreatment blood samples were collected (Lilongwe N=208, Machinga N=156 and Nkhotakota N=152). Samples were analyzed for PfCRT K76T and mutations in DHFR codons 59 and 164 and DHPS codon 540. A randomly selected subset of 50 samples was analyzed for mutations at the DHPS 437 and DHFR 51 and 108 codons. Genotyping was done by allele specific restriction analysis of PCR-amplified parasite DNA. K76T genotyping was completed for 476 of 517 samples. No K76T mutations were detected. The prevalence of the highly SP resistant quintuple mutant (DHPS 437 and 540, DHFR 51, 59, and 108) was 88% (44/50). None of the 270 samples genotyped to date has carried the DHFR I164L mutation that confers the highest degree of resistance to pyrimethamine. In conclusion, this study confirms a complete absence of the K76T chloroquine resistance marker in isolates collected outside of Blantyre and closer to Zambia and Mozambique, suggesting that the withdrawal of chloroquine led to the disappearance of chloroquine resistance throughout Malawi despite continued chloroquine use and high rates of chloroquine resistance in neighboring countries. The high prevalence of DHFR and DHPS mutations is consistent with the high SP treatment failure rates recently measured in Malawi.

(ACMCIP Abstract)

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DNA MISMATCH REPAIR IN *PLASMODIUM FALCIPARUM*: POSSIBLE MECHANISM FOR ACCELERATED DRUG RESISTANCE

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Resistance to antimalarial drugs is spreading at an alarming rate. Understanding the mechanisms that lead to resistance is important for the discovery of new treatments. The relationship between drug resistance and genomic maintenance is unexplored territory in *Plasmodium falciparum* biology. The inability of cells to maintain genomic integrity leads to the rapid accumulation of DNA mutations, the underlying cause of many diseases. In particular, DNA mismatch repair (MMR) facilitates genomic fidelity by approximately a thousand fold and targets mispaired bases that arise through errors in replication, recombination or due to DNA damaging agents. Ablation of MMR activity has been linked to carcinogenesis, microsatellite instability, and resistance to chemotherapeutic drugs. Cells containing mutations in MMR genes were shown to be resistant to DNA damaging agents used in cancer

chemotherapy. This is thought to arise due to DNA damage tolerance, where cells acquire non-lethal, highly mutagenic lesions, which persist in the genome due to aberrant repair mechanisms. Many antimalarials are DNA damaging agents and may select for malaria parasites that possess a drug resistance phenotype. This resistance may be directly associated with a loss or decrease of parasite MMR activity. Given *P. falciparum's* rapid development of drug resistance and its unusually A-T rich genome, characteristic of a mutator phenotype, it is not difficult to imagine that the parasite has diminished post-replication repair efficiency. Human homologs of key MMR genes have been identified within the *P. falciparum* genome. Results from our studies have shown that drug sensitive *P. falciparum* lysate has the ability to recognize and repair DNA mismatched substrates. Furthermore, repair efficiency of drug resistant *P. falciparum* lysate is reduced as compared to drug sensitive *P. falciparum* lysate, confering a correlation between defective mismatch repair and increased resilience to antimalarial therapy.

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A TWO-STAGE MODEL OF MALARIA TRANSMISSION AND ITS IMPACT ON THE SPREAD OF RESISTANCE

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Despite substantial attention spent over the years understanding the selection and emergence of *de novo* resistance to antimalarial drugs, there has been relatively less understanding of how resistance spreads geographically. The transmission of antimalarial resistant parasites must be studied within the context of the ecology and epidemiology of the parasite. An earlier epidemiological model concluded that immunity was unimportant for the evolution of resistance, but in that model, immune individuals were not infectious to mosquitoes. Here, we present a new epidemiological model and re-examine how immunity impacts the spread of resistance. The model implicitly models clinical disease, and it considers two immune stages, called naive and immune. Unlike previous models, immune humans remain infectious to mosquitoes, but with reduced infectivity. They are also less likely to develop clinical malaria and use antimalarial drugs. We simulate the evolution of resistance across a range of transmission conditions using rates of antimalarial drug use from recent studies that explores the relationship between the entomological inoculation rate (EIR), clinical immunity, and the incidence of clinical malaria. Because the immune system limits the incidence of clinical disease, it plays an important role in selection because sensitive phenotypes have a natural ecological refuge. Reduced infectivity limits the effect of the refuge, but it does not completely eliminate it. Thus, immunity allows sensitive parasites to persist even when population biological models without explicit immune structure would predict they should go extinct. Thus, immunity does, indeed, play an important role in the evolution of resistance to antimalarial drugs.

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ASSESSING THE ORIGIN AND SPREAD OF DIHYDROFOLATE REDUCTASE AND DIHYDROPTEROATE SYNTHASE MUTANT ALLELES IN *PLASMODIUM VIVAX* POPULATIONS

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Plasmodium vivax is a serious health concern in many regions; inadvertent treatment with sulfadoxine/pyrimethamine (S/P) is common. Mutations in *dihydrofolate reductase* (*dhfr*) confer resistance to pyrimethamine and diminish clinical responsiveness to S/P; the effect of *dhps* mutations on clinical responsiveness to S/P is less clear. Studies in *P. falciparum* have suggested that resistance-conferring mutations in *dhfr* spread via selective sweep. However, mutations in *P. vivax dhfr* and *dhps* may have arisen and spread in a different manner, as *P. vivax dhfr* and *dhps* are highly polymorphic, with a variety of point mutations and insertions/deletions (indels) observed. Single nucleotide polymorphisms (SNPs) and indels both within and flanking *dhfr* and *dhps* provide information about the manner in which resistance-conferring alleles have moved through natural populations. We amplified *dhfr-ts* and a portion of the flanking intergenic region (793 bp upstream/683 bp downstream) from 81 contemporary global isolates; the entire upstream flanking intergenic region (an additional 2513 bp) was amplified from a subset. We found a range of polymorphisms both within the *dhfr* coding region and in the flanking intergenic regions. Based on our haplotype assessment, we have found that the highly resistant *dhfr* quadruple mutant has two distinct origins, one in Thailand and one in Indonesia. The somewhat resistant *dhfr* double mutant 58R/117N, conversely, is associated with at least 10 distinct haplotypes. As well, *pppk-dhps* and its entire flanking intergenic region (497 bp upstream/631 bp downstream) were amplified and sequenced from all isolates; a variety of polymorphisms were found. These polymorphism data are useful in understanding the way in which resistance-conferring mutations have arisen and spread in natural *P. vivax* populations.

(ACMCIP Abstract)

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A DECISION TREE MODEL FOR ESTIMATING THE COST-EFFECTIVENESS OF RECTAL ARTESUNATE TREATMENT FOR SEVERE CHILDHOOD MALARIA AT THE COMMUNITY LEVEL

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While 20% of hospitalized children with cerebral malaria die, 25% of survivors may develop neurocognitive deficits. Severely ill malaria patients cannot tolerate oral treatment. In remote malarious areas, access to health facilities providing parenteral treatment is poor and the quality of care is variable. In such settings, use of artemisinin suppositories is recommended by the World Health Organization to reduce mortality and prevent sequelae from severe malaria. A randomized, double-blinded, placebo-controlled trial has recently showed a significant survival benefit of a single dose of rectal artesunate in young rural children (unpublished). We estimate the incremental cost-effectiveness of pre-referral treatment with artesunate suppositories in comparison to the standard practice of parenteral treatment. A decision-tree model is used to estimate and compare the outcomes and costs for a hypothetical cohort of febrile children under 5 years of age living in an area with perennial malaria transmission. We assume that 45% (range: 30%-60%) of fevers are due to malaria. We use the Piot model, employing a probabilistic framework, to account for factors affecting treatment-seeking behavior of caregivers from the onset of illness to the completion of treatment. We estimate that only 17% (7%-32%) of these children receive antimalarials within 48 hours of fever onset. Treatment alternatives following decisions on oral antimalarial treatment are presented as event pathways in the decision-tree model. Preliminary results assessing the community effectiveness of oral antimalarial treatment indicate a malaria cure rate of 6% (2%-14%). It is estimated that 2%-5% of the treatment failures would progress to severe malaria.

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KNOWLEDGE, AVAILABILITY AND UTILIZATION OF MALARIA PREVENTION MEASURES DURING PREGNANCY IN JHARKHAND, INDIA

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Previous malaria in pregnancy (MIP) studies in India have demonstrated the important role of malaria in maternal and neonatal morbidity and mortality. India currently has a national policy recommending chloroquine (CQ) prophylaxis in pregnancy. However, it is not known whether this policy is widely implemented and whether other effective malaria prevention strategies are routinely utilized in pregnancy. This yearlong cross-sectional study was conducted to evaluate the burden of disease due to MIP at 3 hospital sites, 1 urban and 2 rural, in an area with moderate intensity malaria transmission in the state of Jharkhand, India. One component of the study involved observation of antenatal visits between health care workers (HCWs) and pregnant women (PW) by trained study personnel followed by exit interviews to assess availability and utilization of malaria prevention measures in pregnancy. Detailed facility assessments also were conducted to determine availability of antimalarial medicines, laboratory supplies and bednets. Assessments of 3 facilities and 120 paired HCW observations/ PW exit interviews were conducted during 2007. These observations were noted: 1) Asks about presence of fever 24/120 (20.0%); 2) Assesses for signs/symptoms of anemia 49/120 (40.8%); 3) Measures temperature 21/120 (17.5%); 4) Obtains blood smear 17/120 (14.2%); 5) Checks hemoglobin 65/120 (54.2%); 6) Prescribes antimalarial prophylaxis 1/120 (0.8%); 7) Recommends bednet use 0/120 (0%). Exit interviews included: 1) Malaria a major concern in pregnancy 92/120 (76.7%); 2) Reports bednet in household 109/120 (90.8%); 3) Sleeps under bednet most nights 98/109 (89.9%); 4) Bednet insecticide treated 4/109 (3.7%); 5) Taken chemoprophylaxis in pregnancy 0/120 (0%); 6) Government ever sprayed house w/ insecticide 61/120 (50.8%). The facility assessment showed: 1) Bednets available for sale 1/3 (33.3%); 2) CQ stockout in past 6 months 0/3 (0%); 3) SP stockout in past 6 months 1/3 (33.3%). In conclusion, while bednets are widely available and used by PW in this district in Jharkhand, other malaria prevention measures known to be effective, such as chemoprophylaxis and insecticide-treated bednets, are underutilized. For improved prevention of MIP, policymakers should target these gaps.

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EFFECTIVENESS OF FREE AND MARKET-BASED DISTRIBUTION STRATEGIES FOR ACHIEVING COMMUNITY-WIDE COVERAGE AND PROTECTION WITH INSECTICIDE-TREATED NETS IN RURAL TANZANIA

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Insecticide Treated Nets (ITNs) are a key malaria prevention measure for countries across Africa. National and international policies correctly target subsidies towards infants, under-fives and pregnant women because of their vulnerability to severe disease. However, fewer national malaria control programs promote or aim for broader coverage of the population as a whole even though this is required to achieve a community level mass effect. In 2006 we conducted a household survey in 31 villages in Rufiji District. In each of 2000 selected households, every member available on the day of the interview (6,326 individuals) responded to a structured questionnaire about net use the previous evening. Net use by the population as a whole (62.6 %) was sufficient to support community-level protection. Use was highest for infants (0 - 1 year; 87 %), followed by children one to five years (under-fives; 80.1 %), then over fifteen (adults; 59.6 %). Children between five and fifteen had the lowest proportion of net use, 53.7%. Even though large numbers of nets had been provided for free through vaccination campaigns (28.4%) the majority of nets in use were obtained through private commercial outlets either with (14%) or without (45.7%) a voucher subsidy. More infants slept under nets subsidized through a discount voucher scheme (42%) than any other source whereas under-fives most commonly used nets acquired free during the campaign (53 %). Older children and adults largely used nets purchased at full market price (42.8 and 60.4 %, respectively). In conclusion, although high insecticide treatment levels remain to be assured, Rufiji Disrict has already achieved the Roll Back Malaria targets for coverage of children with bednets. Importantly, voucher subsidies, full-cost market systems and mass distribution campaigns targeting vulnerable groups appear to be mutually compatible. Crucially, free net campaigns had not compromized the commercial sector which extends coverage to untargeted groups, enabling coverage levels that could achieve equitable mass effects which benefit users and non-users alike.

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PATIENT-TO-PATIENT TRANSMISSION OF NOSOCOMIAL MALARIA IN KOREA

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On January 24, 2007, it was reported that *Plasmodium falciparum* was found in a 57-year-old man who had been hospitalized because of phthisis. Neither the man, nor his family had traveled to areas endemic for any malaria recent 30 years. His house was far from an airport or a harbor. Since *P. falciparum* or even vector mosquitoes mediating falciparum malaria have never appeared in Korea, an epidemic intelligence survey was conducted by Korea Center for Disease Control and Prevention. First, the possibility of transfusion malaria was dealt in out after the survey of donors' blood. Under epidemic intelligence survey against the hospital, an important clue was found that a foreign crew had died from *P. falciparum* in the same an emergency ward of the hospital on December, 2006. In the present study, we described nosocomial transmission of malaria from the foreign crew to the Korean man via blood exposure. Genomic DNA was extracted from the two thin blood smears of the foreigner crew and 2ml whole blood samples from the Korean man. The extent variable blocks 2 and 17 regions at merozoit surface protein-1 gene of *P. falciparum* were typed by allelic type-specific PCR and sequenced. DNA extraction and PCR amplification of two samples were conducted at different place and in different time. The sequence analysis revealed that two sequences from the foreign crew and Korean man were exactly same. Both sequences showed same variable patterns in terms of repeat number and position (311111131111121) in block 2 and the same pattern of SNP (Q-KNG-L) in block 17. In order to assume the origin of *P. falciparum*, the full length of MSP-1 gene was cloned by using the DNA from Korean man, and is now under analysis. In this study, strong possibility of nosocomial infection by some reason such as medical staffs or equipments was deduced. The further studies to clarify this infection based on epidemic intelligence are on the way.

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TRENDS IN MALARIA DISEASE BURDEN AT HEALTH FACILITIES IN ZAMBIA

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Malaria is the leading cause of morbidity and mortality in Zambia, with an estimated 3.5 million cases annually. An integrated approach has been adopted to mitigate the burden of malaria in Zambia, with major focus areas being prompt and effective treatment, vector control, environmental management and public education. In 2003, Zambia changed its malaria treatment policy from using Chloroquine as the first line treatment for uncomplicated malaria to the more efficacious Artemisinin combination therapy (ACTs), Artemether-lumefantrine. This antimalarial drug was deployed to all health facilities in Zambia by last quarter of 2004. The efficacy of ACTs has been proven (currently 99% -100%), however the overall effect on health outcomes has not been explored. Currently, in Zambia ACTs are the most widely deployed malaria interventions in the country. To supplement improved case management, preventive measures are being applied (these include, IRS, ITNs, IPT, etc). Survey estimates show that 44% of Zambians own at least one ITN, 62% pregnant women received 3 doses of IPT. Unlike the preventive measures, ACT use is being implemented countrywide. Trends in malaria disease burden before and after drug policy change coupled with intensified vector control strategies were explored. The variables reported on included, malaria incidence, anemia, blood transfusions, malaria admissions and malaria deaths by age group over time (2000-2006). The study conducted a critical review of all health facility records from selected centers and then the same review was conducted for Zambia total disease burden based on HMIS data.
Results: Current trends show that, post drug policy change (including vector control scale up), there has been a reduction in malaria disease burden and the associated health outcomes explored in this study at health facilities. A direct relationship between the health outcomes and malaria incidence was observed. In conclusion, the current integrated approach to control malaria in Zambia has shown to yield positive results. Increase in financing of the programme has improved capacity to implement the action plans in line with RBM, MDG and NHSP goals. Thus with the current tools for malaria control coupled with increase in financing, it is possible to achieve impact.

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MATHEMATICAL MODELS OF IN-HOST MALARIA REGULATION, AND INDIVIDUAL BASED APPROACH TO COMMUNITY TRANSMISSION IN HETEROGENEOUS ENVIRONMENT

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Individual based modeling of malarial transmission offers an attractive alternative to conventional SIR-type methodology (Ross-Macdonald, Diezt et al). It has many advantages, among them relative ease to accommodate various heterogeneities in host, vector and parasite populations. One starts with a host model where single or mixed parasite species interact with host immunity. Immune regulation in a human/mammalian host involves a complex web of multiple cell types, signaling/ effector proteins and reaction pathways. A full account of these components and processes in a mathematical model can be difficult and often impractical. One successful strategy employs a simplified 'feedback-control' regulation mechanism of immunity. We shall outline a few sample models for single and mixed infections. They resemble some familiar patterns of population biology: predation/ competition/ cooperation. Mathematically, they take the form of coupled continuous (differential) or discrete/stochastic equations. We study their basic dynamic patterns (equilibria, stability, recrudescence), and establish their relation to the available data on long-term disease histories. The latter includes both malaria-therapy data of Boyd-Kitchen, and the population survey data obtained in PNG studies. Then we outline several applications of such individual-based communities and 'immune-modulated competition' to (i) long term effect of ant-malarial therapy and the onset of drug-resistance; (ii) effect of preventive interventions (drug therapy and vaccination) on disease severity in young ages; (iii) evolution of parasite virulence through imperfect vaccination.

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HUMAN RESERVOIR OF *PLASMODIUM VIVAX* IN THE LOW TRANSMISSION VILLAGE OF PERUVIAN AMAZON

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Plasmodium vivax is the most common malaria in the Peruvian Amazon where transmission is hypoendemic. We hypothesize that internal migration to areas of high transmission related to logging and fishing appears has an important role in the geographic expansion of areas where malaria occurs. In this way, socioeconomic factors define human reservoirs of infection. A community census was done in Mazán. Active surveillance was conducted with twice weekly home visits. Clinical symptoms and travel information have been systematically collected. Mosquito collections using human landing catches were carried out 2 times monthly. Thick smears were taken every 3 months in all enrollees and if the person developed fever or symptoms compatible with malaria or traveled outside a 10 km radius of the village. The census revealed a highly mobile population. 21% of individuals had traveled outside the village in the month prior to census. The number of prior lifetime infections was greatest among loggers an fisherman.
Monthly surveillance in 317 households revealed an incidence rate of vivax of 0.25 infections/person-year. 2076 febrile episodes were detected, 8.4% of which were positive for *P. vivax* and 2.1% for *P. falciparum*. 62.1% of parisitemic individuals were febrile, 13.4% were afebrile but with other symptoms of malaria, 14% were asymptomatic individuals returning to the village in the last 72hrs, and 10.4% were asymptomatic habitants without recent travel. In all, 24% of people with *P. vivax* parasitemia were asymptomatic. Individuals traveling outside the 10km perimeter around the town in the last 72 hours were 3.63 times more likely to be parasitemic with *P. vivax* and twice as likely to have asymptomatic *P. falciparum* parasitemia as non-migratory residents. The most abundant species of Anopheline mosquito collected was *Anopheles* (Nys.) *darlingi* (84.8%). Peak biting of darlingi took place between the hours of 8p and 9p (0.086 bites/person*hr). Human and entomologic data support our hypothesis that persons infected with malaria are likely to have contracted it outside the limits of the village. Migratory labor appears to be the major determinant of human reservoirs of *Plasmodium vivax* in Mazán. Malaria control in such areas could be achieved most efficiently by the deployment of a targeted intervention to definable high risk groups using either ITN, chemoprophylaxis or a transmission blocking vaccine

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LIMITED SEQUENCE VARIATION IN THE *PLASMODIUM FALCIPARUM* SPOROZOITE THREONINE-ASPARAGINE-RICH PROTEIN AMONG CLINICAL ISOLATES

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The sporozoite threonine-asparagine-rich protein (STARP) of *Plasmodium falciparum* is a promising component for a pre-erythrocytic stage malaria

vaccine because both naturally acquired and experimentally induced antibodies against STARP protein can block sporozoite invasion into hepatocytes. To date, population-based sequence analysis has not been available to elucidate the extent of sequence variation in this gene. Here, we recruited 101 isolates of *P. falciparum* from diverse endemic areas in Thailand which contained single genotype as determined by analysis of the merozoite surface protein-1 and -2 genes. The complete STARP sequences of these isolates, encompassing 2 exons and an intron, were directly determined from PCR-amplified products generated from a proof reading DNA polymerase. Results revealed that the 5′ non-repeat region contained 4 nonsynonymous substitutions while perfect sequence conservation occurred in region M. Both microheterogeneity of sequence and insertion/deletion of repeat units have been observed in the tandem 45 and 10 amino acid repeat regions among Thai isolates. However, sequence of the 3′ non-repeat region was highly conserved except a few codon changes. Nucleotide diversity and haplotype diversity (±S.D.) were 0.0024±0.0001 and 0.936 ±0.011, respectively. Analysis of polymorphism in the STARP gene excluding the repeat regions using Tajima's D, Fu and Li's D and F parameters yielded negative but not significant values. Likewise, the McDonald and Kreitman test for these regions did not show significant departure from neutrality. Alternatively, the paucity of nucleotide substitutions in the STARP gene could compromise the sensitivity of these tests. Meanwhile, the rate of nonsynonymous substitutions per nonsynonymous site (dN) significantly outnumbered that of synonymous substitution per synonymous site (dS) ($p<0.05$) in the 5′-non-repeat region, suggesting that positive selection would operate in this part. Whether immunogenic epitopes locate in the N-terminal portion of STARP protein would require further investigation. Nevertheless, the highly conserved STARP sequences as determined from a large number of clinical isolates encourage the incorporation of this protein into a malaria vaccine.

(ACMCIP Abstract)

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DETERMINANTS OF INSECTICIDE-TREAT NET (ITN) USE AMONG CHILDREN UNDER FIVE YEARS OF AGE IN ZAMBIA: RESULTS OF A NATIONAL MALARIA INDICATOR SURVEY

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Zambia is aggressively scaling up malaria interventions to meet national coverage targets. During the rapid scale up interval, we examined the availability and use of insecticide-treated nets (ITNs) and examined factors associated with ITN use among children under 5 years of age in Zambia. Data from the 2006 Zambia National Malaria Indicator Survey (MIS), a nationally-representative household survey based on a two-stage cluster design, were used in the analysis. Households were surveyed on the availability and use of ITNs through a full net roster based on the Demographic and Health Survey (DHS) malaria module and the Roll Back Malaria (RBM) Monitoring and Evaluation Reference Group (MERG) recommendations. Odds ratios were used to associate desired ITN usage behavior with background household (rural/urban, asset-based wealth index), children (age, gender) and net characteristics, overall measures of net availability and mother's knowledge of malaria and prevention methods. Nearly 50% of Zambian households reported having at least one mosquito net, with 47% of households reporting having at least one ITN. The mean net-to-occupant ratio was 0.19 and 0.20 for rural and urban areas, respectively. Of available nets in households, only 70% were used by someone the night before the survey, with slightly higher usage of nets reported in rural areas (71.3%) than urban areas (67.1%). Forty percent (40%) of nets were long-lasting insecticidal nets (LLINs) as identified by their brand by survey respondents. Among children under 5 years of age, 23.4% of children in rural areas and 21.4% of children in urban areas slept under an ITN the night before the survey among rural households (42.7%) and urban households (48.9%) that have have at least one ITN. Having at least two ITNs in the household, being the youngest child, and the mother's knowledge of ITNs as way to protect oneself from malaria, were significantly associated with higher usage among children under five. In conclusion, results support current national and international efforts to scale-up malaria control interventions, specifically ITNs, and emphasizes the need to focus on barriers to consistent ITN use among children. National malaria control programs must integrate ITN use promotion strategies into ITN delivery programs to bridge the gap between ITN ownership and use and to ensure that national and regional targets are met.

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TREATMENT SEEKING BEHAVIOR OF PATIENTS WITH *PLASMODIUM FALCIPARUM* OR *P. VIVAX* INFECTION IN PAPUA, INDONESIA

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Although infection with malaria is common in Indonesia, treatment seeking behavior in patients with either *Plasmodium falciparum* or *P. vivax* infection has not been well documented. The aim of this study was to ascertain the treatment seeking behavior of patients with fever to facilitate appropriate therapeutic interventions to control malaria. A cluster randomized survey of treatment seeking practices was conducted on 825 households in Timika, Papua Indonesia. Malaria infection was diagnosed by microscopy and by symptoms described by the respondents using a recall period of one month. The prevalence of asexual parasitaemia was 16.3% (634/3,890) with the rate slightly higher for *P. falciparum* (7.5%) compared to *P. vivax* (6.4%). Overall 35% (1813/5255) of people questioned reported a febrile episode in the preceding month, with further details on treatment seeking behavior available in 65% (1177/1813) of cases. In total 26% (302/1177) of people did not seek treatment for their febrile illness, primarily because they did not feel unwell enough (62% 198/302). Of those seeking treatment 353 (40%) went to a public or malaria control clinic, 239 (27%) went to a pharmacy or drug store, 197 (23%) to a private clinic and 86 (10%) took treatment at home. Of those patients seeking treatment 60% (521/875) received an antimalarial drug and 399 (46%) had a blood test for malaria which was confirmed in 313 (78%) of cases. Although there were no significant differences between age groups, there were according to ethnicity; only 57% (340/601) of Papuans sought treatment outside the home for fever compared to 78% (449/576) of non Papuans (OR=2.7 [95%CI 2.1-3.5]; $p<0.001$). In conclusion, there were significant differences in treatment seeking behavior between different ethnic groups in this region. More than half of patient sought treatment at private clinics or from purchasing over the counter medicines.

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MORPHOLOGIC AND MOLECULAR ANALYSIS OF MALARIA AND MALARIA-LIKE PARASITES IN WILD MACAQUES, SOUTHERN THAILAND

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Southeast Asian macaques are considered to be natural hosts for a number of nonhuman primate malaria, some of which can establish infection and produce symptoms in humans upon natural, accidental or experimental transmission. We have conducted a cross-sectional survey of malaria in wild macaques in Ranong Province 570 kilometers from Bangkok, southern Thailand using morphological study of malaria by giemsa-stained blood smears and analysis of the small subunit ribosomal RNA (SSU rRNA) sequences. Blood samples were collected from 21 macaques, morphologically identified as *Macaca fascicularis aurea*, by capture-and-release method without an uneventful consequence. Results of giemsa-stained thin and thick blood smears revealed 8 positive samples (38%) for malaria and malaria-like parasites while 6 isolates (29%) yielded positive test by PCR targeting the SSU rRNA sequences, spanning 2.2 kilobases. However, it was not possible to determine the malaria species unequivocally based on morphology in blood smears. Identification of the A- and S-types of the SSU rRNA genes was obtained from sequencing of the PCR-amplified subclones from each isolate. Comparison of the A-type sequences in this study with those in the GenBank database has identified *Plasmodium inui* in 4 macaques, two of which were co-infected with *P. coatneyi* and *Plasmodium* spp. Interestingly, microheterogeneity occurred in the A-type SSU rRNA sequences of *P. inui*, suggesting that strain variation exists in this malaria species. Phylogenetic analysis revealed that the homologous sequences of malaria-like parasites found in 2 other macaques displayed a distinct cluster from those of plasmodia species. We further determined the mitochondrial cytochrome b sequences of these malaria-like parasites and confirmed them to be *Hepatocystis* spp., a hemoprotozoan parasite of nonhuman primate transmitted by *Culicoides* spp. Consistent results were obtained when analysis was done using the S-type SSU rRNA sequences. Importantly, *P. inui* has previously been reported to cause accidental infection in laboratory workers. Whether wild macaques can transmit this malaria species to humans under natural transmission cycle would require further surveillance.

(ACMCIP Abstract)

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BEDNET OWNERSHIP IN KENYA: THE IMPACT OF 3.4 MILLION FREE NETS

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In July and September 2006, 3.4 million long-lasting insecticide-treated bednets (LLITNs) were distributed free in a campaign targeting children under 5 years (CU5s) of age in 46 districts with a high burden of malaria in Kenya. We conducted a survey to evaluate who received campaign LLITNs, who owned insecticide treaded bednets (ITNs), and how these nets were being used. We evaluated the impact of a distribution strategy aimed at a single target population (CU5s) on ITN ownership and usage in other populations such as pregnant women, women of reproductive age, and ownership in all households. One month after the conclusion of the campaign, we conducted a multi-stage cluster survey of 100 enumeration areas in 20 of the 46 districts, reaching 2059 households. Handheld computers (PDAs) with attached global positioning systems (GPS) were used to develop the sampling frame, guide interviewers back to chosen households, and collect survey data.The distribution increased ITN ownership in households with CU5s by 28.7 %: to 74.4 % (95% CI: 71.8, 77.0). 51% of CU5s reported sleeping under an ITN during the previous night versus 4.6% reported in the 2003 Kenya DHS survey. 39.1% of all households received a campaign net, elevating overall household ownership of ITNs to 50.7% (95% CI: 48.4, 52.9 %), versus 30.5% prior to the survey. One third of homes receiving LLITNs in the distribution owned no other nets. 36.3% of pregnant women, a group not targeted in the distribution, slept under an ITN during the previous night (95% CI: 29.0. 43.7%). ITN ownership for HH with women of reproductive age increased by 18.6% (from 40.3% to 58.9%). 48.3 % of all households did not have a CU5 and therefore were not eligible to receive campaign nets. The distribution was successful in reaching a high proportion of the target population, families with children under 5, the risk group most vulnerable to malaria. Free distributions of LLITNs targeting pregnant women have been scheduled. Further work remains to understand and reduce non-usage of owned nets. The targeted distribution strategy substantially increased ITN ownership and usage in non-target populations.

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EVALUATION OF OXIDATIVE STRESS AND ANTI-MSP1-19 IMMUNOGLOBIN G RESPONSE TO MALARIA INFECTION IN PREGNANCY

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Although immunity to malaria is reduced in pregnancy, the maternal immune system still continues to respond to malaria infection by the production of antibodies. IgG has been reported to play significant role in immune response against *Plasmodium falciparum*. Pregnancy and malaria have also been reported to induce oxidative stress by the secretion of reactive oxygen species. The malaria parasite is sensitive to oxidative damage. Anti-MSP1-19 antibodies and reactive oxygen species have been reported to be protective against malaria in children. We therefore investigated the effect of malaria infection on the level of antibody (IgG) response against MSP1-19 (a promising blood stage vaccine candidate antigen) and oxidative stress in pregnancy. A total of 250 pregnant women were studied in Ibadan, Nigeria. Thick and thin blood smears were prepared from each patient for parasite identification and quantification. Anti-MSP1-19 IgG was determined in plasma using ELISA technique. Oxidative status was monitored by estimating levels of malondialdehyde (MDA) as an indicator of lipid peroxidation and reduced glutathione (GSH) using standard spectrophotometric methods. 30% of 115 and 60% of 135 pregnant women were positive for malaria by microscopy during the dry and wet seasons respectively. Parasite density was higher in the wet than dry season. Mean anti MSP1-19 IgG and MDA levels were significantly higher in the dry than wet season. Among primigravidae, MDA and IgG levels were significantly higher among malaria positive relative to negative in both wet and dry seasons. Among multigravidae, IgG level was also significantly higher among malaria positive than malaria negative but the higher MDA level was not significant. GSH level was significantly lower in malaria positive relative to negative in both seasons. Similarly, GSH level was lower among primigravidae compared with multigravidae and increased with gravidity.in both seasons. In conclusion, IgG and MDA levels were positively associated with malaria infection. The result indicates that malaria infection induces immune response and oxidative stress which may be protective in pregnancy.

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RELATIONSHIP OF IL-18 PROMOTER POLYMORPHISM (-137 G/C) WITH SEVERE MALARIAL ANEMIA AND HYPER-PARASITEMIA IN INFANTS AND YOUNG CHILDREN

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Interleukin (IL)-18 is an important cytokine that regulates both innate and acquired immunity. IL-18 promoter polymorphisms have been associated with several autoimmune and infectious diseases. However, association of IL-18 polymorphisms with malaria disease severity is still largely unexplored. To examine the role of genetic variation in the IL-18 gene in conditioning severe malarial disease outcomes in children from a holoendemic *Plasmodium falciparum* transmission area, the relationship of the IL-18 -137 G/C polymorphism, severe malarial anemia (SMA, Hb less than 6.0 g/dL), and hyper-parasitemia (HP >50,000 parasites/μL) was investigated. Children with acute malaria (n=459) were enrolled at Siaya District Hospital in western Kenya. IL-18 -137 G/C genotyping was carried out using a Taqman 5' allelic discrimination by real time PCR. Prevalence of the genotypes were: GG, 79.9%; GC, 17.8%; and CC, 2.3%, with G and C allele frequencies of 0.89 and 0.11, respectively. Multivariate logistic regression revealed that relative to the GG genotype, homozygous C alleles had a non-significantly increased risk of developing SMA (OR 2.37, 95% CI 0.59-9.54, P=0.22). However, relative to homozygous G alleles, heterozygous individuals were non-significantly protected against HP (OR 0.72, 95% CI 0.38-1.36, P=0.31). Taken together, these analyses demonstrate that variation in the IL-18 promoter at -137 may not be strongly associated with malarial disease severity in young children residing in holoendemic areas of malaria transmission.

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DECREASED IL-10 PRODUCTION IS ASSOCIATED WITH LYMPHOCYTOSIS IN CHILDREN WITH SEVERE MALARIAL ANEMIA

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Hematological derangements are common phenomena of acute malaria among children in regions with *Plasmodium falciparum* holoendemicity. Our previous studies showed that lymphocyte count and malaria pigment-containing monocytes were important predictors of severe malarial anemia (SMA) in children. Additional studies have shown that lymphocytes are important mediators of protective immunity during blood-stage malaria. Moreover, IL-10 is associated with lymphocytosis in individuals with Epstein Barr virus and Chagas disease. To further explore the role of IL-10 in modulating lymphocytosis during SMA, we investigated the relationship between lymphocytosis and IL-10 in children (n=174, aged 3-31 mos) presenting with acute malaria at Siaya District Hospital, western Kenya. Complete blood counts were performed by an automated hematological analyzer, while parasitemia was determined on Giemsa-stained blood films. Lymphocytosis, monocytosis and granulocytosis were defined by an absolute count above 5500/μL, 0.8/μL and 8.6/μL respectively. Circulating IL-10 levels were measured using enzyme-linked immunosorbent assay. Levels of IL-10 in plasma were inversely correlated with the lymphocyte count (r=-0.307, P<0.0001). In addition, lymphocytosis was associated with significantly lower IL-10 levels (P=0.002) in children with acute malaria. However IL-10 levels did not differ among children with or without either monocytosis or granulocytosis. Additional analyses revealed that lymphocytosis was most prevalent among children with SMA (P<0.0001). Results presented here suggest that reduced levels of IL-10 production may be important for promoting lymphocytosis in children with SMA.

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THE ROLE OF PFRH INVASION LIGANDS AS TARGETS OF ANTIBODIES THAT PROTECT AGAINST *PLASMODIUM FALCIPARUM* MALARIA

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After repeated exposure to *Plasmodium falciparum* malaria effective immunity eventually develops that influences the outcome of the infection and protects from severe complications. However, the mechanisms of naturally acquired immunity are still poorly understood. Antibodies play an important role, but their targets are largely unknown, and antibody effector mechanisms are not well defined. *P. falciparum* expresses different ligands for invasion of erythrocytes; these include the family of reticulocyte binding homologues (PfRh proteins), which have been recently identified. PfRh2 and PfRh4 have been shown to play an important role in sialic acid-independent invasion of erythrocyte, but their role as targets of acquired immunity has not been established. Our aim is to investigate the importance of PfRh invasion ligands in protective immunity as targets of antibodies that inhibit erythrocyte invasion and protect against malaria. To this end we expressed regions of PfRh2a, PfRh2b and PfRh4 proteins and evaluated antibodies to recombinant proteins by ELISAs using sera samples from longitudinal cohort studies Papua New Guinea and Kenya. By testing different parts of the respective proteins in ELISAs we have identified the most immunoreactive regions and have used corresponding peptide arrays to identify relevant antibody epitopes. We are also examining the relationship between antibodies to specific regions and acquired invasion-inhibitory antibodies. Our results indicate that antibodies to PfRh proteins are acquired with increasing exposure to *P. falciparum* and antibodies to specific regions are associated with protection from symptomatic malaria among children. These results reveal important insights into the acquisition of protective immunity and will contribute to vaccine development.

(ACMCIP Abstract)

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THE IMPORTANCE OF THE ANTIBODY ISOTYPE RESPONSE TO *PLASMODIUM FALCIPARUM* MEROZOITE ANTIGENS IN PROTECTION FROM CLINICAL MALARIA

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Antibodies to merozoite antigens of *Plasmodium falciparum* are thought to play an important role in protection from malaria. Effective immunity is likely to depend on sufficient levels of antibody of the correct specificity, but may also depend on antibody subclass. It has been observed that for some antigens there is a change in the IgG isotype profile with increasing

age and exposure to malaria, and that different antigens appear to induce IgG of different subclasses. This may determine if a response is protective as the subclasses have different biological functions. IgG1 and/or IgG3 appear to be the predominant subclasses to most merozoite antigens. However, the significance of IgG subclasses against merozoite antigens in protective immunity or for antibody function is not clearly understood. To address these issues, we measured IgG isotypes in a cohort of 206 PNG children. All children were initially treated for malaria and subsequently followed by active surveillance over 6 months for re-infection (determined by PCR) and symptomatic episodes of malaria due to *P. falciparum*. Antigens used include the vaccine candidates AMA-1, MSP1-19, and MSP2, as well as other merozoite invasion ligands. We have examined isotype responses to a number of *P. falciparum* merozoite antigens to i) determine the nature of isotype responses to different antigens in the same population, ii) examine whether specific IgG subclass responses are associated with protection from malaria and whether the protective isotype response is consistent across different antigens, iii) determine the effect of age, prior exposure, or active infection on the nature of isotype responses, and iv) examine whether polymorphisms in antigens influence the IgG isotype response. Our findings provide important insights into the nature of the acquired immune response and are highly relevant for vaccine development.

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IN UTERO HUMORAL IMMUNE RESPONSES TO *PLASMODIUM VIVAX* AND *P. FALCIPARUM* ANTIGENS IN PAPUA NEW GUINEA

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In malaria endemic areas, many infants are born to mothers infected with malaria during pregnancy. It has previously been demonstrated that immunity to blood stage *Plasmodium falciparum* may be initiated *in utero* by exposure to malaria infected erythrocytes or parasite products transferred across the placenta. This fetal exposure may result in either the induction of fetal immune priming or tolerance *in utero*. A recent study has shown that the nature of this induced response can affect the development of functional immunity during infancy, and may therefore also contribute to malaria susceptibility. We aimed to examine the extent of *in utero* exposure to *P. vivax* and *P. falciparum* by determining the frequency of prenatal B cell sensitization in infants born in an area of perennial malaria transmission in Papua New Guinea. A cohort of women was followed during pregnancy, with sampling occurring at multiple time points including delivery. Malaria infection during pregnancy was determined by PCR. Humoral responses (IgG and IgM) in cord blood to *P. falciparum* and *P. vivax* antigens were examined. Responses were examined to several antigens as responsiveness may be differentially regulated and be dependent on differing levels of exposure to specific antigens. The presence of IgM was indicative of a fetal immune response as this isotype does not cross the placenta from the maternal circulation. Placental alkaline phosphatase (PLAP) levels were also determined in paired maternal and cord samples to exclude admixture of maternal and cord blood. Our findings provide a unique insight into the extent of *in utero* exposure to *P. vivax* as well as contributing to the growing number of studies examining fetal responsiveness to different *P. falciparum* antigens.

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WHAT WOULD EXPLAIN THE DIFFERENCES IN SUSCEPTIBILITY OF DCS SUBSETS TO MALARIA INFECTION DURING PREGNANCY?

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The importance of dendritic cells (DCs) for the initiation and regulation of immune responses against foreign organisms has raised considerable interest in the qualitative and quantitative analysis of these cells and their activation-maturation in various human diseases. During pregnancy, *Plasmodium falciparum* accumulates in the placenta and is associated with altered immune function and poor birth outcomes. This study, therefore, investigated the impact of *P. falciparum* infection on DCs maturation in West African primi-secondigravid malaria. Circulating dendritic cells in peripheral, cord and placental blood, and their maturation were analysed using flux cytometry technics. In infected pregnant women, plasmocytoid DCs showed aignificant decrease in all compartments; their count was (8,3 ± 3% versus 20,8 ± 3%) in peripheral blood, (17,11 ± 3 % versus 24,5 ± 2 %) in placental blood and (10,86 ± 2% versus 20,19 ± 2%) in cord blood. The HLA-DR expression was also severely impaired both plasmacytoid DCs subsets and in all compartments when compared to uninfected pregnant women. For example, in peripheral blood, HLA-DR expression was (662,01 ± 98 UA versus 1301,24 ± 218 UA) for mDCs ($p < 0,005$); (415,8 ± 40 UA versus 672,9 ± 69 UA) for pDCs ($p < 0,005$ in placental blood, the expression was (662,89 ± 110 UA versus 1421,9 ± 108 UA) for mDCs ($p < 0,005$); (270,05 ± 39 UA versus 438,64 ± 41 UA) for pDCs ($p < 0,005$ and in cord blood the expression was (887,85 ± 118 UA versus 1182,30 ± 83 UA) for mDCs ($p < 0,005$); (306,7 ± 47,8 UA versus 412,35 ± 39 UA) for pDCs ($p < 0,005$). These results suggest a difference in DCs susceptibility to *Plasmodium falciparum* infection and probably a difference in the biology of these two subsets. Our study suggests deep investigations on DCs biology in case of malaria in order to better understand their interaction with *P. falciparum* and malaria immunology. A crucial fact in the control of malaria infection

(ACMCIP Abstract)

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THE ACQUISITION AND MAINTENANCE OF ANTIBODIES AGAINST *PLASMODIUM FALCIPARUM* MEROZOITE ANTIGENS IN CHILDHOOD

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Antibodies to merozoite antigens of *Plasmodium falciparum* are believed to be an important component of acquired protective immunity and are thought to act by inhibiting erythrocyte invasion by merozoites. However, the acquisition, maintenance, and specific targets of naturally acquired humoral immunity are still poorly understood. Associations between antibody responses and clinical parameters or protective immunity have been previously evaluated using data from measuring antibodies at a single time point, but few studies have investigated antibodies to merozoite antigens in longitudinal cohorts with extended follow-up. We have examined the acquisition and maintenance of antibodies to merozoite antigens among a cohort of 300 children aged 0-10 years followed over a 3 year period. Active weekly surveillance for malaria was performed over the entire study period and serum was collected for antibody testing every 6 months. Antibodies to recombinant AMA1

(3 different alleles), MSP1-19, MSP2, and schizont protein extract were measured among all children at each time point by ELISA. AMA1, MSP1, and MSP2 are leading vaccine candidates and are believed to be important, or essential, for erythrocyte invasion by merozoites. By examining antibodies at multiple time points over an extended period our results provide important insights into the acquisition and maintenance of key immune responses. Longitudinal analysis reveals antibodies measured at a single time point may not reliably reflect a person's immune status or immune responsiveness as antibody levels can vary substantially over time. Antibody levels wane quickly and are poorly maintained in many individuals, raising questions about the adequate development of B cell memory. Maintenance of high antibody levels is influenced by exposure, parasitization, age, and genetic factors, and may differ for different antigens. These results have implications for understanding acquired immunity and how it is maintained and may assist the development and evaluation of candidate vaccines.

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ESTROGEN AND PROGESTERONE SYNERGISTICALLY AFFECT RESPONSES TO *PLASMODIUM CHABAUDI* IN FEMALE C57BL/6 MICE

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Previous data from our laboratory illustrate that intact C57BL/6 males are more likely to die during *Plasmodium chabaudi* infection, recover from weight loss and anemia slower, and have lower IFNγ and IL-10 responses than intact females. Removal of the ovaries and, hence, the primary production of sex steroids in females reverses these differences. Thus, sex differences in response to *P. chabaudi* may be mediated by differential transcription and translation of IFNγ and IL-10 that are influenced by estrogen, progesterone, or both. In the present study, female mice were ovariectomized (ovx) and implanted with pellets containing 0.1 mg estradiol (E2), 10 mg progesterone (P4), 0.1 mg E2 + 10 mg P4, or cholesterol (placebo). Females were inoculated ip with 10^6 *P. chabaudi* infected erythrocytes, and body mass, body temperature, anemia, parasitemia, and cytokine production were monitored at various time points post-inoculation. Adminstration of E2, alone or in combination with P4, mitigated infection-induced weight loss, anemia, and hypothermia as compared with ovx females that received placebo pellets. Hormone treatment did not affect levels of parasitemia. Females administered E2 in combination with P4 produced significantly more IFNγ during peak parasitemia than females implanted with either placebo pellets or pellets containing E2 or P4 alone. The effects of estrogen and progesterone on IL-10 production is currently under investigation. Taken together, these data suggest that estrogen and progesterone act synergistically to enhance IFNγ and, possibly, protect females during malaria infection.

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TEMPORAL STABILITY OF BLOOD STAGE MALARIA IMMUNE SURROGATES OF PROTECTION IN A MALARIA HOLOENDEMIC AREA

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In malaria endemic areas, individuals acquire immunity through natural exposure to the parasite. In order to establish solid immune endpoints for human malaria vaccines, it is essential to understand naturally acquired humoral and cellular immune responses to malaria antigens. Functional anti-malaria antibodies are increasingly being evaluated as important correlates of protection, yet little is understood regarding their temporal stability and relation to malaria exposure. We evaluated the temporal stability of functional anti-malaria antibodies and compared them to traditional measures of serology and T cell immunity. We examined responses against merozoite surface protein-1 (MSP-1), the most abundant blood stage malaria protein and vaccine candidate. Blood samples from 16 asymptomatic Kenyan adults living in a malaria holoendemic region were sampled at 6 time points over the course of 9 months. T cell IFN-γ responses to MSP-1 were determined by ELISPOT and antibodies to the 42 kD and 19 kD C-terminal processed fragments of MSP-1 were determined by serology and by two functional assays that quantify a) overall anti-malaria growth inhibitory activity (GIA) and b) invasion inhibition antibodies directed specifically against MSP-1_{19} (MSP-1_{19} IIA). The kappa test of agreement was used to determine immune stability over specified time intervals (3 weeks, 6 weeks, 6 months, and 9 months). Anti-malaria antibodies determined by serology were most consistent over time, followed by T cell IFN-γ and GIA. MSP-1_{19} IIA was the least consistent over time. On the other hand, a temporal boost in MSP-1_{19} IIA levels correlated with increases in rainfall and malaria transmission whereas other measures of MSP-1 immunity did not vary over time. In conclusion, MSP-1_{19} IIA is a transient but potentially sensitive indicator of immunity that reflects recent blood stage infection.

(ACMCIP Abstract)

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SELENIUM LEVELS, MALARIA AND ENDEMIC BURKITTS LYMPHOMA IN WESTERN KENYA

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Endemic Burkitt's lymphoma (eBL) is the most prevalent pediatric cancer in Equatorial Africa. The etiology of this lymphoma is multi-factorial involving early-age infection with Epstein Barr Virus (EBV) and frequent exposure to *Plasmodium falciparum* malaria. Since these co-infections are common in African children living in malaria endemic areas, other eBL risk factors are being investigated. One potential co-factor could be selenium deficiency. Selenium is an essential micronutrient and is an integral component of the antioxidant enzyme glutathione peroxidase (GPX). Many populations worldwide exhibit selenium deficiencies but little is known about selenium deficiency within African populations. Moreover, there is emerging data to suggest the selenium deficiency potentiates viral infections. We hypothesized that deficiencies in selenium could contribute to increased risk for eBL by decreasing host ability to control EBV infection. To test this hypothesis, a cross sectional survey was conducted in children living in a region of Western Kenya that has high incident rates of eBL. Blood was collected from 79 children ages 1 to 11 years, to determine selenium levels using pl*GPx enzyme immunoassay for selenium GPX. In addition, EBV viral loads were measured by RTQ-PCR. The mean selenium levels were 2.4 µg/dl ± 0.7 (range 0.63-3.38 µg/dl). This level is lower than those reported for developed countries where levels range from 6-8 µg/dl (New Zealand and Finland) to 14-25 µg/dl (USA). To test whether the selenium levels correlated with EBV viral load, we measured EBV viral load in the same study participants. Although several children had elevated EBV viral loads, we did not find a correlation of selenium levels with EBV viral load. Our data demonstrates that there is evidence of selenium deficiency in children living this region in Kenya but more studies are needed to determine what impact selenium deficiency has on immune function.

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STERILE PROTECTION AGAINST MALARIA INFECTION REQUIRES TAP IN SPITE OF COMPLETELY OPERATIVE TAP-INDEPENDENT VACUOLAR CROSS-PRESENTATION PATHWAY

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Induction of long-lasting protective immunity to malaria by repetitive immunization with *Plasmodium berghei* γ radiation-attenuated sporozoites (γ-spz) requires MHC class I-restricted $CD8^+$ T cells, implying that γ-spz/liver stage derived antigens have to be presented through one of the cross-presentation pathways. Based on accumulation of proliferating and IFN-γ producing effector/memory $CD8^+$ T cells in the livers of $TAP^{-/-}$ mice, we showed that TAP-independent vacuolar pathway efficiently operates *in vivo* during priming and subsequent boosts with γ-spz. Likewise, infectious sporozoite challenge increased the number of IFN-γ^+ $CD8^+$ T cells in γ-spz immune $TAP^{-/-}$ mice and induced IFN-γ production by wt γ-spz immune $CD8^+$ T cells transferred into $TAP^{-/-}$ environment suggesting that γ-spz/liver stage derived MHC class I specific peptides generated in a TAP-independent manner participate in response to infectious sporozoites. Nevertheless, the pattern of IFN-γ secretion, parasitemia and survival data upon infectious sporozoite challenge clearly indicated that TAP-associated antigen processing is indispensable for sterile protection.

(ACMCIP Abstract)

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LEVELS OF MANNOSE-BINDING LECTIN DURING PREGNANCY COMPLICATED WITH *PLASMODIUM FALCIPARUM* INFECTION IN CAMEROONIAN WOMEN

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During pregnancy, Plasmodium falciparum-infected erythrocytes sequester in the placenta where they induce pathology and increase risks of low birth weight (LBW) babies. In response to the infection, maternal macrophages also accumulate in the intervillous space (IVS) of the placenta where they secrete cytokines and phagocytose parasites. The innate immune mediator mannose binding lectin (MBL) enhances phagocytosis via opsonization. MBL has been shown to bind to malaria-infected erythrocytes. Thus, we hypothesized that pregnant women might produce MBL upon infection and would aid in parasite clearance. Previously, we showed that women who had a genotype for lower production or a nonfunctional form of MBL had an increased risk of delivering LBW babies. In this study, we measured the level of MBL in plasma of 56 Cameroonian women during the course of pregnancy who lived in the city (low transmission) or village (high transmission). Results showed that, in the city, MBL levels were elevated during pregnancy compared to non-pregnant women but remained high in the village. Primigravid women in the city who were blood-smear positive for malaria had higher median MBL levels during the first trimester and at delivery than non-infected women.. Thus, MBL was most likely triggered by microbial infections in addition to malaria in these women. Finally, MBL levels were significantly higher in the IVS and fetal cord blood than in the peripheral blood at term ($p = 0.05$) independently of malaria infections. Thus, it is likely that MBL does not play an important role in clearance of parasites from the IVS.

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DISTRIBUTION OF *PLASMODIUM FALCIPARUM MSP1* ALLELIC VARIANTS IN THE ARTIBONITE VALLEY OF HAITI, 2006

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Both multiplicity of infection and genetic diversity (number of parasite genotypes per infection) can serve as proxy indicators of the intensity of malaria transmission. In addition, genetic diversity may correlate with disease, including severe malaria. The purpose of this study was to evaluate the genetic diversity of *Plasmodium falciparum* isolates from Haiti using the 3 allelic families in Block 2 of msp1 as molecular markers (K1, RO33, Mad 20). *Plasmodium falciparum* isolates were obtained from: 1] Individuals included in a population-based survey to estimate the prevalence of malaria parasite infections in the population (active case detection) and 2] Individuals attending the Hôpital Albert Schweitzer (passive case detection). 29% of the "cases" included in the study were children 11 years of age or younger. A total of 9 distinct msp1 Block 2 amplicons (differentiated by size and allelic type) were detected in 34 individuals. 70% of the analyzed samples were RO33 positive; RO33 was the most frequent and least polymorphic allotype found, with only one genotype of 120 bp. In contrast, K1 was the most polymorphic allotype with 6 genotypes based on amplicon size (ranging from 150 - 250 bp). 59% of the infections harbored 2 or 3 allelic types. The mean number of parasite genotypes per infection was 1.7, with a minimum of 1 and a maximum of 3 genotypes per infection. The island of Hispanola is the only island in the Caribbean where *P. falciparum* malaria is still endemic and chloroquine-susceptible. To our knowledge, this study represents the first examination of genetic diversity in Haiti. The results suggest that there is relatively little genetic diversity. This observation may reflect a low intensity of transmission in Haiti, together with low levels of importation and/or mixing with parasites from other malaria-endemic regions which may help to explain the absence of chloroquine resistance in Haiti.

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HUMAN INSULIN REGULATES OXIDATIVE STRESS AND AGING IN THE MALARIA VECTOR *ANOPHELES STEPHENSI*

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Observations from nematodes to mammals indicate that the insulin signaling cascade (ISC) regulates lifespan. As in other organisms, the ISC is conserved in mosquitoes and signaling occurs in multiple tissues. During bloodfeeding, mosquitoes ingest human insulin. This simple observation suggested that exogenous insulin could mimic the endogenous hormonal control of aging in mosquitoes, providing a new model to examine this phenomenon at the organismal and cellular levels. To this end, female *Anopheles stephensi* mosquitoes were maintained on diets containing human insulin provided daily in sucrose or three times weekly by artificial bloodmeal. Regardless of delivery route, mosquitoes provided with insulin at $1.7x10^{-4}$μM and at $1.7x10^{-3}$μM, doses 0.3-fold and 3.0-fold higher than non-fasting blood levels, died at a faster rate than did controls. In mammals, insulin signaling induces the synthesis of reactive oxygen species and down regulates antioxidants, events which increase oxidative stress and which have been associated with reduced lifespan. In the mosquito, insulin treatment of cells *in vitro* induced hydrogen peroxide synthesis while dietary supplementation reduced total superoxide dismutase (SOD) activity and manganese SOD activity relative to controls. The effects of insulin on mortality were reversed when diets were supplemented with manganese (III) tetrakis (4-benzoic acid) porphyrin (MnTBAP), a cell-permeable SOD mimetic agent, suggesting that insulin-

induced mortality was due to oxidative stress. We have also demonstrated that dietary insulin activates Akt/protein kinase B and extracellular signal-regulated kinase in the mosquito midgut, suggesting that, as observed in *Caenorhabditis elegans*, the midgut may act as a "signaling center" for mosquito aging.

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DISRUPTION OF A PUTATIVE ABC TRANSPORTER IN *PLASMODIUM FALCIPARUM* ALTERS PARASITE GROWTH AND RESPONSES TO ANTIMALARIAL DRUGS

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ABC transporters play important roles in drug resistance and nutrient transport. In the malarial parasite *Plasmodium falciparum,* a homolog of the human multiple drug resistant gene (*Pfmdr1*) has been shown to be involved in various drug responses; and multiple transporters were associated with higher IC_{50} levels in responses to chloroquine (CQ) and quinine (QN) in field isolates. However, subsequent studies could not confirm the associations, although inaccuracy in drug tests in the later studies could have contributed to the lack of associations. Here we performed targeted gene disruption of a putative ABC transporter (PfABC-C2) that was associated with responses to CQ and QN. Parasite clones with disrupted PfABCC2 were obtained from two *P. falciparum* isolates (W2 and Indo). Parasites with disrupted PfABC-C2 grew slower than the wild types and did not grow to a parasitemia higher than 5% under standard culture conditions, possibly due to lower efficiency in removing toxic metabolites produced by the parasites. Compared with wild type parasites, the PfABC-C2 knockout parasites showed changes in sensitivity to the antimalarial drugs QN and mefloquine (MQ), but showed minimal changes in sensitivity to CQ and artemisinine (ART). This study suggests that PfABC-C2 plays an important role in transport of toxic metabolites and antimalarial drugs in *P. falciparum*.

(ACMCIP Abstract)

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DIFFERENTIAL CHANGES IN *PLASMODIUM FALCIPARUM VAR* TRANSCRIPTION DURING PARASITE ADAPTATION TO *IN VITRO* CULTURE

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Plasmodium falciparum erythrocyte membrane protein 1 (PfEMP1), encoded by the *var* multi-gene family, is expressed on the surface of *P. falciparum* infected erythrocytes and has been implicated in many of the complications associated with falciparum malaria. Transcriptional switching of *var* is commonly investigated using *in vitro* cultured parasites, as parasite material from patients is very limited. To ensure these *in vitro* cultured parasites adequately represent parasites within patients, we investigated the affect that short term *in vitro* cultivation has on *var* gene transcription in patient samples. A significant reduction in overall abundance of *var* transcripts was observed during the first ~10 days of culture. The rate of down-regulation was not constant among all *var* genes with certain groups of genes having significantly faster rates compared to others. Transcriptional changes in *var* induced by different external stresses such as parasite density and drug pressure have been considered in an effort to elucidate factors pertinent in generating the observed changes. Until the mechanism/s underpinning the transcriptional changes are better understood the results of this study have significant implications for investigating associations between *var* transcription and clinical manifestations using parasites that have been enriched by *in vitro* culture.

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POPULATION STRUCTURE OF *PLASMODIUM FALCIPARUM* IN THE PHILIPPINES

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Molecular analysis is a powerful tool in the epidemiology of malaria. In the present study, we attempted to estimate levels of genetic diversity and differentiation of *Plasmodium falciparum* populations in the Philippines, and also tried to evaluate a correlation of the extent of genetic diversity of the populations with their malaria endemicity. We used 9 microsatellite DNA loci of the parasite genome to analyze 67 *P. falciparum* isolates from 3 malaria endemic provinces (Kalinga, Palawan, Davao del Norte) in the Philippines. Expected heterozygosities of the populations in Kalinga, Palawan and Davao del Norte were 0.35, 0.56 and 0.50, respectively. Effective population sizes of the populations in Kalinga, Palawan and Davao del Norte were 800, 3312 and 2309, respectively, by stepwise mutation model. These results indicated that the Kalinga population was genetically less divergent than the Palawan population. To estimate the genetic differentiations among the 3 populations, we calculated F_{ST} values among them. They were 0.11 (Kalinga - Palawan), 0.14 (Palawan - Davao del Norte) and 0.18 (Davao del Norte - Kalinga), suggesting that the 3 populations were relatively isolated from each other. We also examined multilocus linkage disequilibrium in each population using a standardized index of association (I_A^S). Significant linkage disequilibrium was observed in the Kalinga ($I_A^S = 0.1103$: $P < 0.01$) and Davao del Norte ($I_A^S = 0.1153$: $P < 0.001$) populations, and not in the Palawan population. These results are consistent with the previous reports that, in the regions where malaria transmission is low, the genetic diversity is low and significant linkage disequilibrium is observed in the parasite population, and vice versa. Parasitological survey also revealed that malaria transmission in Kalinga is low (annual parasite incidence (API): 3.6) and the transmission in Palawan is intermediate (API: 16.3). Elucidating the genetic structure of the parasite will provide a useful knowledge for understanding the epidemiology of a certain malarious area.

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DEVELOPMENT OF A *PLASMODIUM* GENERIC, *FALCIPARUM*, AND *VIVAX* SPECIFIC REAL TIME PCR BASED ON 18S RRNA

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Despite decades of pain-staking research in drug and vaccine development, malaria remains a world wide infectious threat infecting over 500 million and killing 1.5-3 million per year. The current "gold standard" assay used for diagnosing all four species of human malaria, *Plasmodium falciparum, malariae, ovale,* and *vivax*, is microscopic identification of parasitized red blood cells using giemsa stain. However, good malaria microscopy requires extensive training, vast technical knowledge, and years of experience without which accuracy and precision are compromised producing false positive and negative results. Poor microscopy can slow time of diagnosis, as well as, development of new drug therapeutics and vaccines. To address these limitations, we have developed a real time polymerase chain reaction (RTPCR) assay from publicly available data capable of detecting the 18S rRNA of *Plasmodium* generic, *P. falciparum*, and *P. vivax*. The assay conditions have been optimized ([MgCl2], [Probe], and [Primer]), the limits of detection/quantification for *P. falciparum* and *vivax* were measured, and the specificity of all primer/probe sets against both *P. falciparum* and *P. vivax* were assessed. Our limit of detection was 1 parasite/µl. Co-efficient of variance was measured above 20% at 10 parasites/µl. Our *Plasmodium*

generic probe/primer set was able to detect both *P. falciparum* and *P. vivax*, while *Pf* and *Pv* specific sets only detected their respective targets.

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IDENTIFICATION AND CHARACTERIZATION OF A NOVEL ASPARAGINES RICH MEROZOITE APICAL PROTEIN THAT IS INVOLVED IN ERYTHROCYTE BINDING AND INVASION BY THE MEROZOITE

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Proteins that coat *Plasmodium falciparum* merozoite surface and those secreted from its apical secretory organelles are considered promising candidates for the vaccine against malaria. In the present study, we have identified an asparagine rich parasite protein (AARP) that harbors a predicted signal sequence, a C-terminal transmembrane region and its transcription and translation patterns are similar to known merozoite surface/apical proteins. Using GFP-targeting approach using an inducible, schizont-stage expression system and by immunofluorescence, AARP was localized to the apical end of the merozoites. Red blood cells binding assays with COS cell surface expressed protein showed that it binds to red blood cells through its N-terminal region with a receptor on RBC surface that is sensitive to trypsin and neuraminidase enzyme treatments. Sequencing of this gene from different *P. falciparum* strains as well as field isolates showed that this N-terminal region is highly conserved. Recombinant protein corresponding to N-terminal region of AARP was produced in its functional form in *E. coli*. Recombinant AARP showed reactivity with immune sera from individuals residing in *P. falciparum* endemic area. The anti-AARP antibodies significantly inhibited parasite invasion *in vitro*. Our data on localization, functional assays and invasion inhibition, clearly suggest role of this protein in erythrocyte binding and invasion by the merozoite.

(ACMCIP Abstract)

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DNA VACCINE TRIALS USING 3 BLOOD STAGE ANTIGENS OF *PLASMODIUM VIVAX* KOREAN ISOLATES

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Since *Plasmodium vivax* malaria re-emerged in the Republic of Korea in 1993, studies have focused on identifying the genotypes of Korean isolate. In order to reveal the protective immune characteristics of *P. vivax* surface proteins in Korean isolates, genomic DNA was prepared and three major vaccine candidate proteins were cloned. That is, DNA fragments of PCR products of DBP, MSP-1, and AMA-1 were subcloned into TA vector and the genes encoding these candidate antigens were sequenced. These confirmed antigens were cloned into pcDNA 3.1(-) vector and expression plasmids were finally constructed. The vector used for expression, pcDNA 3.1(-), were inserted with mutant ubiquitin genes, afterwards, the expression plasmids including each candidate antigen are expected to be generated by the ubiquitin-proteasome pathway *in vivo*. COS-7 cells were transfected with expression plasmids using lipofectamine and these proteins were successfully expressed *in vitro*. AMA-1 was 56.8 kDa and DBP 37.8 kDa in western blotting. Our vector contains mutant ubiquitin and induces the antigen preparation to MHC class I molecules. DNA vectors carrying 3 kinds of major vaccine candidate DNA were inoculated into mice. Our results showed the inoculation of AMA-1-incorporated pcDNA vector induced the increase of IFN-r mRNA signals and total IgG and IgG2a titers. In the same manner DBP and MSP- incorporated pcDNA vector induced the increase of IFN-r mRNA signals. Our results suggest that DNA vector carrying DNA of *P. vivax* Korean isolates induce the immune responses in experimental mice although we don't know whether the immune responses of vaccine are enough inducing the protection after infection or not.

(ACMCIP Abstract)

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THE USE OF MICROSATELITES AND TANDEM REPEATS IN GENETIC POPULATION ANALYSIS OF FIELD *PLASMODIUM VIVAX* ISOLATES FROM BRAZILIAN ENDEMIC AREAS

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In Brazil, 600.000 malaria cases were reported last year and around 70% of these were caused by *Plasmodium vivax*. The strategies for parasite control depend on the understanding of their genetic variability and population structure. The molecular markers are important tools that can help in this understanding. Only a few markers have been described for *P. vivax*, including microsatellites and tandem repeats (TRs). The aim of this work is to search molecular markers that are polymorphic in the Brazilian isolates for populations studies. We selected some polymorphic molecular markers have already described in literature, being 5 TRs. It has been done the variability analyzes of these molecular markers in *P. vivax* - infected individuals populations from four different Brazilian regions (Manaus/AM, Cuiabá/MT, Macapá/AP, Belém/PA). Each locus was amplified using specific primers and DNA extracted from whole blood of the patients as a template. The amplified products were visualized first in agarose gel, and after that they were analyzed in MegaBACE 500 using the software MegaBACE™ Genetic Profiler Software Suite v2.2 and some specific fragments were sequenced. The genetic population analyzes are being done with software Fstat. Until the moment, we observed that all studied loci were polymorphic among brazilian populations. The locus MN25 is been the most variable and the locus MN23 showed the higher number of alleles being the second most variable. The locus MN7 is been the least variable one, showing only two alleles in the studied populations. The *P. vivax* subpopulation from Manaus/AM is been the most polymorphic ones, due to the higher average of alleles. Some samples were selected for DNA sequencing to confirm the size polymorphism showed on agarose gel. They exhibited deletion or insertion of some nucleotides, including the repeat units. Moreover, amplified fragments of the same size showed point mutations, also within of the repeat units. The analysis of several sequences will help to better understand the mechanisms generating the variability in TRs.

(ACMCIP Abstract)

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THE FREQUENCY OF SP AND CQ RESISTANCE MARKERS IN SEVEN DISTRICTS IN ZAMBIA

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Chloroquine (CQ) and sulfadoxine-pyrimethamine (SP) are both failing antimalarials in Zambia as was evidenced by upward surge in all epidemiological indicators of the malaria disease. Chloroquine and SP treatment failures of 52% and 32.6% respectively were encountered in Zambia. Many studies suggest an association between treatment failures to CQ, SP and frequency of resistance markers. The frequency of SP and CQ resistance markers was determined. Molecular typing of point mutations in the *dihydrofolate reductase* (*dhfr*), dihydropteroate

synthase (*dhps*) and *chloroquine resistance transporter* (*crt*) genes was analyzed among children with uncomplicated malaria for the presence of mutations conferring resistance in the *Plasmodium falciparum.* The prevalence of molecular markers for SP and CQ resistance from all sites was high. Most resistant *dhfr* encountered were the triple and double mutants. Double and single mutations were dominant for dhfs. Patients carrying both sensitive and resistant strains to CQ were frequently encountered. Sensitive alleles for SP and CQ were relatively rare or absent. This molecular analysis demonstrates that resistant strains of *Plasmodium falciparum to SP and CQ* exist in Zambia in high propositions. This is the first data demonstrating the alleles responsible for resistance to SP and CQ in Zambia and is essential for policy direction in managing malaria in pregnancy and children weighing less than 5Kg and the return of CQ. In onclusion, in Zambia, SP and CQ treatment failure rates are attributed to high prevalence of SP and CQ resistance molecular mutations in the parasite gene pool detected in multiple sites.

(ACMCIP Abstract)

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RECOMBINATION GENERATING HAPLOTYPE DIVERSITY IN THE LIGAND DOMAIN OF *PLASMODIUM VIVAX* DUFFY-BINDING PROTEIN

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Plasmodium vivax is the human malaria species with a most widely world distribution and is highly endemic in Brazilian Amazon region. The establishing of the disease in the host depends on the merozoite invasion of new blood cells. In *P. vivax*, this invasion is exclusively mediated by interaction of Duffy-binding protein (DBP) to the cognate erythrocyte receptor. Since the immunity against DBP has been demonstrated being allele-specific and the variability being region-specific, it is very important to identify the DBP variants among different malaria endemic areas and the genetic structure of the parasite field populations. In a previously published study, we analyzed the presence of variants in some polymorphic loci identified among Papua New Guinea isolates, however little is yet know about the DBP genetic structure of Brazilian parasites among different geographical sub-populations. The current investigation undertakes a comprehensive analysis of genetic diversity at the binding domain of DBP (DBPII) from different Brazilian populations using DNA sequencing and analyzing the impact of these polymorphisms in the structure of the protein. We report that genetic diversity varies between Brazilian regions, which may reflect differences of transmission intensity, geographic isolation and possible divergent selection between populations. Comparisons of interpopulation genetic divergences reveal that the *dbpII* haplotype repertoire in Brazilian regions overlap only slightly. We also find that meiotic recombination seems to play an important role in generating haplotype diversity at this locus. Mapping of diversity onto a 3D structural model of the protein indicates that some polymorphisms lie close to the binding site and may have functional importance for erythrocyte binding, while others may be likely to bear epitopes for antibody recognition. Further investigations are needed to determine the role of these residues in erythrocyte binding and the importance of allelic diversity for immune evasion.

(ACMCIP Abstract)

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PLASMODIUM VIVAX TRAP: IMMUNOGENICITY AND PROTECTIVE EFFICACY IN RODENTS AND *AOTUS* MONKEYS

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The thrombospondin related adhesion protein (TRAP) is a malaria pre-erythrocytic antigen currently pursued as malaria vaccine candidate to *Plasmodium falciparum*. In this study, a long synthetic peptide (LSP) representing a *P. vivax* TRAP fragment involved in hepatocyte invasion was formulated in both Freund and Montanide ISA 720 adjutants and administered by IM and SC routes to BALB/c mice and *Aotus* monkeys. We measured specific humoral immune responses in both animal species and performed a sporozoite challenge in *Aotus* monkeys to assess the protective efficacy of the vaccine. After immunization both mice and *Aotus* seroconverted as shown by ELISA, and the specific anti-peptide antibodies cross reacted with the parasite in IFAT assays. Only two out of six immunized animals became infected after *P. vivax* sporozoite challenge as compared with four out of six animals from the control group. These results suggest that this TRAP fragment has protective potential against *P. vivax* malaria and deserves further studies as vaccine candidate.

(ACMCIP Abstract)

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A PHASE I/IIB RANDOMIZED, DOUBLE-BLIND, CONTROLLED CLINICAL TRIAL OF THE SAFETY, IMMUNOGENICITY AND EFFICACY OF RTS,S/AS02D, A CANDIDATE MALARIA VACCINE IN MOZAMBICAN INFANTS

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Results from a major Phase IIb trial of GlaxoSmithKline's malaria vaccine candidate in children aged 1 to 4 years in Southern Mozambique showed that the vaccine has a good safety profile and is immunogenic. Efficacy against clinical and severe disease was demonstrated, as previously reported. In accordance with the clinical development plan, we conducted the first trial in infants of this candidate vaccine. The RTS,S-based candidate malaria vaccine is being developed to be integrated into the Expanded Program on Immunization (EPI) in malaria endemic regions of Sub-Saharan Africa. The primary objective of this study was to assess vaccine safety of RTS,S/AS02D when administered two weeks post DTPw/Hib vaccine in young infants. It also provided key development information on the non-inferiority of the hepatitis B response induced by RTS,S/AS02D compared to a licensed hepatitis B vaccine, and proof-of-concept of efficacy. RTS,S/AS02D or the comparator vaccine (*Engerix-B*™ hepatitis B vaccine [GSK]) were administered at 10, 14 and 18 weeks of age staggered with the administration of *TETRActHib*™ (DTPw/Hib [Sanofi Pasteur]) at 8, 12 and 16 weeks of age. After each vaccination, infants were followed up at home for 7 days to evaluate reactogenicity. Hematology, renal and hepatic function were measured at one week post Dose 1, one month post Dose 3, 3½ months post Dose 3 and 12 months post Dose 3 of RTS,S/AS02D or *Engerix-B*™ vaccines. Anti-HBs and anti-CS antibody titers were measured one month post Dose 3. Cases of malaria infection by *P. falciparum* were monitored by active detection of infection up to 12 weeks after the third dose of study vaccine (RTS,S/AS02D or *Engerix-B*™) and by passive case detection at health facilities in the study area. Serious adverse events were reported throughout the study period. Children will be followed up to 14 months after their first dose of *TETRActHib*™. Results corresponding to the safety, immunogenicity and efficacy analyses up to 6 months after the third dose of RTS,S/AS02D or *Engerix-B*™ vaccines will be presented.

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EFFECT OF CPG ON STABILITY OF BSAM-1/ALHYDROGEL FORMULATION

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BSAM-1/Alhydrogel, a tetravalent candidate vaccine against the blood-stage malaria parasite, is composed of AMA1-FVO, AMA1-3D7, $MSP1_{42}$-FVO and $MSP1_{42}$-3D7 formulated on Alhydrogel (aluminum hydroxide) at a ratio of 1:1:2:2. The immunogenicity of Alhydrogel-based vaccines can be greatly enhanced by the addition of the CpG oligodeoxynucleotide. However, the biochemical stability of BSAM-1/Alhydrogel in the presence of CPG 7909 has not been reported. In this study, we assessed the effect of CPG 7909 on the stability of BSAM-1/Alhydrogel by determining the level of protein dissociated from the Alhydrogel using SDS-PAGE/silver staining. AMA1, $MSP1_{42}$ and total proteins detected in the supernatant of BSAM-1/Alhydrogel+CPG 7909 formulation following centrifugation were considered dissociated. Protein band density was quantified by the ImageQuant software. CPG 7909 at a final concentration of 1.02 mg/mL was added to 120 µg/mL and 480 µg/mL BSAM-1/Alhydrogel formulations, and the protein dissociation was determined following 0 or 6h incubation at 4°C or 37°C. Protein dissociation was undetectable either in formulation without CPG 7909, or in 120 µg/mL formulation with CPG 7909 under the test conditions. However, protein dissociation was detected in 480 µg/mL formulation in the presence of CPG 7909 at time 0 and increased with higher incubation temperature and longer incubation time. Total protein dissociation increased from 147 ng (3.1 %) at time 0 to 194 ng (4.0 %) at 4°C and to 337 ng (7.0 %) at 37°C over the 6h incubation period. Following 6h incubation, the amount of AMA1 dissociation was approximately 4 or 8 times greater than that of $MSP1_{42}$ at 4°C (155 vs. 40 ng) or at 37°C (366 vs. 43 ng), respectively. CPG 7909 addition did not significantly modify protein integrity at 4°C; however, lower molecular weight fragments were also detected in 480 µg/mL formulation incubated at 37°C for 6h and were identified as degradation products of $MSP1_{42}$-3D7. The results of the present study indicate that low levels of protein are dissociated from BSAM-1/Alhydrogel+CPG 7909 and the biochemical stability and integrity of the vaccine were not significantly altered.

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NOVEL SPOROZOITE ANTIGEN DISCOVERY OF *PLASMODIUM FALCIPARUM* SCREENED USING HUMAN IMMUNESERA

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The pre-erythrocytic malaria vaccine is aimed to block sporozoite invasion and/or liver stage development and therefore to protects humans from the infection. Only small numbers of candidate antigens in this type of vaccine are currently being studied for their potential to induce protective immunity, including CSP (circumsporozoite protein), SSP2 (sporozoite surface protein 2), and LSA1 (liver stage antigen 1). In order to identify novel vaccine candidates, we set up a post-genome strategy as follows. First we selected and cloned 96 genes which are expected to be expressed in sporozoite stage based on *Plasmodium falciparum* genome database, then prepared transcription template through PCR-based high throughput procedures, followed by protein expression by the wheat germ cell-free system. Using this approach, we succeeded in obtaining 88 (92%) recombinant proteins, with the majority of proteins expressed in the soluble form. All of these proteins were then screened by ELISA for reactivity to panels of immune sera from naturally immune individuals in Thailand. Following affinity purification of the selected recombinant proteins, we raised polyclonal mouse and rabbit sera for antigen characterization studies such as parasite stage expression and subcellular localization. This characterization by immuno fluorescence assay (IFA) have identified 7 novel proteins expressed in sporozoite stages, having 5 expressed on the surface and the other two in the cytoplasm of the sporozoite. Future studies will help to qualify the potential of these antigens to serve as vaccine candidates.

(ACMCIP Abstract)

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TRANSMISSION-BLOCKING ACTIVITY OF DNA VACCINE ENCORDING *PLASMODIUM VIVAX* GAMETOCYTE PROTEIN, PVS230

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The aim of the transmission-blocking vaccine (TBV) is to interfere the development of malaria parasite in the mosquito and prevent next infection to the host. The target molecule of TBV that is under the clinical development is currently Pvs25 alone in *Plasmodium vivax*. However, since the Pvs25 molecule is expressed mainly in the mosquito vector, we would not expect the boosting effect by the natural infection after the immunization. Here, we identified gametocyte protein, Pvs230, may be the basis for an infection-boostable TBV. Pvs230 has seven cysteine motif domains that were conserved among orthologs in *Plasmodium* spp. Transcription of Pvs230 gene was specifically detected in gametocyte-stage parasites. We produced mouse antiserum against the first and the second cysteine motif domains of Pvs230 by DNA immunization. This antiserum reacted with native protein of *P. vivax* gametocytes by western-blotting analysis, and Pvs230 was localized on the gametocyte surface determined by confocal microscopy. In addition, feeding Anopheles dirus mosquitoes with a mixture of this antiserum and gametocytemic blood derived from *P. vivax* patients in Thailand resulted in interference of oocyst development in mosquito midguts. Accordingly, Pvs230 is involved in the transmission-blocking, and could be expected as the novel TBV candidate in *P. vivax*.

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A PLATFORM FOR GENERATING CONJUGATED MALARIAL VACCINES TO *PSEUDOMONAS AERUGINOSA* EXOPROTEIN A

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The potential of conjugate vaccines to significantly increase antibody responses is well established and has recently been demonstrated for the *Plasmodium falciparum* transmission blocking vaccine candidate Pfs25 when conjugated to the *Neisseria meningitidis* serogroup B outer membrane complex or *Pseudomonas aeruginosa* ExoProtein A (rEPA). Based on the findings for Pfs25 conjugated with rEPA, we aimed to develop a platform for generating conjugate malarial vaccines. $MSP1_{42}$ is a leading blood-stage malaria vaccine candidate but it is increasingly evident that the best of our current vaccine formulations do not induce sufficient levels of antibodies to markedly interfere with parasite development *in vivo*. We selected $MSP1_{42}$FUP for conjugation to rEPA

due to the presence of a free cysteine residue, which provides a free thiol for chemical conjugation to an activated maleimide derivatized rEPA containing either an aliphatic spacer (APA) or a hydrophilic spacer (PEO). The conditions for rEPA modification were developed using a design of experiment for multivariant analysis of temperature, linker concentration and incubation time. The activated rEPA was characterized by an Ellman's reaction, reverse-phase HPLC and maldi-mass spectroscopy. Conditions were selected such that the molar ratio is approximately 1 rEPA with 4 to 5 linkers. The conjugation reaction was conducted by mixing modified rEPA with $MSP1_{42}$FUP at a 1: 6.2 molar ratio, which was empirically optimized by small-scale conjugation studies. The conjugated proteins were purified by size exclusion column chromatography and analyzed using SDS-PAGE, Western blot, SEC-MALS-HPLC, sedimentation velocity and amino acid analysis. The ratios of $MSP1_{42}$FUP to rEPA for $rEPA_{APA}$ and $rEPA_{PEO}$ conjugates were 3.9 and 3.8 based on amino acid analysis, respectively. The overall recovery of $MSP1_{42}$FUP was about 40%. The results of small animal immunogenicity studies with these conjugates will be presented. This platform is suitable for conjugating rEPA to other malarial antigens containing an innate or bioengineered free cysteine.

(ACMCIP Abstract)

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PLASMODIUM VIVAX VACCINE: IMMUNOLOGICAL CHARACTERIZATION OF NEW CANDIDATE VACCINE USING GENOMIC AND PROTEOMIC DATA

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The recent explosion of genomic sequencing has made available a wealth of data that can now be analyzed to identify protein as potential targets for vaccine development. Actually, more than 70 proteins from *Plasmodium falciparum* had been discovering to use as a development vaccine however in *P. vivax,* only 5 candidate vaccines had tested in clinical trial. We decide to study new candidate vaccine using genomic/proteomic tools, to asses the antigenicinity using sample from individual endemic area and immunogenicity of DNA vaccine in murine model. We standardized an eukaryotic system to express new antigen and evaluate their immunological recognized by IFA test with serum from endemic area and immunized mice. To evaluate the cellular response we carried out the *ex vivo* assay to determined IFN-γ production using cells from exposed and unexposed individual samples. Additionally, spleen and node lymphatic cell from mice were evaluated. We identified, ten new genes/proteins have the following characteristics: a gene length <5 Kb; they are encoded by a single exon; the presence or absence of predicted signal peptides, signal anchors, or transmembrane domains; orthology to genes in other *Plasmodium* spp. for which there is proteomic or transcriptome data on the stage specificity. All genes/proteins were expressed in eukaryotic system, and all sequences were verified using automatic sequence and their expression with mab by IFA. The total genes were recognized and correspond to predicted sequence. Sera from 16 exposed individuals recognized three new genes/proteins that corresponding pre-eritrocitic stage. Eight of them recognized one gene/protein from erythrocytic stage, six sera recognized eight genes/proteins that expressing erythrocitic stage, and two sera samples recognized six genes/proteins that corresponding to liver and erythrocitic stage. No genes/proteins were recognized by the sera samples from unexposed individuals. Three genes/proteins were classified with high immunity reactivity since were recognized by all sera. Maybe can used as *P.vivax* vaccine antigens possibly these genes/proteins are important for the hepatic stage *P. vivax* development. We describe a novel strategy to mine genomic sequences databases for the identification and prioritization of novel target antigens and the immunological recognized by naturally exposed humans and mice. Three new antigens could be used to design a multigene malaria DNA vaccine.

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IMPACT OF RTS,S/AS02A AND RTS,S/AS01B ON MULTIPLICITY OF INFECTIONS AND CSP T-CELL EPITOPES OF *PLASMODIUM FALCIPARUM* IN ADULTS PARTICIPATING IN A MALARIA VACCINE CLINICAL TRIAL

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RTS,S is a recombinant protein expressed in yeast, and contains a part of the circumsporozite protein (CSP) sequence of *Plasmodium falciparum* linked to the hepatitis B surface antigen in a hybrid protein. The RTS,S antigen is formulated with GSK Biologicals' proprietary adjuvant systems AS02A or AS01B. A recent trial conducted in semi-immune adults in Kombewa District, western Kenya, tested safety, immunogenicity and efficacy of the RTS,S/AS02A and RTS,S/AS01B vaccines. In this study we sought to determine the molecular impact of these two vaccines on the surviving malaria parasites. The distribution of *csp* sequences and the multiplicity of infecting strains were examined at baseline (199 isolates) and in break-through infections in vaccine trial participants. Comparisons of the multiplicity of infection and of the distribution of *csp* genotypes in parasite isolates from vaccinated individuals and from those receiving a non-malarial vaccine were consistent with similar genotyping analyses conducted in the context of previous trials of the RTS,S vaccine in African adults and children. The cohort in the RTS,S/AS01B arm contained significantly fewer break-through genotypes parasites as revealed by MSP-1 microsatellite markers than the control arm ($P = 0.0258$). This was not the case for subjects in the RTS,S/AS02A arm ($P = 0.1353$). Although some findings at the level of individual amino acids (Q339 in th2r and D371 in th3r) were in opposite directions, analysis of the overall sequence combining both polymorphic t-cell epitopes did not reveal specific strain specificity for either of the vaccine formulations.

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A NOVEL POPULATION GENOMIC APPROACH FOR IDENTIFYING VACCINE TARGETS

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We report a new metric that specifically identifies genetic loci subject to selective pressure from the human immune system. The test is based on the identity of segregating polymorphic amino acid residues in a pathogen population rather than counts of nonsynonymous and synonymous nucleotide mutations, and relies on empirical knowledge of peptide binding affinity to common HLA alleles. We find that this novel metric yields results that are concordant with traditional polymorphism/divergence analyses used to identify immune (diversifying) selection at pathogen genes. For example, previously reported studies found through population genetic analysis that the malaria protein TRAP exhibits strong evidence of immune selection relative to the CSP protein, though both are leading vaccine candidates. Our metric confirms that TRAP is likely subject to much greater immune pressure than CSP, making it a potentially more effective vaccine target. As our method does not require an outgroup genome sequence, nor a complete survey of polymorphism at each gene, it may be more practical as well as more sensitive in some cases for detecting immunogenic genes in pathogen genomes. We further use the approach to demonstrate that human immune pressure is an important driver of genome-wide functional divergence across populations of the malaria parasite *Plasmodium falciparum*. Given the increasing availability

of genome-wide SNP data from pathogen resequencing projects and genotyping analyses, we expect population genomic analyses of immune selection to have an important impact on the future prioritization of genetic loci in vaccine development pipelines.

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E.COLI-EXPRESSED AND REFOLDED VAR2CSA DOMAINS INDUCE ANTIBODIES AGAINST NATIVE STRUCTURAL EPITOPES ON THE SURFACE OF CSA-BINDING PARASITES

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VAR2CSA is the leading candidate antigen for a vaccine against pregnancy malaria (PM), but its large size and polymorphisms pose a challenge for vaccine development. We expressed one GST-tagged (DBL6) and four His-tagged (DBL1, DBL3, DBL4, and DBL5) domains of this antigen from 3D7 parasite in *Esherichia coli*. All His-tagged domains were purified by affinity chromatography followed by reverse-phase HPLC from inclusion bodies in denaturing conditions and then refolded. The GST-tagged domain was expressed and affinity purified in soluble form. After immunization, rabbit and mouse sera were tested for reactivity with 3D7 and FCR3 parasites selected for adherence to the placental adhesion receptor chondroitin sulfate A (CSA). Sera raised against 3D7 DBL5ε recognized VAR2CSA on the surface of parasitized erythrocytes (PE) of both laboratory strains, while sera raised against DBL6ε recognized only 3D7 PE. Reactivity of these sera with the surface of CSA-binding isolates collected from pregnant women is currently being evaluated. This is the first report showing that refolded *E.coli*-expressed VAR2CSA domains are capable of inducing antibodies against native VAR2CSA, and these antibodies cross-react with heterologous VAR2CSA of different parasite strains, an important step in developing a PM vaccine.

(ACMCIP Abstract)

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DEVELOPING DNA-PRIME ADENOVIRUS-BOOST VACCINES FOR THE PREVENTION OF MALARIA

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Malaria vaccine development has been challenging due to the parasite's complex life cycle, large genome, and ability to evade the host immune response. Genetically-based vaccines such as DNA plasmids and viral vectors induce higher levels of CD4+ and CD8+ T cells in humans than recombinant protein-based vaccines, particularly when administered in prime-boost combination. Because strong cell-mediated immunity has been associated with protection against the pre-erythrocytic stages of malaria in animal models, these vaccine platforms may improve vaccine efficacy. Pre-clinical data have demonstrated that priming with DNA and boosting with adenovirus serotype 5 (Ad5) is safe, immunogenic and protective. Based on these findings, Naval Medical Research Center, GenVec Inc. and USAID are developing a DNA prime-adenovector boost regimen for clinical testing, sponsored by US Army. Vaccine constructs encode the *P. falciparum* circumsporozoite protein and apical membrane antigen 1 (mammalian codon optimized), in hope to induce protection against both pre-erythrocytic and erythrocytic stages. In the initial clinical trial, malaria-naïve adults will be divided into study groups based on level of antibody to the Ad5 backbone, defined as an Ad5 neutralizing antibody titer of ≤ or >500, denoting negative (-) and positive (+) status respectively. Our hypothesis is that DNA priming will enhance immune responses relative to Ad5 alone, circumvent the inhibitory effects of pre-existing immunity to Ad5 and induce protection against sporozoite challenge. Groups 1 and 2 [Ad5 (-) and (+) respectively, n=12/group] will receive a heterologous prime-boost regimen containing 3 doses of DNA at months 0, 1 and 3, followed by 1 dose of Ad5 at month 9, while groups 3 and 4 [Ad5 (-) and (+) respectively, n=12/group] will receive a homologous prime-boost with 2 doses of Ad5 at months 3 and 9; all groups will be challenged 28 days after the last immunization. Safety, tolerability, immunogenicity and efficacy will be assessed. The supporting pre-clinical data and rationale for the design of the trial will be presented.

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VECTOR COMPETENCE OF FLORIDA *AEDES AEGYPTI* AND *AE. ALBOPICTUS* TO LA RÉUNION STRAIN (LR2006 OPY1) OF CHIKUNGUNYA VIRUS

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Chikungunya virus (CHIK) is responsible for ongoing large-scale epidemics in the Indian Ocean basin and southern India, raising the possibility of a global CHIK epidemic. These epidemics are due to a new, emergent strain of CHIK, which may be more effective at being vectored by *Aedes albopictus*, previously described as a CHIKvector of secondary importance to *Ae. aegypti*. *Aedes albopictus* is an invasive species in the eastern United States, and can achieve high densities in some locales. In order to characterize the potential for this virus to cause an outbreak in the southeastern United States, we examined the vector competence to CHIK (strain LR2006 OPY1) of Florida strains of *Ae. aegypti* (common in urbanized areas) and *Ae. albopictus* (common in suburban and rural areas), and two well characterized laboratory strains (*Ae. aegypti* Rockefeller and *Ae. albopictus* Lake Charles). Using quantitative real time RT-PCR, we determined infection, dissemination, and total body titer for blood fed mosquitoes 7 days post infection (p.i.) (*Ae. albopictus*) and 3, 7 and 10 days p.i. (*Ae. aegypti*). We found F_1, Florida *Ae. aegypti* to be the most refractory to the virus, although all mosquito strains were highly susceptible to both infection and dissemination. Percent disseminated increased from days 3 to 10 for both strains of *Ae. aegypti*, as did body titer for F_1 Florida *Ae. aegypti*. Our results suggest the southeastern United States would be susceptible to an emergence of CHIK, and that both urban and rural areas are vulnerable. In addition, the comparison of *Ae. aegypti* strains demonstrates the necessity of using recently field-derived material to determine vector competence.

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FIELD COMPARISON OF ANOPHELINE COLLECTION METHODS: CO_2-BAITED CDC LIGHT TRAPS VERSUS HUMAN LANDING CATCHES IN BELIZE, CENTRAL AMERICA

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There is increasing concern over the ethical use of volunteers to conduct human-baited mosquito collections for vector behavior research within disease endemic settings. A pilot study was performed in February 2007 in Belize, Central America to gather preliminary data on tent entry behavior of the target malaria vector, *Anopheles albimanus*, for future insecticide evaluation. Collections were conducted over four nights from 1800-1950 h inside and outside of tents (1 m x 2 m) that were placed adjacent to a known *An. albimanus* habitat (i.e., *Eleocharis* marsh). Two tents contained CO_2-baited CDC light traps and two tents contained Belize Ministry of Health human volunteers. An additional two persons were situated outside at either end of the designated field site. All anophelines

landing on the exposed lower leg of volunteers during a 50-min sampling period were aspirated and maintained in individual cartons. During a 10-min resting period, collectors rotated between tents and inside/outside positions. Trap collection bags were also removed and new ones replaced. A total of 1,686 anophelines were captured including: 492 *An. albimanus*; 455 *An. crucians*; 433 *An. punctimacula*, and 306 *An. vestitipennis*. Similar numbers were collected inside and outside of the tents; however, there was a species-specific difference in the densities collected between the CDC light traps compared to human-landing catches. Overall, human volunteers collected the highest totals of *An. albimanus* (99%) and *An. vestitipennis* (88%), both important malaria transmitters in Belize, as well as *An. punctimacula* (73%). In contrast, 64% of *An. crucians*, a non-vector, were collected from traps. Results from this pilot study indicate that human volunteer catches are more productive at collecting malaria vector species in Belize than CO_2-baited CDC light traps.

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MOSQUITOES IN SPACE AND TIME: METEOROLOGIC AND EDAPHIC FACTORS AFFECTING *CULEX TARSALIS* ABUNDANCE IN CALIFORNIA

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Culex tarsalis varies substantially in its abundance and phenology across the diverse ecological regions of California. Previous publications on the distribution of this species only briefly addressed temporal variations in abundance and were based on the experience of investigators and reviews of published accounts rather than spatially extensive and semi-continuous mosquito trapping records. Using a 10-year surveillance dataset from 868 trapping sites located throughout California, we developed Bayesian Poisson regression models to examine the effects of meteorologic and edaphic factors on the abundance and phenology of *Culex tarsalis*, an important vector of several viruses in the western United States. We also characterized and accounted for spatial and temporal dependence among trap counts, two aspects typically ignored in other studies. Temperature, precipitation, snowpack, land use, and human population density were associated with trap counts, but the direction and magnitude of these associations differed among ecological regions and over time. The predictive value of the models and the relative importance of the predictors will be discussed. Effects of these factors on mosquito distribution and abundance have important implications for transmission of endemic mosquitoborne viruses as well as other viruses with the potential for introduction.

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AUTOMATED DETECTION AND RECORDING OF MOSQUITOES FLYING THROUGH EAVES OF AN AFRICAN VILLAGE HUT

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APTIV Inc's FAST-ID™ technology is designed to count and identify individual flying insects. When an insect flies between a light source and photosensor, a flight signature is captured by a datalogger. Individual insects are automatically identified by comparing their flight signature with a library of signatures using a statistical classifier or an artificial neural network. The APTIV system consists of three components: a light source (sun or artificial), a photosensor and a data acquisition system. These are integrated into a single compact, light-weight and field proven unit. A field trial in Mbita, Kenya in January 2006 was established to test the application of ground based optical sensors (FAST-ID) for malaria early warning and research. The aims of this field trial were to 1. Build a library of recorded flight signatures for the principal mosquito species that vector malaria in western Kenya; 2. Demonstrate the capability to automatically identify malaria vectors using their flight signature; 3. Demonstrate the concept of sentinel huts; and 4. Demonstrate the capability to monitor vector behaviour in the field. A typical village hut in Lwanda, Kenya was fitted with the FAST-ID sensor system. Each wall was fitted with a FAST-ID sensor and IR light source to monitor mosquitoes, entering through the eave gaps, as they passed through a sensed area that ran the length of the wall. The FAST-ID unit, including an IR illuminator, was activated at sunset (about 1800 hours) and turned off at sunrise (about 0600 hours). Immediately after each FAST-ID unit (4 per hut) was shut down at sunrise, the digital flight recording was transferred to a laptop computer and a one hour search for resting mosquitoes in the hut was conducted by two persons. Illuminators were checked at the same time. Recordings in the village hut were undertaken every night for almost three weeks. Individual mosquitoes were recorded flying through the eaves on several different nights. This is the first report of unattended mosquito flight activity recording in a village hut.

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THE EVIDENCE OF INCREASING LEVEL ON SUSCEPTIBLE TO PYRETHROID OF *AEDES AEGYPTI* IN PANG MAI DAENG VILLAGE IN NORTHERN THAILAND

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DDT was used for indoor residual spraying (IRS) to control malaria vectors in Thailand from 1949-1995. In 1995, DDT was terminated from the national malaria control programme with the favor of synthetic pyrethroids. The reason for this removal is unclear but are due mostly to public concerns of its adverse effects on the environmental and health issues. In spite of long term use of DDT, several anopheline mosquitoes remain physiological susceptible to DDT particularly the main malaria vectors in Thailand. In contrast, most *Aedes aegypti* populations from Thailand were found physiological resistant to DDT. In addition, resistance to other organophosphate and carbamate insecticides were also documented in those *Ae. aegypti* populations. Recently, *Ae. aegypti* was reported to resistant to pyrethroids ie. permethrin and deltamethrin. This may possibly due to the increase use of synthetic pyrethroids and/or cross-resistance. During 2001-2007, the susceptibility status of *Ae. aegypti* population from Pang Mai Deang District, Chaing Mai Province was monitored by the World Health Organization (WHO) standard susceptibility test kit. In this assay, the immature mosquitoes were collected from various containers inside the houses and reared to adults. Results revealed that *Ae. aegypti* adults demonstrated the increase in susceptibility level to permethrin with the mortality rate of 69.9 % in 1997 to 88%, 99% and 100% in 2001, 2006 and 2007, respectively. Conversely, high resistance to DDT in *Ae. aegypti* during the period of observation (2001-2007) was documented. The reasons of these phenomena are discussed based on several factors, including the application of insecticides in both private and government sectors. Results of this study would be most benefit to the insecticide management for the vector control in the future.

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LOGISTICS OF LARGE SCALE LARVAL *ANOPHELES GAMBIAE* CONTROL: TRACKING INSECTICIDE APPLICATION WITH DIGITAL TECHNOLOGY

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Attempts to control larval mosquitoes in habitats that are scattered across large rural landscapes are often hampered by the challenge of determining whether every patch of the landscape is visited by control teams. To address this operational issue, we employed personal digital assistants (PDAs) to record movement patterns and frequency of insecticide application by control teams during the course of an Anopheles gambiae larval control trial in western Kenya. The PDAs (Dell Axim X50 with Global Sat GPS) were configured with Navio Global Satellite and Position Tracker software and Visual CE data acquisition software. Locally hired individuals, each assigned a 2 x 2 km rural area for pump sprayer application of larvicide but with little or no experience in use of computers, were easily trained to utilize these devices during the course of their assigned weekly spray rounds, recording land area visited, amount of time on task, and amount of insecticide applied. The workers used the devices to create tracking files consisting of geographic co-ordinates and time intervals documenting movement patterns of each individual, which were dumped into Microsoft Access files for spatial display of data. This process allowed project managers to quantify effort of field teams and monitor quality control of their work.

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LIGHTS, CAMERA, ACTION: A METHOD TO STUDY MALE *ANOPHELES GAMBIAE* MATING BEHAVIOR IN THE FIELD

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The behavior of male mosquitoes is poorly understood and knowledge of the factors that determine male mating success is almost non existent. Understanding these is key to the success of mosquito control strategies based on the release of males (e.g. sterile male release and transgenics), but studies of male An. gambiae and their mating behavior are typically hard to conduct under field conditions. Problems include identifying swarm locations and how to perform noninvasive data collection. To date few studies have quantitatively addressed hypotheses relating to male swarms due to such factors. We present a video graphic and computer assisted method which can be used to analyze questions about male mosquito swarming behavior by tracking their movement in space. The system allows us to examine the movement of individual mosquitoes within a swarm in a two dimensional plane, enabling us to test novel hypotheses about mating behavior. Preliminary results of tests with other species are presented.

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LABORATORY OVIPOSITION RESPONSES OF *AEDES AEGYPTI* TO VOLATILES FROM PLANT INFUSIONS AND CULTURED BACTERIAL ISOLATES FROM PLANT INFUSIONS

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Oviposition responses of gravid *Aedes aegypti* to native and sterile infusions made from the senescent leaves from white oak trees were evaluated in behavioral bioassays. Native infusions were significantly more attractive than sterilized infusions. Bacteria from bamboo and white oak leaf infusions were cultured in R2A medium. Bacterial species were purified and identified through sequencing of 16S rDNA PCR amplicons. The responses of gravid mosquitoes to bamboo bacterial isolates was evaluated in behavioral bioassays as single-species isolates and mixtures of species. Gravid females exhibited a significant response to bacterial cultures composed of a mixture of species. However, the response of gravid females varied markedly between single-species isolates with some isolates being highly attractive while other isolates were repellent.

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IDENTIFICATION OF MOSQUITO PROTEINS INVOLVED IN THE MOSQUITO-BORNE FLAVIVIRUS LIFECYCLE

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Little is known about the specific interactions between pathogens and their vector hosts. We previously reported several common and unique proteins that are differential expressed during dengue virus and West Nile virus infection in the larval derived *Aedes albopictus* cell line, C6/36. Our analysis has expended to include a third flavivirus, Yellow Fever virus, allowing for the identification of common mosquito proteins which may play an important role in the replication of flavivirus from all three of the major mosquito-borne flavivirus clades. Two dimensional differential fluorescence gel electrophoresis was used to identify proteins that are differentially expressed during flavivirus infection. Further confirmation was obtained by RT-PCR in C6/36 cells and *Ae. aegypti* mosquitoes. We group these potential anti-flavivirus targets into one of two categories: antiviral vector proteins and pro-viral vector proteins. We are currently overexpressing specific transcriptional targets and using RNA interference against others in the C6/36 cell line and in *Ae. aegypti* mosquitoes. Our aim is to identify which of these differentially expressed proteins are essential for or help defend against flavivirus in mosquitoes by inhibiting viral replication and reducing transmission of dengue virus and West Nile virus from mosquitoes.

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COMPLEXITIES IN THE RECOGNITION AND DIFFERENTIATION OF VECTORS AND NON-VECTORS OF MALARIA IN SOUTHERN ZAMBIA

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Anopheles arabiensis and *A. funestus* are considered to be the primary vectors of *Plasmodium falciparum* malaria in the Southern Province of Zambia. In addition to these species, routine indoor spray catch, CDC light trap and human landing catch collections for mosquitoes reveal an array of anophelines that either are not known to be involved in malaria transmission or may be easily morphologically mistaken for these confirmed vectors of *Plasmodium*. These observations expose the dearth of knowledge concerning many anopheline species, their feeding behavior and their vector competence. Incorrect identification of "vectors" may lead to misdirection of limited control resources. As an example, our investigations of *A. longipalpis*, a non-vector species easily mistaken for *A. funestus*, has revealed a complex group of molecular types that may make the differentiation of vector and non-vector species even more complex.

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MEASURING THE IMPACT OF UNPREDICTABLE ENVIRONMENTS ON MOSQUITO VECTORS AND IMPLICATIONS FOR DISEASE RISK MODELING

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It is well known that the immature stages of mosquito vectors face many challenges during their development, such as continuously changing temperatures and ephemeral aquatic habitats. Most disease risk models, however, use development and survival data of mosquito vectors under constant temperature regimes, and water is often provided *ad libitum*. We investigated and compared how the variable environment of two

important disease vectors, the tropical dengue vector *Aedes aegypti* L. and the temperate West Nile vector *Culex pipiens pipiens* L., impacts development and survival. More specifically, we studied the impact of alternating temperature regimens by comparing their effects with those at constant temperatures. For rainfall, we investigated if immature populations of both species (eggs, larvae and pupae) are flushed out due to heavy rainfall. A rain simulator was used for these experiments. Egg rafts and pupae of *Cx. p. pipiens* were highly sensitive to heavy rainfall, whereas larvae of the same species, as well as the eggs, larvae and pupae of *Ae. aegypti* were not. Alternating temperatures affected development and survival of both species, but the effect (enhancing or detrimental) was different over the various temperatures tested. Mosquito population models that incorporate these new parameters will be presented and discussed.

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IS VERTEBRATE BLOOD QUALITY CORRELATED WITH THE HOST SPECIES SPECIALIZATION OF AFRICAN MALARIA VECTORS?

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The *Anopheles gambiae* s.s. mosquitoes that transmit malaria in Africa can produce eggs from the blood of many vertebrate species but generally specialize on humans; a behaviour that makes them highly efficient vectors. The ecological and evolutionary factors that have led to this specialization are largely unknown, and identifying them may suggest novel methods for how this behaviour could be changed to reduce transmission. Here I tested one potential explanation for the specialization of *An. gambiae*: that human blood yields higher fitness benefits than obtained from blood of other host species readily available in their environment. Cohorts of *An. gambiae* were provided with human, cow, goat or chicken blood every four days until death by membrane feeder (n= 366), and their survival and total lifetime egg production compared. The hemoglobin content of chicken blood was higher than other hosts ($F_{1,16}=8.71$, $p<0.01$), and mosquitoes consumed a greater mass of blood (as indexed by hematin excretion) from this species than others ($p<0.01$). Mosquitoes fed cow blood had the highest probability of producing at least one egg before death (2.7 times more likely than human blood, $\chi_2=5.77$, $p=0.02$). However, amongst mosquitoes that did produce eggs, the total number was highest on human blood ($F_{3,78}=7.84$, $p<0.01$). The survival of mosquitoes fed human and chicken blood was similar; with these groups living an average of 3-4 days longer than those fed goat or cow blood. Goat-fed mosquitoes consistently had the poorest survival and reproduction. Overall *An. gambiae* that fed on human blood had high fitness, but their survival and reproduction did not consistently rank above mosquitoes fed on all other blood types. Thus while human blood may be a high quality resource for *An. gambiae*, it does not provide a unique benefit above all other readily available hosts that could account for specialization on people. Alternative hypotheses for host species specialization, including variation in defensive behaviour and availability, are currently being investigated in the field.

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FUTURE THREAT FROM VIVAX MALARIA IN THE UNITED KINGDOM

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Malaria was common in the coastal marshes of southern England in the nineteenth century 1900s. Because of the increasing threat from new and emerging diseases, and the likelihood that climate change will expand areas suitable for transmission of vector borne diseases, we explored the likelihood of malaria returning to the United Kingdom. We generated climate-suitability maps for vivax malaria using a simple process-based model driven by temperature alone. The processed-based model was linked with future UKCIP02 climate change scenarios to generate future risk maps for malaria. Since Anopheles atroparvus, the historical vector of malaria, breeds largely in brackish water, we over-laid our climate suitability maps for malaria on areas of saltmarsh to demonstrate where both climate and habitat could support malaria. The process-based model showed excellent correspondence with the clinical data. Our climate-suitability maps show that large areas of central and southern England could support malaria transmission now and increase in extent in the future. Places at greatest risk will be salt marshes lying within these areas. However, the real threat of disease transmission needs to be considered at the local level where data are woefully inadequate. Nonetheless on the available evidence, climate change is not considered a serious threat to increase the risk of vivax malaria returning to the U.K.

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A PREDICTIVE LANDSCAPE MODEL OF *ANOPHELES GAMBIAE* LARVAL HABITATS IN LOWLAND WESTERN KENYA

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A landscape model predicting habitat distribution for *Anopheles gambiae* larvae was developed from a supervised landcover classification of IKONOS satellite images and topographic data in a 10.34 sq. km area of rural, lowland western Kenya. The study area was divided into 12 x 12 meter cells, numbering 71,824. A reconnaissance of potential larval habitats in the area in 2006 revealed 1,198 of which 86% were occupied by Anopheles larvae (19,776 sampled quantitatively) including An. gambiae s.l. Only 1,120 (1.56%) of the 12 x 12 m cells contained habitats, thus a very small area of land was producing malaria vectors in this landscape. A logistic regression model was developed and verified using randomly chosen cells as sampling units. Explanatory independent variables included elevation (habitats were more common at lower elevations); landcover type (positive associations were with maize fields and wet, grassy fields); distance from stream beds (most [76%] larval habitats were within 200 m of a stream); slope of land; and water flow accumulation. The model correctly predicted habitats to occur in 77.6% of randomly chosen cells, and correctly predicted habitats not to occur in 66.8 % of randomly chosen cells. A landscape model like this one could guide efforts to reduce sources of malaria vectors, and could inform other models of malaria risk relative to aggregated distribution of infectious bites. Current work aims to refine the model and to include other local *Anopheles* species.

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STUDIES ON INSECTICIDE USAGE PATTERN AND RESISTANCE STATUS OF *ANOPHELES GAMBIAE* S.S IN THE ASHANTI REGION OF GHANA

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Information on insecticide usage was obtained from seven localities in the Ashanti Region, Ghana in a questionnaire survey from September 2004 to February 2006. Also, blood-fed *Anopheles gambiae* s.l. *(*for *Plasmodium falciparum* infectivity studies) and larvae (reared to adults in the laboratory for bioassays) were collected from the same localities during the same period. Susceptibility levels to some pyrethroid, organophosphate and organochlorine insecticides; were assayed using WHO-guidelines for testing adult mosquitoes. PCR was used to identify *An. gambiae* s.l. to species level, M and S molecular forms of *An. gambiae* s.s and the presence of the knockdown resistance (*kdr*) mutation. 330 farmers, 9 Agric Extension Officers, 47 Agro-chemical Sellers, 468 householders and 39 domestic insecticide sellers were enrolled into

the survey. The survey revealed high levels of agricultural insecticide application (49.7% Lambda-cyhalothrin-, 27.1% Endosulfan-, 12.5% Cypermethrin-, 7.3% Cyfluthrin-, 2.8% Deltamethrin-, 0.3% Bifenthrin-, 0.3% Cypermethrin-Dimethoate-based; *n=288*). Domestic aerosols were found to contain various insecticides but insecticide coils were 100% D-Allethrin-based. Bioassay results revealed overall mortalities of 17% (DDT), 20% (Cyfluthrin), 42% (Permethrin), 48% (Lambda-cyhalothrin), 65% (Propoxur), 75% (Deltamethrin), and 100% (Malathion). Except for Malathion, there was a high level of resistance in the *An. gambiae* populations to all the insecticides tested. The resistance levels correlated with the high insecticide usage at the study sites except for carbamate. All *An. gambiae* s.l. identified were *An. gambiae* s.s (96.8% S-form and 3.2% M-form; *n=1734*). The *kdr* allele was found in 86% of bioassay survivors indicating the *kdr* mechanism as the basis for the observed resistance. The *kdr* phenotypes were found only in the S-form. The overall *P. falciparum* infectivity rates were 0.083 for *An. gambiae* s.s S-form (*n=1172*) and 0.031 for *An. funestus* s.s (*n=32*). None of the *An. gambiae* s.s M-form (*n=50*) was found to be infected.

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MALARIA TRANSMISSION RISK AND IRRIGATION IN NORTHERN GHANA

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Many governments in Africa have sought ways of improving food production by initiating large-scale irrigation projects, involving reclamation of semi-arid and arid areas for cultivation of crops. The introduction of irrigation has substantially prolonged the season during which adult malaria vectors can be found in areas with seasonal rainfall as mosquitoes have access to larval habitats perennially. In general, it has often been assumed that the prevalence of malaria increases in local communities following the development of irrigation and this has been a perennial subject of debate. However, contradictory results are reported on the effect of irrigation on malaria risk in Africa, however, the general trend seems to favor the belief that development of rice irrigation in Africa has seldom resulted in increased malaria transmission A recent study in northern Ghana has shown that prevalence of parasitaemia of children was greater among those residing in the irrigated area than the surrounding non-irrigated areas. We conducted concurrent entomological studies to determine the level of malaria transmission in some communities served by an irrigation system and the surrounding non-irrigated areas in the Kassena-Nankana district in the northern part of Ghana. Although the sporozoite rate in the irrigated area was lower than the non-irrigated area, the high biting rate in the irrigated area, gave a much higher annual entomological inoculation rate (EIR) of 629 than 277 in the non-irrigated area. Factors contributing to the high risk of malaria transmission in the irrigated compared to the non-irrigated areas are discussed against those found in other parts of Africa.

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VECTOR COMPETENCE OF SELECTED KENYAN MOSQUITO (DIPTERA: CULICIDAE) SPECIES FOR RIFT VALLEY FEVER VIRUS

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Rift Valley fever (RVF) continues to be a significant problem in Kenya as well as in Egypt, Yemen, and Saudi Arabia. Mosquitoes collected in the Lake Naivasha region of Kenya were evaluated for their potential to transmit RVF virus (RVFV) under laboratory conditions. After feeding on a hamster with a viremia of $10^{9.7}$ plaque-forming units of virus/ml of blood, *Culex zombaensis* Theobald were highly susceptible to infection with RVFV, with 89% becoming infected. In contrast, *Cx. quinquefasciatus* Say that were fed on the same hamsters, were marginally susceptible, with only 20% becoming infected. Differences in percentages of mosquitoes that developed a disseminated infection were equally disparate, with 55 and 8%, for *Cx. zombaensis* and *Cx. quinquefasciatus,* respectively. Forty-eight percent of the *Cx. zombaensis* with a disseminated infection that fed on a susceptible hamster transmitted virus by bite, indicating a moderate salivary gland barrier. However, the presence of a salivary gland barrier could not be determined for *Cx. quinquefasciatus* because none of the 18 mosquitoes that took a second blood meal had a disseminated infection. These studies illustrate the need to identify the ability of individual mosquito species to transmit RVFV so that correct decisions can be made concerning the application of appropriate control measures during an outbreak.

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TEMPORAL AND SPATIAL PATTERNS OF WEST NILE VIRUS TRANSMISSION IN SAGINAW COUNTY, MICHIGAN, 2003-2005: EVIDENCE FROM MOSQUITO POOLS, DEAD BIRDS, AND SENTINEL PHEASANTS SUGGEST HUMAN RISK FACTORS

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West Nile virus (WNV) was first recognized in Michigan in 2002, where transmission has become endemic, with at least 711 human cases having been recognized. Michigan's Saginaw County Mosquito Abatement Commission (SCMAC) has maintained a mosquito monitoring system since 1982, and began testing these mosquitoes for WNV infection in 2002. We analyzed these data to identify how WNV transmission varies in time and space by integrating laboratory testing of mosquito pools, submitted dead birds, and sentinel pheasants. Mosquitoes were collected by New Jersey Light Traps at 22 sites during May-September, 2003-2005. Specimen preparation and tests for WNV followed CDC guidelines using ≤25 mosquitoes in species-specific pools. Wild dead birds were reported by area residents and an oral swab was taken from each for virus testing. Sentinel pheasants were systematically bled each week for WNV antibodies. Standard, spatial and time-series statistical analyses were undertaken. Totals of 1,471, 2,308 and 704 mosquito pools were tested during 2003-2005, respectively. *Aedes vexans* was most abundant, but had a low minimum infection rate (MIR) (0.06-2.11) compared to less abundant *Culex pipiens* (1.75-4.66) and *Cx. restuans* (1.22-15.63). Annual average wild dead bird infection was 27.5% (11/40), 46.5% (59/127), and 49.2% (31/63) during 2003-2005, respectively. Positive Crows appeared earlier than Blue Jays, but incidence for both species peaked around mid-August. Spatial clusters were found in different locations each year. Sentinel pheasant incidence was 6.6% (33/502), 7.5% (21/279), and 2.7% (9/330) during the same period. Different analyses of time-lagged variability in mosquito abundance during 1989-2005, in relation to precipitation and temperature in that period, showed significant associations with species-specific mosquito abundances. The high WNV infection incidence among mosquitoes and wild birds demonstrates significant transmission. Both *Cx. pipiens* and *Cx. restuans* appear to be important vectors. Our results suggest that transmission to people may be limited by spatial and/or behavioral factors, and that risk varies within the transmission season and over years.

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A MODIFIED Y-TUBE OLFACTOMETER TO INVESTIGATE THE HOST DENSITY-DEPENDENT BEHAVIORAL RESPONSE OF MOSQUITOES

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The ratio between host-seeking mosquitoes and hosts is a critical parameter of mosquito-borne transmission because it affects the number of mosquitoes that will become infected by an infectious host. Host-seeking behavior in mosquitoes is, at least partly, odor mediated. As host odor intensity is a function of host density, the behavioral response of mosquitoes to simultaneous presentation of two odor intensities has potentially important epidemiological implications. We will present results of a series of experiments in which we investigate the behavioral response of female *Culex pipiens* mosquitoes to simultaneous presentation of two host odor concentrations. We use the prototype of a modified Y-tube olfactometer that uses live chicks as odor source and discuss potential future applications of this experimental setup.

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MOSQUITO AND BITING MIDGE MIDGUT STRUCTURES AND PROCESSES THAT MAY AFFECT ARBOVIRUS INFECTION AND DISSEMINATION

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Females of several species of mosquitoes (Diptera: Culicidae) and biting midges (Diptera: Ceratopogonidae) are competent to transmit arboviruses to vertebrate hosts following ingestion of a viremic blood meal, infection of midgut epithelial cells, dissemination of virus into the hemocoel, and finally infection of the salivary glands. Based on histological and immunocytochemical studies, we propose three midgut structures/processes which may influence arbovirus midgut infection and dissemination: (1) the formation and persistence of a remnant of the non-cellular larval foregut intima (LIR) at the foregut-midgut junction in several mosquito species and the biting midge, Culicoides varipennis; (2) early events in the formation of the non-cellular peritrophic matrix (PM); and (3) midgut cell sloughing into the lumen. Each of these, while perhaps not completely blocking virus, is likely to influence which midgut cells become infected and to what extent. The LIR and early-formed layers of PM may act as physical barriers relative to the midgut cell membrane surfaces. The LIR is located in the proventriculus and could influence virus contact with the cardial epithelium. This is the first report of a LIR in biting midges. The idea of PM acting as a physical barrier to arbovirus infection has been challenged on the basis that its formation is not complete until long after virus would have engaged receptors on the midgut cell surfaces. However, histological studies, e.g. of Aedes triseriatus (Diptera: Culicidae), have revealed a well-defined layer of PM, perhaps not completely formed, but a distinct layer none-the-less, in the region of the anterior-posterior midgut junction and in the region of the pyloric valve. By removing infected cells from contact with the rest of the midgut epithelium, cell sloughing may have a modulating influence on midgut cell infection and the rate of cell sloughing may very between mosquito strains and species.

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EFFECTS OF LANDSCAPE PATTERNS AND BIRD COMMUNITY COMPOSITION ON WEST NILE VIRUS TRANSMISSION PATTERNS IN CT

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The intensity of West Nile virus (WNV) transmission is highly variable within and among urban areas in southwestern CT. The most important enzootic vector for WNV in the northeastern US is Culex pipiens, an urban/suburban mosquito that feeds predominantly on birds, especially the American robin. We examined whether differences in Cx. pipiens infection rates were associated with certain landscape patterns, the abundance of reservoir-competent bird species, and bird diversity in 21 sites in CT. We found that the best fitting negative binomial regression model included mosquito abundance, three landscape metrics (number and shape of commercial patches and patch diversity), and the proportion of three bird species (European starlings, Mourning doves and Gray catbirds). In addition, in a subset of four sites, we examined the degree of host specialization of Cx. pipiens by comparing the proportion of Cx. pipiens blood meals from a particular bird host with the proportion of that host in the bird community. We found that Cx pipiens preferentially fed on American robins and that the preference was higher in the most urbanized sites, where the proportion of robins was actually lower. Our research showed that landscape and bird community are highly predictive of Cx. pipiens infection rates and should therefore be included in models estimating entomological risk. The finding that landscape patterns may affect the degree of Cx. pipiens host specialization may have important implications for WNV transmission dynamics and merits further study.

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COMPARATIVE CLUSTERS OF ORTHOLOGOUS GENE ANALYSIS OF *BABESIA, PLASMODIUM* AND *THEILERIA*

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At 8.2 Mb, *Babesia bovis* has one of the smallest apicomplexan genome sequenced to date. Structural features of the *B. bovis* and *Theileria parva* genomes are remarkably similar, and extensive synteny is present. In contrast, *B. bovis* and *Plasmodium falciparum* have major differences in genome size, chromosome number, and gene complement. Similarity clustering using the predicted proteomes of *B. bovis*, *T. parva* and *P. falciparum* created 1,945 three-way Clusters of Orthologous Groups (COGs). The data were then used to search for *B. bovis* orthologs of proteins that have been characterized in *T. parva* and *P. falciparum* as targets of protective immune responses, as well as those that play a role in stage-specific parasite biology. Many *P. falciparum* sexual stage-specific genes do not share Jf-COGs with *T. parva* or *B. bovis*. Likewise, *P. falciparum* sporozoite genes that are expressed initially in the mosquito also do not cluster with *T. parva* or *B. bovis*. Large blocks of synteny are evident between *B. bovis* and *T. parva* chromosomes although synteny rarely extends to telomeres. At a gross level *B. bovis* chromosomes 2 and 4 primarily consist of sections of *T. parva* chromosome 4 and 2, and 3 and 1, respectively. *B. bovis* chromosome 3 contains sections from all four *T. parva* chromosomes, while *B. bovis* chromosome 1 contains DNA from *T. parva* chromosomes 3, 1 and 2. Closer examination of syntenic blocks indicates that inversions in gene order have also taken place. The completion of the *Babesia bovis* genome project allows for a detailed comparison between *Plasmodium* and *Theileria*. Findings generated from this study will aid our understanding in mechanisms of antigenic variation employed by these organisms and in identifying targets for chemotherapies.

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IN VIVO EFFECTS OF PYRIMETHAMINE AND ARTESUNATE ON ACUTE AND CHRONIC TOXOPLASMOSIS

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Acute and chronic *Toxoplasma* infections were assessed in mice using stage-specific antibodies and immunocytochemistry. The effects of pyrimethamine and artesunate treatment were investigated at various time points post-drug administration. During acute toxoplasmosis, in the untreated toxoplasmosis group, tachyzoites were initially identified on the surfaces of the liver and spleen. There was a rapid increase in the number of tachyzoites associated with invasion of the liver from the surrounding connective tissue, resulting in formation of inflammatory lesions containing increasing numbers of tachyzoites. Similar increases in tachyzoites were also observed within the spleen. In contrast, only a few individual tachyzoites were seen in the brain at the final time point. Similar results were observed in the artesunate treated group although the mice survived 3 days longer. In contrast in the pyrimethamine treated group, only a few parasites were observed at the first time point post-infection; thereafter, no parasites were observed and all mice survived. Pyrimethamine was very effective in treating acute toxoplasmosis, even when a virulent parasite strain was used. In chronic infections, the brains of the untreated group contained an average of 310 ± 2.06 tissue cysts with an average size 45.85 ± 12.08 µm. No significant differences in either number or size of tissue cysts were found in the brains of mice treated with pyrimethamine or artesunate. This study showed that pyrimethamine and artesunate were ineffective against tissue cysts in mouse brains. Double immunocytochemical labelling confirmed the exclusive presence of tachyzoites during the acute phase, and bradyzoites during the chronic phase.

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CHARACTERISATION OF THE EFFECTS OF PENTAMIDINE-DERIVATIVES IN *NEOSPORA CANINUM*- AND *TOXOPLASMA GONDII*-INFECTED CELL CULTURES

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Pentamidines have a long-standing history as anti-trypanosomal, anti-leishmanial and anti-malarial drugs. In this study, we report on the effects of a series of pentamidine-derivatives against the apicomplexan parasite *Neospora caninum* and *Toxoplasma gondii*. For this, HFF monolayers were infected with tachyzoites, at 1 h p.i. drugs were added once at concentrations of 0.05 µg/ml, and proliferation of tachyzoites was measured by real-time PCR at day 3 p.i.. Of 20 pentamidines tested, the best efficacies against N. caninum were noted for DB768 and DB750 (IC_{50} between 0.150 and 0.237µM), with maximal proliferation inhibition at 1.5 µM. These two drugs were chosen for further studies. Tachyzoite proliferation was also stopped when treatments were initiated at days 2 or 3 p.i.. At 1.5 µM, a treatment duration of 24 h was suifficient to exert a true parasitocidal effect. Electron microscopy of treated infected cultures revealed distinct alterations in parasite ultrastructure including vacuole formation, electron-dense cytoplasmic inclusions and membrane stacks, distortion of the PV tubular network, and lipid droplet formation. Pentamidines acted exclusively upon intracellular parasites, since pretreatment of tachyzoites for 1 hour had no effect on infectivity. Treatments of uninfected HFF with DB768 and DB750 did affect host cell proliferation/viability. However, 24 hour-pretreatment of uninfected HFF monolayers with both drugs, followed by infection with *N. caninum* tachyzoites and subsequent culture, resulted in complete inhibition of parasite prolfieration for a period of 2-3 days. This suggests that either these compounds or respective active metabolites were still present after removal of the drugs, or that drug treatments impaired some functional activities in HFF, which were essential for parasite survival. Since the effects seen upon pretreatment of host cells suggest an involvement of the host cell, the respective targets must be identified. DB750 has been coupled to epoxy-agarose, and affinity chromatography using parasite extracts and/or HFF lysates is being performed in order to identify pentamidine-binding proteins, and thus potentially novel pentamidine drug targets.

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EFFECTIVENESS OF A RIBOFLAVIN AND LIGHT BASED PATHOGEN REDUCTION TECHNOLOGY (PRT) SYSTEM TO ELIMINATE *BABESIA MICROTI* FROM APHERESIS PLATELETS AND PLASMA

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Babesia microti is an intraerythrocytic parasite transmitted naturally to vertebrate hosts by ixodid ticks. In recent years, *B. microti* has also emerged as a critical blood safety issue, with > 60 cases of transfusion-transmission reported, but appropriate interventions are lacking. As a result, we evaluated the ability of Mirasol PRT system, developed by Navigant Biotechnologies, to reduce the presence of *B. microti* in apheresis plasma and platelets. In this study, apheresis plasma and platelets units were spiked with high titer *B. microti*-infected hamster blood. Subsequently, riboflavin was added to the infected plasma and platelet units and illuminated with 6.24 J/ml of ultraviolet light. Various samples were collected at different stages during the Mirasol PRT treatment protocol: a positive control (after injection of parasite), a riboflavin control (after injection of the riboflavin into the infected unit, but before illumination) and test sample (after illumination). Also, sample aliquots were stored at appropriate condition (5 days at 22-24°C for the platelets and 7 days at -20°C for the plasma) for later evaluation. To detect the presence of viable parasites, each sample was injected into groups of 6 hamsters. Four weeks post-inoculation, hamster blood was tested for *B. microti* infection by blood smear and real time PCR (RT-PCR) analysis. To date, we tested two units of plasma in separate experiments and one unit of platelet concentrate. Plasma positive controls showed over 90% parasitemia by blood smear while the parasite was not detectable in the blood smears of the hamsters receiving treated samples. By RT-PCR, one treated unit was negative while the second one showed a 5 log reduction in parasite load. The platelets unit tested gave similar result, with negative blood smears and a 4 log reduction by RT-PCR in the treated sample. Freezing of samples and the presence of riboflavin in the absence of UV light activation also had pronounced negative effects on parasite viability. The data indicate that use of riboflavin and ultraviolet light has the potential to greatly reduce the risk of transfusion-transmitted *B. microti* from plasma and platelets. This ability may be conferred in part by a direct inactivation of *Babesia* parasites by riboflavin alone. Given the absence of *B. microti* screening of the blood supply, this process represents an efficient option for improving blood safety.

(ACMCIP Abstract)

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THE PROINFLAMMATORY CYTOKINE EXPRESSIONS WERE SUPPRESSED BY TLR2 IN MACROPHAGES TREATED WITH *TOXOPLASMA GONDII LYSATE*

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Toll like receptors (TLRs) are important transmembrane molecules for recognition and signaling in the immune system. TLRs are capable of recognizing wide range of organisms from bacteria virus, fungi, and protozoa. *Toxoplasma gondii* is an intracellular protozoan parasite and infection of this parasite is in world wide distributed. The infection was mediated by an inflammatory response which IL-12 dependent IFN-γ plays a crucial role. However, little is known about how TLRs mediate innate immunity to eukaryptic pathogens including *T. gondii*. In this study, we evaluated that interactions between the TLR2 and *T. gondii* lysate mediated NF-kB activation and the induction of cytokine mRNA and protein expression were studied. THP-1 cells, HEK293 cells were cultured in culture plates with RPMI1640/10% FBS at 37°C, 5% CO_2 conditions. In 96 well plates, THP-1 cells ($1x10^5$ cells/well) were cultured with LPS (10 μg/ml) or *T. gondii* lysate (50 μg/ml) for 24 h or 48 h. The supernatant was collected and measured IL-12, IL-10 by ELISA. *T. gondii* lysate inhibits LPS-induced IL-12 and IL-10 productions via TLR2. Neutralization experiments with Abs blocking TLR2 demonstrate that this receptor plays a critical role in the signal transduction pathway induced by *T. gondii*. HEK293 cells were transiently transfected with the NF-kB luciferase, MyD88, pCH110 plasmid DNA and expression plasmids for human TLR2, TLR4 or empty control vector. At 24 h following transfection, cells were challenged with 10μg of LPS and/or *T. gondii* lysate for a futher 24h. The cells were then lysed and luciferase reporter levels measured using the Dual-Luciferase System (Promega). *T. gondii* lysate trigger NF-kB dependent expression of IL-8 in HEK293 cells transfection with TLR2. We observed that the expression of TNF-α mRNA was increased with LPS treatment and suppressed with *T. gondii* lysate by RT-PCR. Through transfection studies and the use of blocking monoclonal anti-TLR2 and anti-TLR4 Abs, we have found that *T. gondii* lysate induced the suppressions of IL-12 TNF-a, IL-10 expression and increase of IL-8 via TLR2 and NF-kB activation.

(ACMCIP Abstract)

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IMMUNOSUPPRESSION OF MICE AFTER INTRAPERITONEAL OR GASTRODUODENAL INJECTION WITH A VIRULENT *TOXOPLASMA GONDII* KOREAN ISOLATE (KI-1)

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Toxoplasma gondii KI-1 tachyzoites were isolated from blood of an ocular patient in the Republic of Korea, and have been successfully passaged in the laboratory. The morphology, pathogenicity, infectivity, and genetic and cell culture characteristics of KI-1 were similar to those of RH strain. In the present study, BALB/c mice were infected intraperitoneally with 10^3 tachyzoites, or gastroduodenally with 10^6 tachyzoites of KI-1, and host immune responses were compared according to the two infection routes. The gastroduodenal infection was done using a slender tube inserted into the duodenum of mice, and found successful by confirming the parasite B1 and SAG1 gene expressions in visceral organs of mice. Survival of mice, proliferation of T cells ($CD4^+$ and $CD8^+$ cells), NK cells, and macrophages among the spleen and mesenteric lymph node cells, cytokine signals in the tissues, and cytokine production *in vitro* were examined. Survival of gastroduodenally infected mice was longer than that of intraperitoneally infected mice. However, in both groups, NK cells, macrophages, and CD4+/CD8+ T cells were unchanged during the 9 days of experimental period, and CD8+ T cells were only slightly increased on day 9 post-infection. INF-γ and TNF-α signals were unchanged in the tissues of both groups. In vitro IFN-γ and IL-12 productions were also unchanged. Tachyzoite infection by either route induced immune suppression of mice on the basis of no responses of the spleen and mesenteric lymph node cells to Con A stimulation. The infected mice in both groups eventually died. Our results show that KI-1 tachyzoites induce immune suppression of BALB/c mice during the early stage of infection regardless of the infection routes.

(ACMCIP Abstract)

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SERUM ANTIBODY RESPONSES TO CP27, AN IMMUNODOMINANT *CRYPTOSPORIDIUM* SPP. ANTIGEN IN BANGLADESHI CHILDREN WITH DIARRHEA AND CRYPTOSPORIDIOSIS

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Cryptosporidium spp. are a significant cause of diarrheal disease worldwide. In children in developing countries, cryptosporidial infection under the age of 2 years may result in persistent diarrhea as well as subsequent delays in physical and cognitive development. Currently there is no vaccine available for cryptosporidiosis. Cp27 is a 27 kDa immunodominant *Cryptosporidium* surface antigen that induces protective immune responses in animal models of cryptosporidiosis and is a putative vaccine candidate. The aim of this study was to investigate immune responses to this antigen in Bangladeshi children with cryptosporidiosis. This study is part of a prospective case-control study of children under age 5 years who presented with diarrhea to the ICDDR, B. Children with *Cryptosporidium* spp. detected in the stool by PCR (cases) and age-matched children with diarrhea but no *Cryptosporidium* spp. detected in the stool by PCR (controls) were studied. Sera were analyzed from 49 cases and 40 controls at initial presentation and from 33 cases and 17 controls at follow up 21 days later. Serum IgG, IgA, and IgM levels were measured by ELISA using recombinant (r) Cp27 as antigen. Serum IgA ($p=0.0258$) and IgM ($p<0.0001$) levels to Cp27 were significantly higher in cases compared to controls at presentation. Also, there was a significant increase in serum IgG ($p<0.0001$), IgA ($p<0.0001$), and IgM ($p=0.0056$) levels to Cp27 from the initial to the follow up time points in cases but there was no significant increase in controls. The results of this study indicate that *Cryptosporidium*-infected children develop an increase in IgG, IgA, and IgM levels to Cp27 over time and support development of this antigen as a putative vaccine candidate.

(ACMCIP Abstract)

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OSTEOPROTEGERIN (OPG) PROTECTS *CRYPTOSPORIDIUM* AGAINST DEATH INDUCED BY TRAIL (TNF-RELATED APOPTOSIS-INDUCING LIGAND)

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Cryptosporidiosis is caused by protozoan parasites that infect human intestinal epithelial cells. The mechanisms by which parasites are cleared and the mechanisms used by the parasites to evade the host response are poorly understood. Previously our group has shown that *Cryptosporidium* infection induces the up-regulation of the Osteoprotegerin (OPG), which is

a member of the TNFR family that functions as soluble decoy receptor and known TNF family ligands include TNF-related apoptosis-inducing ligand (TRAIL) and TNF-related activation-inducing cytokine (TRANCE), both of which have been implicated in host defenses. Since TRAIL is an important ligand for OPG, we reasoned that OPG might promote parasite survival by antagonizing an anti-apoptotic effect of TRAIL. We found that Treatment of infected cells with TRAIL induced epithelial cell apoptosis and reduced parasite numbers around 50%. By contrast addition of recombinant OPG blocked these effects. These results suggest a novel TRAIL-mediated pathway for elimination of *Cryptosporidium* infection and a role for OPG in modulating this host response.

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A ROLE FOR AMINO ACIDS $_{212}$KLR$_{214}$ OF EBOLA VIRUS VP40 IN ASSEMBLY AND BUDDING

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The Ebola virus matrix protein VP40 is the most abundant viral protein found in virions and is able to produce virus-like particles (VLPs) in the absence of other viral proteins. Similar to other RNA viruses, VP40 is thought to contain three domains which facilitate budding from the plasma membrane: the late (L) domain, the membrane (M) association domain, and the self-interacting (I) domain. While the L-domain of Ebola VP40 has been well characterized, the exact mechanism by which VP40 mediates budding through the M and I domains remains unclear. Based upon the previously published crystal structure of VP40, we targeted amino acids $_{212}$KLR$_{214}$ for mutagenesis since Lys212 and Arg214 are part of an exposed region in the C-terminal domain and are potentially important for overall structure and/or oligomerization of VP40. To determine if these residues were important for VLP budding and VP40 function, a series of alanine substitutions were generated in the $_{212}$KLR$_{214}$ region of VP40, and these mutants were examined for VLP budding, intracellular localization, membrane association, and oligomerization. We found that the $_{212}$KLR$_{214}$ residues of VP40 are critical for efficient release of VP40 VLPs, with Leu213 being most important. In addition, VP40 KLR mutants displayed altered patterns of cellular localization compared to that of wild-type VP40 and self-assembly of VP40 KLR mutants into oligomers was also altered compared to that of wild-type VP40. Interestingly, the KLR mutants retaining the Leu213 displayed localization and oligomerization patterns that were similar to that of wild-type VP40 while the mutants lacking Leu213 were significantly different from wild-type VP40. These results suggest that the $_{212}$KLR$_{214}$ residues of VP40, particularly Leu213, are important for proper assembly/oligomerization of VP40 which subsequently leads to efficient budding of VLPs.

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CRIMEAN-CONGO HEMORRHAGIC FEVER VIRUS ENCODES AN NSM PROTEIN

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The medium (M) RNA segment of Crimean-Congo hemorrhagic fever virus encodes a relatively large (225 kDa) polytopic membrane protein that undergoes extensive processing by cellular proteases to yield not only the G_N (37 kDa) and G_C (75 kDa) envelope glycoproteins found in virions, but also several nonstructural proteins that are derived from N-terminal cleavage of the G_N precursor. Recently, we found that the G_N precursor also undergoes C-terminal cleavage in the region of its second predicted transmembrane domain. Using a polyclonal antiserum specific to the cytoplasmic loop preceding the signal sequence of the G_C precursor, we identified a novel 15 kDa protein derived from transiently expressed M polyproteins of several CCHFV strains. We also confirmed the presence of this species in lysates of cells infected with the IbAr10200 strain of CCHFV. This protein was not detected in lysates of semi-purified virions, and so we have named it NS_M by analogy to the similarly positioned and topologically identical nonstructural protein found in the M polyproteins of orthobunyaviruses. Currently, we are attempting to identify the protease(s) responsible for generation of NS_M, as well as characterizing its intracellular trafficking within cells and its interactions with viral and cellular factors. We have found that small proteins comprising only the G_N-NS_M junction and some flanking regions can be efficiently cleaved both in transfected cells and in rabbit reticulocyte lysates in the presence of microsomal membranes, thereby providing a convenient system for identifying the protease *in vitro*. Preliminary immunofluorescence microscopy studies with epitope-tagged NS_M proteins in the context of full length M polyproteins suggest that the NS_M traffics to a perinuclear region, possibly the Golgi. Accumulation in the Golgi compartment may implicate the NS_M in glycoprotein trafficking and/or CCHFV morphogenesis. Indeed, we find that NS_M coprecipitates with G_N and G_C in mammalian cells. Finally, we have begun to develop systems to identify novel interactions of the NS_M with both viral and cellular proteins.

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INFECTIOUS CLONES OF CHIKUNGUNYA VIRUS (LA RÉUNION ISOLATE) FOR VECTOR COMPETENCE STUDIES

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The ongoing outbreak of Chikungunya virus (CHIKV) on several islands in the Indian Ocean and in India, and the importation of numerous cases into Europe and the United States has focused attention on this reemerging virus and highlighted the need for the development of new tools to study vector-virus-host interactions. Infectious cDNA clones of CHIKV using a recent isolate from La Réunion Island (LR2006 OPY1), were constructed and characterized *in vitro*, and in *Aedes aegypti* and *Ae. albopictus* mosquitoes. Comparison of the growth kinetics and infection rates of the viral isolate CHIKV strain LR2006 OPY1 (CHIKV-LR) and a full-length infectious clone (CHIKV-LR ic) indicate that the infectious clone retained the original viral phenotypes. Infectious clones that express green fluorescent protein (GFP) were also produced and characterized in cell culture and in *Aedes* mosquitoes. The CHIKV-LR 5'GFP infected *Ae. aegypti* and *Ae. albopictus* mosquitoes at a similar rate to the parent virus and to the full length infectious clone. The CHIKV-LR 3'GFP only infected *Ae. albopictus* mosquitoes at similar rates. To investigate the molecular basis of the recent outbreak, specific mutations have been introduced into the CHIKV-LR ic. Comparative studies to determine the capacity of these mutant viruses to replicate *in vitro*, and to infect and disseminate in mosquitoes are ongoing. The epidemiological significance of our findings will be discussed.

(ACMCIP Abstract)

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MONKEYPOX: ECOLOGICAL AND LABORATORY INVESTIGATIONS OF HOST-VIRUS DYNAMICS

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Over the past several years several examples of human infection with strains of *Orthopoxvirus monkeypox* has garnered significant academic and political attention due to the emergence of monkeypox as a public health threat. Current concerns stem from waning immunity to monkeypox conferred from smallpox vaccinations, an increase in the number of individuals with compromised immune systems, and a paucity of data regarding this historically problematic disease. This presentation

will provide an update on the current state of knowledge regarding the ecological factors contributing to the primary transmission events (i.e., non-human species to human) within the native geographic range of this viral species. The early recognition of the zoonotic nature of this disease led to the inclusion of ecological investigations as a component of even the earliest recognized outbreaks of human disease in Africa. Subsequent to most of these investigations, several laboratory studies have been conducted with the primary goal of identifying animal models to facilitate the development of treatments and prophylaxis. These laboratory data, obtained from our recent studies as well as from historical literature, combined with those obtained from the early and recent ecological investigations, taken together offer insight into the poorly understood dynamics of the monkeypox host-virus relationship.

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A PHYLOGENETIC ANALYSIS OF SOUTH AMERICAN EASTERN EQUINE ENCEPHALITIS VIRUS

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Eastern equine encephalitis virus (EEEV) is an arbovirus belonging to one of seven antigenic complexes of the *Alphavirus* genus, family *Togaviridae*. The EEE complex is further divided into four distinct genetic and antigenic lineages: Lineage I circulating in North America and lineages II-IV circulating in Central and South America. Previous phylogenetic analyses demonstrated the highly conserved monophyletic nature of North American (NA) EEEV with clustering primarily by year of isolation. Alternatively, a less robust analysis including only small regions of the NSP4, E2 and 3' untranslated region sequences of South American (SA) EEEV resulted in considerable diversity and no clear pattern of evolution. This difference in genetic conservation between NA and SA EEEV may be the result of variations in their enzootic transmission cycles and adaptation to different vertebrate hosts. However, while the enzootic transmission cycle of NA EEEV is well-described and primarily involves the *Culiseta melanura* mosquito vector and passerine birds in hardwood swamp habitats, that of SA EEEV remains unclear and a particular amplifying host has not yet been implicated. Limited ecologic and genomic information for SA EEEV constrains assumptions regarding the evolutionary relationships among and between members of each lineage and the transmission of EEEV in Central and South America. Therefore, sequences of all available SA EEEV isolates spanning a diverse geographical and temporal spectrum were expanded and included in a phylogenetic analysis. Multiple methods were used to analyze both nucleotide and amino acid sequences and the resultant phylogenies confirmed the diversity among and within the multiple SA EEEV lineages with robust bootstrap values for many geographic clusters of genetically-conserved isolates. This pattern of evolution supports the local use of ground-dwelling amplifying hosts with limited dispersal in the enzootic transmission of SA EEEV, however a few strong relationships between geographically-distant isolates suggest that avian species could play an important role in dispersal of the virus.

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GENETIC DIVERSITY AND POSITIVE SELECTION IN EASTERN EQUINE ENCEPHALITIS VIRUS

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We used the skyline plot from the BEAST program (Drummond & Rambaut, 2003) to infer the demographic histories of Eastern equine encephalitis virus (EEEV) in North and South America from previously published sequences, and the REL method available at the Datamonkey web facility (Pond & Frost 2005) to check for positive selection. For North America, data sets of both full and partial structural polyprotein genes were used. For South America analyses were based on partial E2 and nsP4 sequences. All sequences were confirmed as non-recombinant. Results for North American EEEV indicated that the level of genetic diversity remained constant from 1933 (when the most recent common ancestor is estimated to have existed) to about 1982 when there was an exponential increase in the number of lineages lasting up to 1990. This was followed by another constant phase. The increase in genetic diversity may not reflect changes in viral population size since we detected positive selection in E2 (A29T/P and R66S) and E1 (G403A). In the case of the sites in E2 both the ancestral and derived states were found in isolates sampled before and after the exponential phase. In contrast, with the exception of a single isolate from 1950, the G to A change in E1 was only seen in isolates sampled after 1990. The skyline plot for South American isolates suggested a constant level of genetic diversity, however, the highest posterior density intervals were large and analyses using constant, exponential and logistic models of population growth indicated that none of these was favored. There was weak evidence for positive selection at position 383 in E2 (I/V to T), a site that showed no variation (V only) in North American isolates. The relevance of the various amino acid changes (if any) is unclear, particularly since the R (+ve) to S (no charge) change is the only non-conservative one. However, a single amino acid change in E2 of Venezuelan equine encephalitis virus (a close relative of EEEV) from an uncharged to positively charged residue has been shown to confer increased virulence.

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GENOTYPE SHIFT AND REEMERGENCE OF CHIKUNGUNYA IN INDIA

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Chikungunya is an emerging arboviral infection of immense public health significance. An unprecedented Chikungunya epidemic was reported from many parts of India in 2006. We report a detailed serological, virological and molecular investigation of this epidemic by collecting 585 clinical samples of suspected patients from all the affected regions. The serological investigation revealed 66% seropositivity for Chikungunya specific IgM and/or IgG antibodies. The RT-PCR analysis using CHIKV specific amplimer revealed the presence of CHIKV specific RNA in 48% of samples. The viral load was found to vary from 0.1 to 10^7 PFU/ml in viremic patient as determined from a quantitative RT-PCR assay. Thirty five Chikungunya viruses were also isolated from representative PCR positive samples. The genotyping by nucleotide sequencing classified these viruses into East Central South (ECS) African genotype. In earlier outbreaks, Asian genotype was implicated as the etiology of major CHIK outbreaks in India. The sequence analysis also revealed many unique substitutions across the genome of Indian Chikungunya viruses. This study conclusively proved the involvement of a novel ECS African genotype in resurgence of Chikungunya in India after a gap of 32 years.

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BIOSURETY AND REGIONAL PREPAREDNESS FOR A POTENTIAL INFLUENZA PANDEMIC AND OTHER THREATS POSED BY BIOLOGICAL SELECT AGENTS AND TOXINS: THE ARMED FORCES RESEARCH INSTITUTE OF THE MEDICAL SCIENCES EXPERIENCE

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Following acts of terrorism in the United States during 2001 that included the release of anthrax spores, the US Congress passed laws to ensure the safe and secure handling of Biological Select Agents and Toxins (BSAT) to prevent their potential use as weapons of mass destruction. Federal regulations prescribe the handling of BSAT in laboratories located within the Continental United States (CONUS); however, these regulations are not applicable to US laboratories located outside CONUS, (OCONUS). The US Department of Defense requires that all military laboratories that possess BSAT regardless of their geographic location establish a biosurety program. Conducting safe and secure BSAT research in OCONUS laboratories pose unique operational and diplomatic challenges and offer unique opportunities. Armed Forces Research Institute of the Medical Sciences is the first US OCONUS laboratory to implement a biosurety program. Armed Forces Research Institute of the Medical Sciences is located in Thailand, a country in which some of its endemic diseases are caused by BSAT, including the Highly Pathogenic Avian Influenza H5N1 virus. Armed Forces Research Institute of the Medical Sciences is preparing to support global Pandemic Influenza Preparedness and other BSAT-related efforts at a regional level by developing organizational and infrastructure assets, including the construction of a state-of-the-art Biosafety Level 3 containment facility, to conduct safe, reliable, accountable, and secure basic and epidemiological research. These efforts require a high level of cooperation and coordination between the US and Thai Governments' agencies, including the US Army, the Royal Thai Army, with the support of the US Embassy, Thailand and the US Department of State. The Armed Forces Research Institute of the Medical Sciences experience can be applicable to other international research laboratories.

(ACMCIP Abstract)

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A SEROSURVEY OF THE KENYAN SOMALI HERDER POPULATION IN NORTHEAST PROVINCE DURING THE RIFT VALLEY FEVER VIRUS EPIDEMIC OF 2006/07

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In November 2006 increased Indian Ocean temperatures caused El Nino-like weather patterns throughout Kenya resulting in wide-scale flooding in Northeast Province. This flooding led to a dramatic increase in the mosquito vector population and subsequent Rift Valley fever virus (RVFV) transmission among the livestock and human population in the province's primarily pastoralist Somali population. A case/control study was initiated in January 2007 in the province (and later in two other) to examine risk factors for severe disease caused by RVFV versus mild or subclinical disease and for RVFV infection versus non-infection. Field teams identified 23 cases and collected 374 samples from 176 households. Only 3 male controls between 15 and 35 years of age were identified even though numerous cases were young males. Because many males in this community within this age bracket spend most of the year herding livestock far away from their home village, they were not captured within our study. To help correct the resulting selection bias, a team visited 4 water-points in Northeast Province and interviewed 102 herders from 16-19 February, 2007. All were male, and the median age was 28. 36% described mild symptoms during the outbreak time period, and 2 described hemorrhagic symptoms. 16% reported bed net use, and 59% reported contact with sick animals. Collected sera have been tested for the presence of IgM and IgG antibodies to RVFV. Because a significant number of subjects were likely infected in early December, determination of recent infection by the presence of IgM antibodies alone will likely cause misclassification because of waning IgM levels in some subjects. Threshold IgM values and IgG values will be analyzed to better classify serum samples as positive or negative. Once disease and infection status is fully determined, logistic regression will be used to determine significant risk factors. The sub-study's data will supplement the larger case/control study but also offers unique insight into the little studied population of Kenyan Somali herders.

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REDUCING MEASLES BURDEN IN NIGERIA: LESSONS FROM THE ANAMBRA STATE INTEGRATED MEASLES IMMUNIZATION CAMPAIGN 2006

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Measles remains a major cause of child mortality and morbidity in Nigeria affecting children aged between 9 months and 15 years. To reduce measles burden in Nigeria and reduce child mortality and morbidity in line with the Millennium Development Goals, Nigeria carried out Integrated Accelerated Measles Immunization campaign which incorporated OPV and Vitamin A. Lessons learnt are highlighted in this presentation. The immunization campaign was carried out from 3rd-17th October, 2006, in all the 21 local governments in Anambra State, Nigeria covering 1050 vaccination posts for a target children population of 1,602,362 between 9 months to 15 years. Each post was manned by six member health team who were either stationed in designated health facilities or moving from school to school or community by community. Measles vaccine, OPV and oral polio vaccine were given to children according to appropriate age. The exercise was preceded by community mobilization and advocacy. Out of a total of 1,797,307 targeted children, 1,602,362 (89%) received measles immunization. For polio 831,855 (97%) out of 855,860 received immunization, while 98% received Vitamin supplement. Vaccine wastage rate for measles was 4% and OPV 9%. Adverse vaccine reaction was less than 1%. Major constraints were - persistent rainfall during the implementation because it was the rainy season resulting in inaccessibility to some areas especially riverine areas and subsequent extension of the programme by seven days. Rejection was fueled by rumour and false information which started in a nearby State, alleging that children were dying as a result of the immunization and needed to drink coconut water to neutralize the vaccine, and that vaccines contained contraceptives for population control. The rumour was so damaging that it necessitated a state wide radio broadcast by the State Governor to debunk. Generated waste management was not adequately planned for. Conclusion, the exercise was very successful as coverage rose significantly from pre-campaign level of 26% to post campaign level of 89%. Future immunization campaigns should hold in dry seasons to allow for accessibility to riverine areas, crowd mobilization and health team movement. Waste management should be emphasized and strengthened. Rumour remain a key issue and should be considered in planning future mass immunization programs.

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EVIDENCE OF METABOLIC RESISTANCE IN PYRETHROID RESISTANT POPULATIONS OF *ANOPHELES GAMBIAE* FROM BENIN, WEST AFRICA

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Widespread pyrethroid resistance in *Anopheles gambiae* populations is threatening the use of ITNs and IRS in West Africa. We set out to determine whether metabolic resistance, involving the genes encoding cytochrome P450s, glutathione transferases and carboxylesterases, is

contributing to resistance in W. Africa. Also if this is the case does different selection pressures e.g. agricultural pesticide use or oil contamination of mosquito breeding sites, in turn lead to different gene expression profiles? We used the *An. gambiae* detox chip to investigate gene expression in field mosquitoes collected from areas of an agricultural setting and from an area contaminated by oil spillage in Southern Benin. Measured against a susceptible population from the same geographical region, P450s and GSTs were differentially expressed between the susceptible and resistant populations. Interestingly a cohort of three genes including two P450s was expressed in both resistant populations. We present evidence that target site resistance such as kdr is not solely responsible for insecticide resistance in Benin and the significance of these findings will be discussed.

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CONTROL OF CULICINES AND ANOPHELINES USING PYRIPROXYFEN - FIELD SCALE EVALUATIONS

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Field comparisons of larvicides in and around the Amazon city of Iquitos showed that pyriproxyfen is an impressive tool for the control of a range of potential disease vectors (*Aedes aegypti, Culex spp, Anopheles* spp, Ceratopogonids). We present the results of trials comparing different larviciding strategies (pyriproxyfen, temephos, and dissemination of larvicides using granule spreaders) against Ae. aegypti involving the treatment of >1500 households. We also present field data that shows that adult female mosquitoes that oviposit in containers dusted with pyriproxyfen can transfer enough larvicide to other breeding sites to affect immature mosquitoes developing therein. Additionally, ecotoxicological data from large water bodies (disused fishponds) shows that pyriproxyfen is suitable for use in sensitive environments. Treatment with pyriproxyfen (at 50 ppb), did not affect the abundance of most non-target arthropods (e.g Odonata, Hemiptera, Coleoptera) but it caused dramatic decreases in the production of dipteran adults. Emergence trap data showed a 53% decrease in the emergence of all diptera, and a 100% decrease in the emergence of culicids, over a 10 week period). We conclude that pyriproxyfen is a cost effective alternative to the larvicides currently in use in Peru, that it can be used in the wider environment without undue risk and that it is amenable for use in combination with oviposition traps to effect a more targeted means of mosquito control.

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A PARATRANSGENIC APPROACH TO CONTROL OF VISCERAL LEISHMANIASIS: AEROBIC GUT BACTERIAL IDENTIFICATION FROM *PHLEOTOMUS ARGENTIPUS*

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Visceral leishmanasis (VL) causes significant global disease with 500,000 reported cases per year. Three-hundred-fifty million people are at risk and incidence is thought to be underreported. Treatments are lengthy, toxic, costly and rendered ineffective by HIV co-infection. Attempts to eradicate phlebotomine sandflies, the vectors of *Leishmania*, are expensive, toxic and select for sandfly resistance. We are developing a paratransgenic approach to control VL. Paratransgenesis involves identification of bacteria living commensally within the gut of the sandfly, genetically transforming the bacteria to release an anti-leishmanial protein, and colonizing sandflies with transformed bacteria to inactivate leishmania within the sandfly. *Phlebotomus argentipes* is the sandfly vector of *L. donovani* in eastern India. Previous studies of *Phlebotomine* vectors demonstrated aerobic gut bacteria. Additionally, breeding sites of *P. argentipes* in the Indian states of Bihar and West Bengal have been characterized. Therefore, we hypothesize that a paratransgenic strategy involving delivery of engineered bacteria to sandfly breeding sites in eastern India will disrupt vectorial transmission of *L. donovani*. Our first study involved collection of 103 sandflies from four endemic regions of Bihar. Sandflies were surface sterilized, homogenized and plated on BHI agar at 28°C. 16S ribosomal gene sequencing was performed to identify the isolated bacteria. Bacteria were isolated from 68% of collected flies and were predominantly environmental Gram positives and *Enterobacteriaceae*. Among the microbes were *Brevibacterium linens* and *Bacillus megaterium*, both non-pathogenic bacteria with wide-reaching industrial applications in Bihar. *B. megaterium* is a component of biofertilizers and *B. linens* is an organism used in cheese production. Both microbes have been transformed with a broad-range plasmid, pRrMDWK6, which encodes a three domain single chain antibody. Engineered bacteria are currently being deployed to laboratory colonies of *P. argentipes*. Studies with paratransgenic flies carrying bacteria which express the antibody will be a precursor to future trials with bacteria expressing anti-leishmanial proteins. Data from ongoing paratransgenic trials will be presented.

(ACMCIP Abstract)

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A LARGE SCALE LABORATORY CAGE INVESTIGATION OF *AEDES* DENSONUCLEOSIS VIRUS (*AE*DNV) AS A SUSTAINABLE BIOCONTROL METHOD OF *AEDES AEGYPTI* MOSQUITOES

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The geographical area in which Dengue viruses (DENV1-4) are now endemic has expanded rapidly in the last two decades and novel methods of reducing DENV transmission are needed. We are exploring the use of *Aedes* Densonucleosis Virus (*Ae*DNV) to increase mortality of *Aedes aegypti* larvae and adults and to reduce the average adult age below the extrinsic incubation period of DENV. *Ae*DNV is transferred among oviposition sites (OPS) by adults. All previous studies have been completed in small population cages (8 ft^3). Herein we report on expansion of these studies into large (>300 ft^3) cages over a 25 week period as a prelude to field greenhouse trials in Tapachula, Mexico. These cages were seeded with *Ae*DNV in a single OPS. Additional OPSs were established at varied distances from the seed OPS. Unlike previous experiments, larvae were allowed to mature in OPSs, egg counts were assessed for each OPS, and quantitive real time PCR of select mRNAs was used to estimate the age structure of mosquitoes. The questions asked were: 1) How efficiently will *Ae*DNV spread from the seed OPS, 2) What *Ae*DNV titers will be reached in newly colonized OPSs 3) Does the availability of uninfected OPSs affect the efficacy of *Ae*DNV on population size and age reduction? 4) Do females prefer infected or uninfected OPSs? 5) Is the average age of mosquito populations reduced below that of the extrinsic incubation period of DENV?

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QTL MAPPING OF GENES CONTROLLING PERMETHRIN RESISTANCE IN *AEDES AEGYPTI*

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Permethrin is an adulticide widely used in dengue control campaigns. As a result of selection pressure, metabolic and target site resistance mechanisms have developed in several locations throughout the world. In this study, Quantitative Trait Loci (QTL) controlling permethrin survival in *Ae. aegypti* were mapped in a F_3 advanced intercross line (AIL). Parents consisted of an insecticide-susceptible strain (New Orleans) and a permethrin-selected strain from Islas Mujeres, Mexico. A total of 439 adult mosquitoes were phenotyped as alive, recovered or dead following a permethrin exposure in a bottle bioassay (1.2 ug/ bottle). Single nucleotide polymorphisms (SNPs) were analyzed at 24 novel cytochrome P-450 (CYP), esterase or glutathione transferase loci and at 10 previously mapped loci. SNPs were detected by melting curve analysis of allele specific primers. Seven insecticide-resistance genes mapped to chromosome I, six to chromosome II and 10 to chromosome III. Two QTLs controlling mosquito survival following permethrin exposure were detected on chromosome III. The first QTL corresponded with the *para* sodium channel gene, the CCEunk0 esterase gene, and the CYP4H32 and CYP325R1 genes. A second QTL in chromosome III included the esterase CCEae1o esterase gene and a CYP9J29, CYP9J19 and CYP6BY1 genes. The para gene accounted for most of the variation in survival. A single point mutation at residue *Val*1,016 of para was strongly correlated with mosquito survival. The mutation consisted in a G-->A transition at the first position of codon 1,016, conferring a valine → isoleucine substitution. We designed an allele-specific PCR system to identify *para* genotypes from genomic DNA. Inexpensive and simple detection assays for insecticide resistant alleles support ongoing dengue control strategies in developing countries.

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IMPREGNATED NETTING SLOWS INFESTATION BY *TRIATOMA INFESTANS*

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Chagas disease affects millions of people across Latin America. In Arequipa, Peru, vector-borne transmission of Chagas disease by *Triatoma infestans* has become an urban problem. Insecticide treated nets have proven invaluable in malaria vector control but are yet to be evaluated as a protective measure against Chagas disease vector infestation. We placed new sentinel guinea pig enclosures with and without protective covers made of deltamethrin treated netting into households of an urban community of Arequipa. We examined these sentinel enclosures biweekly for infestation by *T. infestans*. After five months the community was sprayed with deltamethrin and we performed a timed search for vectors in all households. Using novel statistical methods we estimated the effect of netting on the rate of vector infestation in sentinel enclosures controlling for the density and distance of potential source populations of insects. The rate of infestation of unprotected enclosures was one bug every two weeks and we estimate that impregnated netting decreased this rate fourfold. Surprisingly, the great majority of insects collected from sentinel enclosures were early-stage nymphs. The structure of the triatomine population in the control sentinel enclosures closely paralleled that of the stable stage distribution of *T. infestans* as measured in laboratory conditions. In conclusion, *Triatoma infestans* rapidly infests sentinel guinea pig enclosures in urban households, but can be significantly slowed with insecticide treated materials. Impregnated netting may be a valuable new tool for Chagas disease control.

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CLONING AND CHARACTERIZATION OF A FATTY ACID AND RETINOL (FAR) BINDING PROTEIN FROM THE HOOKWORM *ANCYLOSTOMA CEYLANICUM*

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Nematodes are unable to synthesize fatty acids de novo and must acquire them from their environment to meet their nutritional requirements. It is hypothesized that the two known classes of structurally unique fatty acid and retinol binding proteins that nematodes produce are used to meet this need, making these proteins viable targets for vaccination and chemotherapy against infection. A cDNA corresponding to a putative FAR protein was cloned from the hookworm *Ancylostoma ceylanicum*. The translated amino acid sequence of *A. ceylanicum* FAR-1 (AceFAR-1) shows homology to FAR proteins previously identified in the dog hookworm *A. caninum* and the free-living nematode *C. elegans*. Using RT-PCR, we have shown that *AceFAR-1* is transcribed in all hookworm life cycle stages, with the highest levels of transcription in developing larvae and adult female worms. Immunoblot experiments using a polyclonal anti-AceFAR-1 IgG reveal a protein of the expected size in adult excretory/secretory proteins and adult worm extracts (HEX), with a stronger signal in female HEX than in male HEX. Using *in vitro* binding assays, the recombinant protein (rAceFAR-1) was found to bind the fluorescent fatty acids DAUDA, cis-parinaric acid, and retinol at equilibrium dissociation constants in the micromolar range. *In vitro* data also shows that rAceFAR-1 binds fatty acids with chain lengths of C_{12}-C_{22}, with the greatest affinity seen for C_{15}, oleic acid (C_{18}), and arachidonic acid (C_{22}). Studies using fluorescently labeled C_{16} localized fatty acid uptake in living adult *A. ceylanicum* to the ovaries of female worms, with competitive binding experiments demonstrating that unlabeled C_{16} and arachidonic acid effectively displace uptake of fluorescent C_{16}. Mucosal vaccination of hamsters with rAceFAR-1 is associated with a 40% reduction in adult worm burden following challenge infection, compared to animals immunized with adjuvant alone. These data suggest that AceFAR-1 plays an important role in hookworm development by mediating uptake of fatty acids *in vivo*.

(ACMCIP Abstract)

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CHARACTERIZING THE HOST-INTERACTIVE SURFACE OF SCHISTOSOMES USING RECOMBINANT ANTIBODIES (SCFVS) FROM IMMUNE FISHER RATS

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Schistosoma mansoni infection is rapidly rejected about four weeks post-infection in Fisher rats. Literature reports have indicated that resistance to schistosomes can be passively transferred to mice by injection of serum from immune rats. Partial protection to schistosome infection was observed when serum was infused up to seven days post-infection and this resistance could be removed by absorption of the sera on adult schistosomes. We find that serum from schistosome infected Fisher rats has significantly higher titer and specificity for schistosome adult apical extracts compared to serum from equivalently infected mice. Infected rat serum can stain the surface of adult schistosomes when the worms are gently fixed in formalin or when metabolism is slowed during the antibody incubations. A library of phage was prepared that displays the antibody repertoire of schistosome infected Fisher rats as single-chain Fv domains (scFvs). The quality of the library was validated

by the successful panning and isolation of multiple independent scFvs recognizing the major extracellular loops of two schistosome tetraspanin immunogens, Sm23 and SmTsp2. The library was then panned on both apical membrane extracts and fixed worms, and six unique strong positive scFvs were obtained. All six scFvs displayed high specificity against enriched schistosome apical membrane extracts by ELISA. One of the scFv clones isolated by this scheme was identical to a previously isolated anti-SmTsp2 scFv. Five of the six scFvs (all except the anti-SmTsp2 scFv) recognize the surface of fixed adult schistosomes. Interestingly none of the worm surface binding scFvs recognizes an antigen species on Western blots suggesting the epitope is on a heterogeneous mixture of antigens and/or is non-proteinaceous. Identification and characterization of the schistosome worm surface antigens recognized by these new rat scFvs should lead to new insight into host/parasite interaction and could identify novel and promising vaccine antigens.

(ACMCIP Abstract)

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ROLE OF THE GOLGI GDP-MAN TRANSPORTER LPG2 IN *LEISHMANIA DONOVANI* VIRULENCE AND EVASION OF MACROPHAGE MICROBICIDAL ACTIVITY

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Leishmania spp. are obligate intracellular parasites of macrophages. Parasite surface glycoconjugates such as LPG, proteophosphoglycan and GIPLs participate at various steps in the interactions with host cells. Previous studies have shown that synthesis of LPG and other phosphoglycans including proteophosphoglycan is dependent on the Golgi GDP-Mannose transporter LPG2. However, lpg2$^{-/-}$ mutants of *L. major* are unable to induce acute pathology whereas lpg2- *L. mexicana* retain this ability. Here we probed the role of LPG2 in *L. donovani*. As seen with *L. major, L. donovani*, lpg2$^{-/-}$ mutants had diminished virulence for mice and for hamsters. Either episomal or chromosomal add-back of the LPG2 gene partially restored virulence. Furthermore, lpg2$^{-/-}$ mutants were defective in survival in macrophages *in vitro*, and virulence was restored in chromosomal add-backs. Using CFSE labeling and DHE to detect oxidant production, both the amastigote and the promastigote forms of lpg2$^{-/-}$ mutants generated an increased oxidative burst than wild type organisms upon phagocytosis by macrophages. This was accompanied by early lysosomal fusion of lpg2$^{-/-}$ mutants. The data suggest that, unlike *L. mexicana* but similar to *L. major*, *L. donovani* LPG2 is essential for parasite virulence, possibly through pathways leading to evasion of lysosome fusion and suppression of the phagocyte oxidative response. Thus the contribution LPG2-dependent molecules to parasite virulence in a host varies depending on the species of parasite.

(ACMCIP Abstract)

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KILLED BUT METABOLICALLY ACTIVE (KBMA) *LEISHMANIA* - A NOVEL PROTOZOAN VACCINE TECHNOLOGY FOR VISCERAL LEISHMANIASIS THAT IS ENHANCED BY TOLL-LIKE RECEPTOR ACTIVATION

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Vaccination with live *Leishmania major* (Leishmanization) is effective against leishmaniasis but is not acceptably safe. Subunit vaccines are safer, but so far lack efficacy. We describe a whole cell vaccine for experimental murine visceral leishmaniasis that uses *Leishmania chagasi* (LC) treated with the psoralen compound amotosalen (S-59) and low doses of UVA. This treatment generates covalent DNA crosslinks and parasites termed "Killed But Metabolically Active" (KBMA) that retain a complete antigenic repertoire but cannot replicate. KBMA-LC promastigotes can infect murine bone marrow-derived macrophages (BMDM) *in vitro*, and infect hepatic macrophages *in vivo*. In BMDMs, KBMA-LC induced nitric oxide production in quantities similar to virulent LC but greater than heat-killed LC, suggesting that KBMA-LC might adequately engage innate immune effectors in a vaccine context. We hypothesized that the Toll-like receptor agonists imiquimod (TLR7) and resiquimod (TLR7,8) would enhance protective immunity induced by a KBMA-LC vaccine due to the known roles of this pathway in antigen-presenting cell activation, regulatory T cell (Treg) suppression, and host Th1/Th2 balance. Susceptible BALB/c mice vaccinated with KBMA-LC showed protection similar to mice vaccinated subcutaneously with untreated virulent LC when challenged IV with virulent LC (over 40% reduction in liver parasite load compared to controls, $p<0.05$). Mice vaccinated with the combination of KBMA-LC and topically applied TLR agonist showed marked additional protection (68% for imiquimod, $p<0.0001$). Resiquimod, a more potent TLR agonist with enhanced TLR8 activity, shows potential for profound protection against *Leishmania* challenge. We present a novel vaccine model against intracellular protozoans that maintains the immunogenicity of live cells with the safety of killed cells, and we provide data that support the combinatorial use of TLR agonists. This technology could potentially be used in therapeutic vaccines, in combination with other genetic alterations in *Leishmania*, or with other protozoan parasites.

(ACMCIP Abstract)

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INNATE INFLAMMATORY AND PHAGOCYTIC RESPONSES TO *PLASMODIUM FALCIPARUM*: LINKED PROCESSES OR MOLECULARLY DISCRETE PATHWAYS?

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Effective innate immune responses are important for control of malaria blood-stage infection and in preventing progression to severe malaria in non-immune individuals. Key innate defenses include a tightly regulated inflammatory response -- severe malaria is associated with highly elevated TNFα -- and host clearance of parasitized erythrocytes (PE). Pattern recognition receptors on macrophages mediate these processes: parasite glycosylphosphatidylinositol (GPI) induces inflammation via TLR2 activation, while scavenger receptor CD36 mediates non-opsonic PE uptake. Both pathways are potential therapeutic targets, but it is unclear whether they are interdependent. Findings in other systems implicate CD36 in inflammation and TLR2 in phagocytosis, and recent evidence indicates that CD36 and TLR2 can cooperate. We investigated whether the innate inflammatory and phagocytic responses of macrophages to *Plasmodium falciparum* are separable: does CD36-mediated PE uptake have inflammatory outcomes, and does TLR2 regulate PE uptake? CD36-mediated endocytosis failed to induce TNFα production. As a more representative model of innate PE uptake, α-CD36 EBABs (Erythrocyte-Biotin-Avidin-Biotinylated antibody) were generated; macrophages internalized EBABs in a CD36-specific manner via a signaling pathway similar to that of PE uptake. Compared to controls, neither PE nor EBAB internalization induced TNFα release, indicating that the inflammatory consequences of CD36 engagement are ligand dependent. Regarding TLR2 regulation of PE uptake, wild type and *Tlr2*$^{-/-}$ macrophages showed no differences in EBAB or PE uptake. When wild type macrophages were pre-treated with *P. falciparum* GPI to stimulate TLR2, EBAB internalization

was enhanced; however, this effect was CD36-independent and generalizable to other TLR ligands. These results suggest that the innate inflammatory and phagocytic responses of macrophages to malaria are indeed discrete. Thus, therapeutic augmentation of CD36-mediated PE uptake should not exacerbate inflammation, nor should inhibition of the TLR2 pathway compromise CD36-mediated PE clearance. The role of TLR-enhanced uptake in malaria will be further examined.

(ACMCIP Abstract)

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USING A DEMOGRAPHIC SURVEILLANCE SYSTEM TO ENHANCE DETECTION OF ADVERSE DRUG REACTIONS TO MALARIA TREATMENT AND ASSOCIATED COSTS IN RURAL TANZANIA

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In 2001, Tanzania changed its first line malaria treatment policy from chloroquine to sulfadoxine pyrimethamine (SP). SP is associated with a rare risk of severe cutaneous reactions (SCR). Following the policy change public concern about such reactions was high and may have contributed to low demand for malaria treatment. Active and passive surveillance systems were established within 56 demographic surveillance site (DSS) villages (total population 135,000) to document actual numbers of severe cutaneous reactions resulting from SP use and their associated treatment costs. Clinicians were then tasked to follow up each case to confirm SCRs and also inquire on associated treatment costs. A total of 498 potential adverse drug reaction cases were reported of which, 430 were identified through the active surveillance system and 68 were detected through the passive surveillance approach. Of all these, 56 cases were confirmed as likely SCR linked to recent use of SP or related drugs while 64 were categorized as probable SCR cases. Costs associated with treating the genuine SCR cases ranged widely from less than USD 3.00 to more than 125. DSS sites offer an excellent platform for monitoring various health intervention programs on communities. The routine passive detection of potential adverse drug reactions was enhanced by the introduction of active case detection within the study communities. Cases detected through the passive approach were more likely to be confirmed or probable SCR than those reported through active surveillance system. Both approaches improved on the surveillance mechanism outside the DSS areas. DSS can serve as a critical foundation for collecting Phase IV safety information.

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MONITORING THE EFFICACY AND SAFETY OF ARTESUNATE+AMODIAQUINE (AS+AQ) OVER SIX YEARS USING THE WHO *IN VIVO* PROTOCOL AND A SIMPLE PHARMACOVIGILANCE STUDY IN THE DISTRICT OF OUSSOUYE, CASAMANCE, SOUTHERN SENEGAL

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The recommendation of using artemisinin-based combination therapies (ACTs) as 1st line treatment for uncomplicated falciparum malaria is supported by clinical trials but limited deployment studies in Africa and SE Asia. We deployed over five years artesunate+amodiaquine (AS+AQ) in health posts in Oussouye district [moderate (EIR-25 bites/person/year) perennial transmission], Casamance (southern Senegal) and monitored its efficacy and safety. Only non pregnant, slide proven *P. falciparum* infected patients of all ages were treated. Target doses were 12 (AS) and 30 (AQ) mg/kg over three days. *In vitro* sensitivity was tested on 242 isolates for CQ, 236 (QN), 250 (AQ), and 183 (AS) taking 1997 as a reference year (no AS+AQ combination). 3037 patients, half aged between 6-15 years, were treated. 966 patients were followed clinically and parasitologically for 28 days; 323 (haematology) and 169 biochemistry) were blood sampled. The remaining 2071 patients were followed up to Day 28 to collect safety information using a pre-established questionnaire; 2050 of them also had a malaria smear on Day3. Parasitological efficacy was consistently high throughout 2000-05: the overall Kaplan-Meier crude (non PCR-corrected) success rate was 94.4% (95%CI 92.8-95.7). AS+AQ was well tolerated. 139 clinical adverse events (AEs) were recorded on 121 patients (104 with one, 16 with two and 1 with three AEs). There was no significant shift in WBCs, renal function tests and no cases of clinically manifest or biochemical hepatitis. *In vitro* sensitivity of isolates remained stable over time. In conclusion, AS+AQ is well tolerated and continues to be effective 6 years into deployment in Casamance. Basic pharmacovigilance is feasible under field conditions. The numbers studied preclude detecting rare, serious toxicities.

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FIXED DOSE ARTESUNATE/SULFAMETHOXYPYRAZINE/ PYRIMETHAMINE COMBINATION THERAPIES COMPARED TO ARTEMETHER/LUMEFANTRINE FOR THE TREATMENT OF UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA ACROSS AFRICA: AN OPEN RANDOMIZED MULTI-CENTRE TRIAL

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The efficacy of Artemisinin based Combination Therapies (ACT) has already been demonstrated in a number of studies all over the world. However, secondary factors like side effects, ease of administration, cost and duration of the treatment can greatly influence the final outcome of any ACT. Therefore, these factors must be handled with equal importance when selecting an appropriate antimalarial treatment. This open randomised multi-centre clinical trial tested the hypothesis that three pills of the fixed dose combination artesunate/sulfamethoxypyrazine/ pyrimethamine (As/SMP fdc), administered over 24 hours is not inferior in efficacy to the same drug administered over 48 hours and that As/SMP fdc, independently of the duration of its dose interval, is not inferior in efficacy to 24 pills of the 60 hours treatment of artemether/lumefantrine (AL, 6-dose) for the treatment of uncomplicated *Plasmodium falciparum* malaria. This multi-centre study was carried out between May 2006 and January 2007 in 4 African countries: Cameroon, Mali, Rwanda and Sudan. Participants at least 6 months of age with uncomplicated *P. falciparum* malaria were randomly assigned to receive As/SMP fdc 3 pills in 24h, As/SMP fdc 3 pills in 48h or AL 24 pills in 60h. Treatment efficacy was assessed using the 28-day protocol of the World Health Organization. A total of 1260 patients were enrolled. Adequate Clinical and Parasitological Responses (ACPR) not PCR corrected on day 28 were 96.3%, 97.3% and 97.9% for As/SMP fdc 3 pills in 24h, As/SMP fdc 3 pills in 48h and AL 24 pills in 60h, respectively. Overall, these results showed an ACPR of 99% on day 28 and reinfection occurred in 35/41 patients . No serious adverse events were noticed. In conclusion, both regimens of As/SMP fdc were as

effective and safe as AL for the treatment of uncomplicated *P. falciparum* malaria. Due to its ease of administration and the shortened treatment duration, the fixed dose one-day treatment regimen with 3 pills only may improve compliance and therefore may be the preferred choice.

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AZITHROMYCIN COMBINATION THERAPY FOR THE TREATMENT OF UNCOMPLICATED FALCIPARUM MALARIA: PRELIMINARY RESULTS FROM AN OPEN LABEL RANDOMIZED CONTROLLED TRIAL IN BANGLADESH

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Spreading multidrug resistance of *Plasmodium falciparum* and the absence of novel antimalarial compounds call for the exploration of currently available agents for their potential use in combination regimens for the treatment of falciparum malaria. Azithromycin is a widely prescribed macrolide antibiotic and has shown intrinsic activity against *P. falciparum in vitro* as well as for treatment and prophylaxis. So far 100 patients aged 8-65 years were enrolled into a randomized Phase II study at the Bandarban District Hospital, Bandarban, Bangladesh, to assess the efficacy of the combination of artesunate with azithromycin in uncomplicated falciparum malaria. Patients were randomly assigned to receive either artesunate plus azithromycin or artemether - lumefantrine (controls) in a 2:1 ratio. Adult patients enrolled in the azithromycin group received 1500mg azithromycin and 200mg artesunate as single daily dose for three days. Preliminary results show an overall 42 days cure rate of 91.1% (95%CI: 79.7-96.9%) and 100% (95%CI: 86.8-100%) after an adjustment by polymerase chain reaction for patients receiving the artesunate - azithromycin combination therapy and the control group, respectively. Two patients were lost to follow-up, 6 were censored in the course of the follow up after presenting with p.vivax parasitaemia. No early treatment failures occurred. Five (9.3%) late treatment failures were seen in the artesunate - azithromycin group and none in the control group ($p>0.05$). Fever clearance time in the azithromycin group was 10 hours as compared to 15 hours in the control group ($p >0.05$) whereas parasite clearance time was found to be just over 27 hours in both arms ($p>0.05$). Both regimens were well tolerated and no serious adverse events occurred during the study. The *in vitro* data showed an IC_{50} of 1910.24ng/ml (2550.79nM) for azithromycin which is comparable to previous data from Thailand. A preliminary analysis of the available data suggests that artesunate - azithromycin is a safe, highly efficacious, economic, and very well tolerated combination therapy and could provide an interesting alternative regimen for the treatment of uncomplicated falciparum malaria. Final data will be presented at the meeting.

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EFFECTIVENESS OF ARTEMETHER PLUS LUMEFANTRINE VERSUS ARTESUNATE PLUS AMODIAQUINE FOR THE TREATMENT OF UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA IN GHANAIAN CHILDREN

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Growing antimalarial drug resistance calls for rapid replacement of failing drugs by artemisinine combination therapy (ACT). Although artesunate plus amodiaquine (AA) and artemether plus lumefantrine (AL) have high efficacy and good safety profiles data on their effectiveness and acceptability are scarce. The aim of this trial was to compare the effectiveness, safety and acceptability of AA and AL in children 6-59 months of age with uncomplicated *Plasmodium falciparum* malaria in Ghana. In a open-labeled, randomized phase IV study children <5 who presented with fever at child welfare clinics of two rural district hospitals in Ghana were screened for eligibility. Participants with *P. falciparum* malaria (parasitemia 2000-200000 asexuell parasites/µl) randomly received either a three-days treatment of AA (Arsucam®) or AL (Coartem®). The first weight-adjusted dose was applied under direct observation and caretakers were instructed on how to complete treatment at home. Children were followed on days 3, 7, 14 and 28 to evaluate clinical, parasitological and hematological response. Re-infections were differentiated from recrudescent parasitemias by analyses of length-polymorphisms of *msp-2* after PCR-amplification from DNA filter paper. Primary endpoint was the clinical and PCR-controlled parasitological cure rate at day 28. Additional endpoints were drug safety (monitoring of adverse events by number, grade and relation to study drug) and acceptability (by standardized questionnaire). Outcome was related to the presence of known molecular markers of drug resistance. The protocol was approved by the responsible Ghanaian and German Ethic Committees and the study was registered at www.ClinicalTrials.gov (NCT00374205). Comparative assessment of the effectiveness of AA and AL and their acceptability will enable policy makers to make evidence-based decisions in selecting an appropriate ACT as first-line treatment for uncomplicated *P. falciparum* malaria. Results of this effectiveness study will be presented.

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EFFECTS OF PIPERAQUINE IN A MURINE MALARIA TREATMENT MODEL

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Piperaquine (PQ) is used widely, particularly in combination with artemisinin drugs, although published preclinical and pharmacokinetic data are limited. The aim of this study was to investigate the pharmacokinetics and pharmacodynamic responses of piperaquine (PQ) in a murine malaria 'treatment' model. Male Swiss mice were inoculated with 10^7 *Plasmodium berghei* parasites and at 2-5% parasitaemia, piperaquine phosphate (PQP; 10, 30 or 90 mg/kg) was administered by intraperitoneal injection. Parasitaemia was determined from peripheral blood smears. For characterisation of PQ pharmacokinetics, healthy and malaria infected mice received 90 mg/kg PQP intraperitoneally. Blood was harvested from groups of mice (n=5) and plasma PQ concentration determined by HPLC. The pharmacodynamic study comprised determination of parasite viability, drug efficacy, re-inoculation responses and parasite resistance at 0, 25, 40, 60, 90 and 130 days after PQP administration. At each time-point after zero, 6 mice were re-inoculated with 10^7 *P. berghei* parasites and blood was harvested from a further 4 mice for viability passage into naïve mice (n=5 for each blood sample). The median survival times were 4, 10 and 23 days for 0, 10 and 30 mg/kg PQP respectively. By contrast, all mice given 90 mg/kg PQP survived >60 days (mean parasitaemia <1%). Pharmacokinetic data for healthy and malaria-infected mice showed similar terminal elimination half-lives of 18 and 16 days respectively. The efficacy study showed that residual PQ concentrations were not adequate to suppress infection after 25 days. Viable parasites were present until 90 days after dosing. Nevertheless, only 50% and 25% of passaged parasites remained viable at 60 and 90 days respectively. We have demonstrated that high dose PQP resulted in long-term survival of *P. berghei*-infected mice. Since PQ concentration in plasma was inadequate for parasite suppression after 25 days, we conclude that the immune system plays

an important role in the maintenance of a persistent, sub-clinical parasitaemia.

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RETHINKING THE DEVELOPMENT OF ANTIMALARIAL COMBINATIONS

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There is a consensus that new antimalarials should not be introduced into clinical use as single agents because of the rapid development of resistance. The rationale for antimalarial combinations is that they should slow the development of resistance and prolong the useful lifetimes of new antimalarials. Recognizing the importance of this goal, the purpose of this abstract is to examine four potential problems with the ways in which combinations are currently studied and developed for clinical use. First, the vast majority of antimalarials (like other biologic agents) have exponential/semilogarithmic dose-response curves. However, the evaluation of antimalarial combinations for synergism, additivity or antagonism typically uses checkerboard testing, which assumes that the agents being tested have linear (arithmetic) dose-response curves. Second, the current approach is usually restricted to antimalarials that are effective alone (low IC_{50}s, *in vivo* efficacies ≥ 90%). However, previous experience in other systems indicates that many effective combinations contain at least one drug which is inactive alone. Third, *in vitro* testing typically cannot address the question being asked - whether use of the combination will reduce the emergence of resistance *in vivo*, in comparison to the use of its components as single agents. Finally, because the number of variables involved in evaluating a combination is enormous, there is as yet no way to predict which combinations will be successful. In contrast, the current practice of linking agents early in the development of combinations is often based on proprietary considerations. As a result, it may inadvertently prevent a broad and thorough examination of large numbers of combinations and permutations. We suggest that careful re-examination of the current approach should facilitate the development of new and more effective antimalarial combinations.

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DISSECTING THE *AEDES AEGYPTI* ANTI-DENGUE IMMUNE RESPONSE

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Dengue virus is the causative agent of dengue fever and dengue hemorrhagic fever, diseases affecting about 100 million people annually. *Aedes aegypti* is known to transmit the virus after an extrinsic incubation period during which the virus is able to infect and pass the midgut barrier, disseminate throughout the hemocoel and infect the salivary glands. Dengue virus infection in the mosquito is thought to induce the expression of a range of immune defenses that are largely uncharacterized. Here we present the *Ae. agypti* global genome expression responses to dengue virus infection and reverse genetic analyses of immune pathway implication in anti-Dengue defense. Our study provides novel insights on how the mosquito utilizes its innate immune system in controlling virus infection.

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QTL MAPPING OF RNAI GENES POSSIBLY RELATED TO DENGUE-2 VIRUS DISSEMINATION IN THE MOSQUITO *AEDES AEGYPTI*

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Aedes aegypti is the most important vector of yellow fever and dengue fever flaviviruses. Vector competence (VC) is defined as the intrinsic permissiveness of an arthropod vector to infection, replication, and transmission of a virus. Researchers have demonstrated global variation in VC for flaviviruses. Several studies have suggested that the RNAi and apoptosis pathways play a significant role in the mosquito innate immune response and VC of dengue-2 virus (DENV-2). Quantitative Trait Loci (QTL) that determine whether mosquitoes with a DENV-2 infected midgut can then disseminate the virus to other tissues was previously identified by crossing a strain selected for high rates of DENV-2 dissemination with a strain selected for low dissemination rates or midgut escape barrier (MEB). QTL were mapped in the F2 and in an F5 advanced intercrossed line. QTL were identified in select regions of chromosome I, II and III. In this study, we developed 6 Single Nucleotide Polymorphisms (SNPs) genetic markers, specific to RNAi and apoptosis, based on melting curve PCR analysis. These markers were then mapped with respect to the existing linkage and MEB QTL maps. R2D2, an RNAi gene critical to the loading of siRNAs into the RISC complex, mapped to chromosome I under the MEB QTL at 31 cM. Argonaute-2 and Dicer-2, other important RNAi genes, mapped to chromosome III. Genes related to apoptosis, inhibitor of apoptosis-1 and viral inhibitor of apoptosis, mapped respectively to chromosome II and chromosome III but not to locations under the MEB QTL. Additional RNAi and apoptosis genes will be mapped and association mapping will follow on genes demonstrating potential contribution to the variation in MEB and vector competence.

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NEGATIVE EFFECTS OF HOST SEROCONVERSION ON MOSQUITO FITNESS

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Vertebrate hosts respond to mosquito feeding by producing antibodies against salivary proteins, a process termed seroconversion. Host seroconversion has been shown to have negative effects on the fitness of other arthropods, including sandflies and ticks. We determined that host seroconversion also negatively affects mosquitoes. Mice were exposed to weekly feeding for a total of five weeks by one of four mosquito species: *Aedes aegypti*, *Ae. albopictus*, *Anopheles gambiae*, and *An. stephensi*. Exposure to biting by any of these mosquitoes resulted in a progressive decline in the volume of blood taken, the number of eggs produced, and the number of those eggs that were viable. Host exposure to biting by one species reduced fitness of the congeneric species, but had no effect on mosquitoes of the alternate genus. For example, exposure to one *Aedes* species decreased performance of the other *Aedes* species, but the two *Anopheles* species were not affected. Host seroconversion and cross-reaction between species was confirmed by Western blot. When mosquitoes (*Ae. aegypti*) were fed on B-cell knockout mice, which cannot seroconvert, no decline in blood meal amount, egg production, or egg fertility was observed, confirming that the fitness-decreasing effects are dependent on host antibody responses. Egg production is significantly reduced early in the seroconversion process, before blood meal amounts are affected, suggesting that reduced fertility involves post-ingestion costs (damage) to the mosquito. We examined the role of the complement system in mediating these effects, and found that the reduction in fertility did not occur when mosquitoes were fed on C3 knockout mice. The blood meal contains salivary antigens (re-ingested saliva), antibodies, and the elements of the complement system; our results suggest these

components interact in the gut of the mosquito in a manner that diverts resources from egg production, reducing fitness. These results suggest that host suitability as a blood-meal source declines as the host is exposed to mosquito biting and develops immune recognition of salivary antigens. Further, the suitability of a host can be influenced by its prior exposure to other related mosquito species. Finally, these effects might be optimized through vaccination strategies that target specific antigens and elicit high titers of complement-activating antibody isotypes, with the goal of reducing mosquito populations.

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A TRANSGENIC "SENSOR" STRAIN OF *AEDES AEGYPTI* FOR IMPLICATING GENES INVOLVED IN THE ANTI-VIRAL RNAI PATHWAY

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The RNA interference pathway is suspected of functioning as a natural defense in mosquitoes against arthropod-borne viruses. This implies that genes which encode for the protein components of the RNAi pathway play a role in determining vector competence. Thus determining the identity of these genes is of paramount importance. In order to generate a phenotypic marker which reflects, or "senses" the status of the RNA interference pathway in *Aedes aegypti*, a series of artificial gene cassettes was assembled. The first two genes encoded for the red and green fluorescent proteins, DsRED and EGFP. The third cassette was an inverted repeat sequence derived from a portion of the EGFP coding sequence. All three cassettes were controlled individually from the eye-specific artificial promoter 3xP3. These cassettes were introduced as part of a single *Mos*1 transposon construct into the *A. aegypti* (*kh*^{w} strain) germline. Transformed "sensor" mosquitoes displayed robust DsRED expression in the eyes, while EGFP fluorescence was mosaic and barely detectable, indicating strong RNAi-based silencing. In order to determine if EGFP expression could be restored, performed knockdown experiments targeting genes potentially involved in the RNAi pathway. When DCR2 or AGO2 were knocked-down, strong EGFP fluorescence was restored in the eyes. Knockdown of AGO3, a gene thought to be involved in the piRNA pathway in the germline, did not restore EGFP expression, indicating that the sensor strain activation is specific for genes involved in RNA interference. Interestingly, knockdown of DCR1 and AGO1 provided a modest increase in EGFP expression, indicating that these genes may provide some level of redundancy to the RNAi pathway in *A. aegypti*. This transgenic sensor strain can now be used to test genes of unknown function, particularly if they are suspected to play a role in the anti-viral innate immune response.

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AN EVIDENCE-BASED STRATEGY TO MITIGATE NEW WATER SUPPLY INFRASTRUCTURE RELATED DENGUE RISK IN RURAL AREAS IN SOUTHERN VIETNAM

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The World Health Organisation recognises that water resource development and management may contribute to the proliferation of dengue and other water-related vector borne diseases by increasing mosquito breeding in infrastructure such as water storage jars, water tanks and wells. In Khanh Hoa province in central Vietnam, 92% of dengue mosquito production was associated with 2000-litre water storage jars supplied by UNICEF. As a direct result of this finding, a new project was established to foster broad inter-sectoral collaboration between the water and health sectors to devise, trial and refine new approaches to dengue control in three provinces in southern Vietnam. A prospective study on household dengue risk was conducted in one southern commune using randomly selected treatment households (households that received new water supply infrastructure) and control households (households that did not receive new water supply infrastructure). Quantitative entomologic sample methods were used to survey for *Aedes aegypti* immatures at each household every 3 months, and questionnaires were used to assess householders' water storage practices and behaviours both before and after installation of new water supply infrastructure. Nearly 20% and 10% of new water supply infrastructure (large 1500-litre containers) were positive for *Ae. aegypti* III/IV instars and pupae respectively, only four months after installation. In terms of numbers of immatures, these new containers were responsible for 71% and 47% of the sampled population of III/IV instars and pupae, respectively. When compared to control households, treatment households were producing 5.7 and 1.7 times more *Ae. aegypti* III/IV instars and pupae, respectively. Reassuringly, 31% of new containers were found to be positive for predacious *Mesocyclops* species despite no intentional *Mesocyclops*-based control programs having been implemented. Over the next 18 months, this project will continue to evaluate the impact that new water supply infrastructure has on vector numbers, and on changes in householders' water storage practices and behaviours as they relate to dengue risk. From this evidence-based approach, suitable interventions will be developed, including augmenting the presence of predacious *Mesocyclops* species. This project will maximise the benefits of water supply programs by ensuring that the water they provide is safe from water-related vector borne diseases.

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SUSTAINED IMPACT OF EVIDENCE-BASED COMMUNITY-DERIVED COMMUNICATION STRATEGIES FOR THE CONTROL OF THE DENGUE VIRUS VECTOR *AEDES AEGYPTI* IN MANAGUA, NICARAGUA

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An ongoing challenge in community-based vector control is to bridge the gap between community knowledge and sustainable practice that reduces mosquito breeding sites. In response, we developed a project using evidence-based community-derived communication strategies for the control of *Aedes aegypti* in Managua, Nicaragua, that is now in its third year of operation. Thirty sentinel sites (132 houses each, for a total of ~3,960 households and ~23,300 individuals) were selected to represent the population of Managua, including 10 intervention and 20 reference barrios. Community members ("brigadistas") generate their own interventions based on evidence from their barrio consisting of "beyond-KAP" questionnaires, detailed entomological surveys, and serological evidence of dengue virus infection in children. Results show that in intervention barrios there is 70% more probability than in reference barrios that residents search for larvae/pupae on their own every 8 days (2005 OR 1.7 (95%CI 1.5-2.1); 2006 OR 1.7 (1.5-1.9)) and that intervention barrios are less dependent on pesticides than reference barrios (2005 OR 1.7 (1.4-2.0); 2006 OR 2.2 (1.9-2.6)), whereas in 2004 (baseline), there was no significant difference in either of these indicators. Notably, 8 intervention barrios now function with minimum input from project facilitators. In terms of entomological indicators, in both 2005 and 2006, the number of homes and discarded containers infested with *Ae. aegypti* pupae was 20% lower in intervention barrios compared to

reference barrios (OR 0.8 (0.6-0.9); OR 0.8 (0.7-1.0), respectively), in contrast with baseline numbers in 2004 (OR homes 1.3 (1.0-1.6); OR discarded containers 1.4 (1.2-1.6)). Additionally, the numbers of larvae and pupae per discarded container were significantly lower in intervention as compared to reference barrios (p<0.01, 2006). Specific "best practices" have been identified that contribute to the succcessful implementation and maintenance of the program, including the voluntary nature of the brigades; the transfer of autonomy, decision-making authority, and technical capacity to the brigadistas; formation of networks of local organizations that support the activities; and the use of community entomological surveys for self-monitoring and planning. An important indication of the sustainability of the intervention is that the behavioral and entomological gains have been maintained over time.

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CULEX PIPIENS AMPLIFIES WEST NILE VIRUS IN NORTHEASTERN UNITED STATES BY VERTICAL AND HORIZONTAL TRANSMISSION

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West Nile virus (WNV) is a tropical virus that has sporadically caused human disease in Europe, but, since its introduction into northeastern United States, it has annually caused human disease where mosquitoes are inactive during winter. We explain how WNV survives winter, how viral amplification is initiated in spring and the role of *Culex pipiens* in amplifying WNV by vertical and horizontal transmission. WNV was vertically transmitted to 2 F_1 female *Cx. pipiens* from a naturally infected female. One vertically infected F_1 female, which was 168 days old, fed, after termination of diapause, on a hamster that subsequently died. WNV was isolated from the female mosquito and from tissues of the hamster. These data suggest that virus survives winter in mated, but unfed diapausing female *Cx. pipiens* that acquired infection vertically in the fall. Amplification is initiated in spring by infected competent females feeding on hosts. Using hamsters and suckling mice as hosts in the laboratory, we demonstrated that *Cx. pipiens* infected *per os* transmits virus (1) horizontally during feeding and subsequently vertically to F_1 progeny, (2) vertically without prior horizontal transmission, and (3) horizontally without subsequent vertical infection. All but one engorged mosquito horizontally transmitted virus 21 or more days post-infection at 26°C. The filial transmission rate was 6 1/2 %. The minimum filial infection rates in hamster and suckling mouse experiments were 1.34/1000 specimens and 0.39/1000 specimens, respectively. Vertical transmission of WNV occurred when females laid eggs 13-33 days after infection and following blood meals 2-4. Six females infected offspring vertically before infecting animals horizontally or without horizontal transmission occurring, and five females transmitted virus horizontally and subsequently vertically. Transmission of virus to F_1 offspring by *Cx. pipiens* before, after, and without horizontal transmission enhances amplification of WNV. These means of transmission by *Cx. pipiens* have enabled WNV to establish in the New World.

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PROTEIN TRAFFICKING TO THE MEMBRANES OF THE RELICT CHLOROPLAST OF APICOMPLEXANS

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Many apicomplexans, including *Plasmodium* and *Toxoplasma,* possess an essential organelle related to chloroplasts, called the apicoplast. Derived from a secondary, as opposed to a primary endosymbiotic event, the apicoplast has four membranes as compared to the two found on chloroplasts. The sequences directing nuclearly encoded proteins to the apicoplast lumen have been well-studied and are composed of a signal sequence followed by a transit peptide. With respect to membrane proteins, to our knowledge the apicoplast is the first secondary plastid for which trafficking to the membranes has been examined. We have analyzed the features and trafficking to two *T. gondii* apicoplast membrane proteins: a plastidic phosphate transporter, APT1, and a protease, FtsH1. Both lack the bipartite targeting sequence characteristic of luminal proteins. These proteins appear to be localized to multiple apicoplast membranes. APT1 is not processed, while FtsH1 is processed at multiple sites. Furthermore, APT1 is also associated with vesicles, particularly when the apicoplast is rapidly enlarging prior to division, indicating for the first time vesicular trafficking of an apicoplast protein. Analysis of FtsH1 deletion mutants show that both the transmembrane domain and peptidase domain contain elements essential for trafficking. These studies, combined with those from the literature on other plastids, allow us to build a comprehensive model for protein trafficking to the apicoplast.

(ACMCIP Abstract)

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HUMAN DEFENSIN α-1 KILLS *TRYPANOSOMA CRUZI* VIA MEMBRANE PORE FORMATION LEADING TO APOPTOSIS

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Mammalian defensins play a fundamental role in the initiation of innate immune responses to microbial pathogens. Here we show that early cellular infection by *Trypanosomaa cruzi,* the causative agent of Chagas' disease, up-regulates the expression and secretion of defensin α-1, which displayed a trypanocidal role. Microarray analysis of the whole human epithelial cell transcriptome upon short infection of cells by *T. cruzi* indicates that the parasite up-regulates the levels of transcripts of defensin α-1. Exposure of defensin α-1, at concentrations not toxic for host cells, significantly reduced the viability of infective trypomastigote forms of *T. cruzi*. Electron microscopic analysis of trypomastigotes exposed to defensin α-1 for a short time revealed pore formation, membrane disorganization and cytoplasmic vacuolization. Furthermore, pre-incubation of trypomastigotes with exogenous defensin α-1 followed by exposure to a primary culture of human epithelial cells significantly reduced trypanosome binding to human cells, including entry and parasite load at 72 hr. Defensin α-1 rapidly induces trypanosome membrane depolarization, membrane blebbing and DNA degradation. These results indicate that the induction of defensin α-1 expression in mammalian cells during early *T. cruzi* infection modulates parasite load as a result of defensin α-1 trypanocidal activity via apoptosis. We conclude that defensin α-1 gene expression and peptide secretion is an effective host innate immune response to control *T. cruzi* infection. We suggest that novel therapeutic approaches, mimicking defensin α-1 mediated innate immune response to early *T. cruzi* infection in host cells, can be used to control Chagas' disease.

(ACMCIP Abstract)

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MOLECULAR CHARACTERIZATION OF A *PLASMODIUM*-DERIVED INFLAMMATORY FACTOR

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Infection of erythrocytes with the *Plasmodium* parasite causes the pathologies associated with malaria, which result in at least one million deaths annually. The rupture of infected erythrocytes triggers an inflammatory response, which is induced by parasite-derived factors that still are not fully characterized. Induced secretion of the pyrogenic cytokine

TNF by these factors has been identified as a major cause of malaria pathogenesis. Here we describe the molecular characterization of a novel pathway that results in the secretion of TNF by host cells. We found that erythrocytes infected by murine (*P. yoelii* and *P. berghei*) and human (*P. falciparum*) parasites accumulate high concentrations of hypoxanthine and xanthine, which are imported by the parasite from the extracellular environment. Degradation of *Plasmodium*-derived hypoxanthine/xanthine results in the formation of uric acid, which triggers the secretion of TNF by murine dendritic cells. We also found that the degradation of *P. falciparum*-derived hypoxanthine results in the secretion of TNF by human dendritic cells and peripheral blood mononuclear cells. Since uric acid is considered a 'danger signal' released by dying cells to alert the immune system, *Plasmodium* appears to exploit this warning system to modulate the host inflammatory response. Identifying the mechanisms used by the parasite to induce the host inflammatory response is essential to develop urgently needed therapies against the disease.

(ACMCIP Abstract)

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REVIEW OF EXPERIMENTAL THERAPEUTICS CHEMICAL INFORMATION SYSTEM FOR ALL COMPOUNDS ACTIVE IN PROPHYLAXIS ANIMAL MODELS

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The primary mission of the Division of Experimental Therapeutics, Walter Reed Army Institute of Research, is to develop new antimalarials for malaria prevention. Large scale screening programs in animal models have been conducted. Most data collected since the 1960s is captured in our Chemical Information System (ETCIS). Tools have been developed to capture summary data for each compound in each test system in flat output side-by-side. The system administrator exported all compound numbers with activity from the three main causal (liver stage active) test systems: single dose mouse mode (RP), single dose chick model (CB), and causal/radical cure rhesus model (DB). In addition, output included drug/chemical name, smile for structure, quantity of drug on hand, efficacy from 14 other test systems, and basic drug information when available. MM is a single dose mouse blood stage efficacy model, and RR and TC are two trypanosome models. Over 300,000 compounds are registered in ETCIS. Of these, 4853 unique Walter Reed numbers were identified that were defined as active or curative in one of the three causal prophylaxis models. Of these, the following numbers were defined in the following models: RP (19 active, 4083 curative); CB (1087 active, 494 curative); DB (42 active, 324 curative); MM (385 active, 1454 curative); RR (5 active, 77 curative); and TC (35 active, 150 curative). The vast majority of these compounds have not been tested *in vitro* against malaria. We are currently in the process of assessing and defining each compound in terms of chemical class, efficacy, toxicity, resistance, and pharmacokinetics based on available information to make a decision and define any actions needed based on that decision. This information will be uploaded in to ETCIS. Several compounds of interest have been identified out of the first 500 compounds assessed. Pipeline Pilot™ has been purchased which will further enhance our ability to understand relationships between these compounds and to evaluate leads. Summary results of this endeavor will be presented. The intent is to publish this database in an electronic format. We seek partners to evaluate and develop the leads identified in this exercise, as well as those from other available test systems.

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REAL TIME ELECTRONIC DATA CAPTURE (EDC) IN PHASE III CDA TRIALS OPTIMISES SAFETY MONITORING CHECKS AND TRIAL CONDUCT

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Antimalarial drug development faces major challenges. The conduct and reporting of clinical trials is always complex, consuming large amounts of time and resource. In an effort to increase the efficiency of research data collection, GSK introduced direct Electronic Data Capture (EDC) via the internet across geographically dispersed sites in Africa for the first time in the CDA (Chlorproguanil/Dapsone/Artesunate) phase III program. The Phase III CDA programme comprises of 2 multi-centre, double-blind 2:1 randomised comparative trials (CDA vs. LapDap, n=900; CDA vs. Coartem; n=1395) with PCR adjusted efficacy at Day 28 as the primary endpoint and characterisation of safety and tolerability as the secondary endpoint. Spanning East and West Africa, these 23 investigational sites have varying infrastructure - from university teaching hospitals to some without access to running water. Despite these differences, the internet based EDC has integrated two important disciplines of clinical research; the conduct of trial at the site with the data analysis and reporting at the Sponsors, (GSK). EDC gives visibility of the trial data at any site, any time. Reporting and analysis which are embedded within the EDC allows GSK to track key operational metrics such as subject enrolment, eCRF status and query closure status enabling earlier detection of trends and anomalies thereby helping to achieve performance targets. This immediate access to information has allowed important insight into evolving laboratory and adverse events data. A central medical monitor and pharmacovigilance unit can review all safety parameters online. This overview has allowed any emergent trends to be communicated rapidly to the Independent Data and Monitoring Committee. We discovered that EDC may be further optimised if all sites have high speed, uninterrupted Internet access. EDC portal allows information exchange and document sharing between site personnel, the Sponsor and external partners such as WHO and MMV through a specific password protected access. On-line web based instructions have been used for the system's software training. The EDC can accommodate protocol amendments and varying IRB requests allowing immediate "bespoke" screens to be set-up without a huge paper trail, printing costs and lead-time. Milestone tracking can be performed expeditiously and routinely. Data from the EDC can be downloaded in many formats ranging from EXCEL based reports to SAS formats for comprehensive statistical analysis. Once the trials are completed, the EDC helps streamline the regulatory submission process by the automated generation of electronic versions of patient data. Taking all these together, EDC will most importantly generate a higher level of safety monitoring compared to the conventional paper based CRF. Thus, by accelerating the

drug development process, EDC may become an essential new business and clinical research tool.

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A MULTI-DIMENSIONAL SCREENING STRATEGY TO DISCOVER NEW ANTIMALARIAL THERAPEUTICS

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New therapeutic agents for the treatment of malaria, the world's most deadly parasitic disease, are urgently needed. The emergence of multi-drug resistant parasites, especially in *Plasmodium falciparum*, has eroded the efficacy of almost all currently available therapeutic agents. The discovery of new drugs, including drugs with novel cellular targets, could be accelerated with a whole-organism high-throughput screen (HTS) of structurally diverse small molecule libraries. We report a whole-organism *P. falciparum* (Pf) growth assay that is technically simple, robust, and compatible with the automation necessary for HTS. The assay uses DAPI staining of DNA as a reporter of blood-stage parasite growth. The whole-organism method is optimal since it will allow all relevant blood-stage targets to be screened simultaneously and will assure that screening positives have desirable properties such as cell permeability and activity in the context of the cell environment. To date, over 80,000 small molecules have been screened and 104 compounds were identified as highly active against multi-drug resistant (MDR) parasites. Compounds were screened at a concentration of 6μM and designated as a screening positive if growth was less than 10 % of that seen in DMSO control wells. The highly active compounds were assayed for mammalian cell toxicity and dose-response. One hundred compounds were identified to have a half maximal inhibitory concentration (IC_{50}) value of ≤ 1μM with a 20-fold selectivity index. To determine the biological activity of these screening positives we implemented a multi-dimensional screening strategy to investigate the effects of the compounds on biological phenotypes (parasite invasion into the host cell erythrocyte, heme polymerization and MDR *P. falciparum* field isolates), putative enzymatic targets (heat shock protein 90, dihydroorotate dehydrogenase, and histone deacetylases) and drug synergistic interactions. Several compounds were found to be effective inhibitors in these various assays; providing insight into their mechanism of action.

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MALARIA-INFECTED MICE ARE CURED BY NEW TRIOXANE DIMERS

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Malaria is a leading cause of death and disease within the developing world affecting people who live in tropical climates the most. Artemisinin is a naturally occuring product from a Chinese plant that has been known for centuries to have antimalarial activity and has shown little to no resistance in modern usage. Short half-life coupled with poor bioavailability have prompted the search for a more potent artemisinin-based drug. We have rationally designed and easily synthesized (4 or 5 high-yielding steps from artemisinin) a series of new artemisinin-derived trioxane dimers. These C-10 carbon dimers were designed to be more robust *in vivo* (i.e. to have longer half-lives) than C-10 acetal trioxanes like sodium artesunate, artemether or arteether. The Swiss Tropical Institute (STI) has tested the *in vivo* efficacy of over 100 of our designer trioxane dimers. Nine of these dimers cured P. berghei-infected mice upon oral administration at a dose of 3 x 30 mg/kg (no parasitemia on day 30 post infection). Several dimers substantially prolonged mouse survival at an oral dose of 3 x 10 mg/kg, and one dimer cured two of three mice at an oral dose of only 3 x 10 mg/kg. Neither overt toxicity nor behavioral change attributable to trioxane dimer administration was observed visually in any of the cured animals.

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ANTIMALARIAL ACTIVITY AND MECHANISM OF ACTION OF ARTEMISININ ANTIMALARIALS: IS THE DIGESTIVE VACUOLE (DV) THE PRIMARY TARGET?

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Artemisinin and derivatives are the most potent antimalarial drugs. However the bioavailability, affordability and toxicity of artemisinin antimalarials need to be improved and the efficiency of production should be enhanced or the compounds replaced with synthetic endoperoxides. A major impediment to the development of novel endoperoxides is the fact that the specific mechanism of action of artemisinin antimalarials is still debated. A proposed mechanism of action involves the build up of activated radical derivatives leading the damage of specific parasite targets such as the Ca^{+2}-ATPase, SERCA or the parasite's mitochondrion. In this study, we synthetised and tested a series of 16 novel endoperoxides and explored the mechanism of action of the most effective compounds. Antimalarial activity and drug interactions were determined by growth inhibition assays (IC_{50}) and isobolograms. Bicyclic *trans*-epoxide endoperoxides show the highest antimalarial activity. Two compounds exhibited significant antimalarial activity (IC_{50} 80-250 nM) against both chloroquine (CQ) sensitive and resistant parasites. Cellular compartments in control and drug-treated parasites were examined and quantified by microscopy using specific fluorescent probes and electron microscopy (EM). Little or no early stage effects of the drugs (at 40x IC_{50}) on the mitochondrial membrane potential (based on Rhodamine 123 uptake) or ER structure (based on ER Tracker staining) were found. Moreover, no antagonistic interaction was observed between thapsigargin, a SERCA inhibitor, and the AA, suggesting different molecular targets. By contrast, the cellular distribution of the pH probe, LysoSensor Blue, is rapidly altered upon AA treatment suggesting early disruption in the DV pH gradient. Additionally EM analysis revealed increased numbers of endocytic vacuoles in some parasites early after the treatment with the novel endoperoxides and total disruption of parasite organelles and membranes after 24 hours treatment with artemisinin. Our findings suggest that early effects on the parasite DV may underpin the mechanism of action of this important antimalarial class.

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TOWARD OPTIMIZATION OF 'REVERSED CHLOROQUINES': IMPROVEMENTS AND NEW SCAFFOLDS

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One response to the proliferation of chloroquine-resistant (CQ^R) *Plasmodium falciparum* malaria has been offered in the form of 'reversal agents', molecules that have little or no antimalarial activity on their own, but affect CQ^R malaria such that it becomes sensitive to CQ (i.e., acting as if it were CQ^S). We have previously reported our work on the development

of "reversed CQs" (RCQs), made by covalently linking CQ and a reversal agent, as reported previously. Should this approach prove to be successful, such 'reversed CQs' (RCQs) would have considerable advantages over CQ/reversal agent cocktails. We now report on an increasing array of molecules that were designed to probe the structural features that are essential for outstanding antimalarial activity against CQ^R, as well as CQ^S, *P. falciparum* malaria. IC_{50} values have been achieved that are about one order of magnitude lower than that of even CQ against CQ^S *P. falciparum* malaria. The RCQ physical/chemical properties can be altered over a broad range, leading to the possibility of independently producing both prophylactic and curative variants; this also means that we should be able to 'tune' the molecules such that they will be very good 'partner' drugs to others, setting-up combination therapies. In addition, some RCQ structural variants, which were originally designed to 'fail', had significant activity against the CQ^S and CQ^R strains. Thus, it appears that we have uncovered new lead molecular scaffolds against malaria, even if we do not yet know their target(s) in the parasite.

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SELECTIVE INHIBITORS OF B-KETOACYL ACP SYNTHASE III (PFKASIII) IN *PLASMODIUM FALCIPARUM* FATTY ACID SYNTHESIS: FROM TARGET VALIDATION TO *IN VIVO* EFFICACY

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Plasmodium falciparum possesses a Type II fatty acid synthesis (FAS) pathway that has become an attractive malaria drug target. We have utilized a structure based approach that integrates biological assays with computational chemistry to select novel chemotypes. Through this process we have identified several inhibitors that are potent against PfKASIII and the parasite. Of approximately 1000 compounds screened, 95 compounds have IC_{50} values in the low micromolar range. These compounds were tested in our in-house *in vitro P. falciparum* assay, and 47 compounds inhibited both the W2 and D6 strains at IC_{50} values of less than 10 micromolar. These compounds exhibit drug-like properties and low toxicity parameters. Selected compounds were further evaluated in the *P. berghei* malaria mouse model for both erythrocytic and exo-erythrocytic activity. Several compounds demonstrated remarkable causal activity suggestive that PfKASIII is important for liver stage development. We also observed suppressive blood stage activity for these compounds that showed better efficacy than triclosan, a malaria FAS inhibitor of the PfENR enzyme. Studies are now underway to validate PFKASIII as a drug target and to establish a true correlation between enzyme inhibition and anti-parasitic activity.

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CLIMATE, EVOLUTION, AND THE TRANSMISSION OF WEST NILE VIRUS IN MOSQUITOES

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The distribution and intensity of transmission of vector-borne pathogens is dependent on their ability to infect, disseminate, and be transmitted when the vector takes a blood meal. West Nile virus (WNV) was introduced into the western hemisphere in 1999 and has subsequently spread throughout much of North, Central, and South America. Here we show that evolution and climate help shape the distribution and intensity of transmission of WNV. A newly emergent genotype of WNV that was first detected in 2001 and subsequently spread across the US was more efficient at infecting, disseminating, and being transmitted by *Culex pipiens* mosquitoes than the original 1999 genotype. The infection and transmission of WNV increased nonlinearly with temperature and the incubation period, and the genotypes sometimes differed in response to incubation temperature. We used a degree-day model with an additional incubation temperature term that accounted for the accelerated infection and transmission at higher temperatures after the same number of degree days to predict the influence of climate on the distributional and seasonal limits of WNV transmission in eastern North America where *Cx. pipiens* is the dominant enzootic vector. There was strong evidence that cold temperatures determined the end of the WNV transmission season along the Atlantic coast. These results highlight the dynamic nature of pathogen-vector interactions and the consequences for spatial patterns of disease transmission.

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INFECTION, DISSEMINATION, AND TRANSMISSION OF A WEST NILE VIRUS GREEN FLUORESCENT PROTEIN INFECTIOUS CLONE BY *CULEX PIPIENS QUINQUEFASCIATUS* MOSQUITOES

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We report the construction and characterization of a full length West Nile virus cDNA infectious clone (ic) that utilizes a recombinant expression cassette to facilitate green fluorescent protein (GFP) reporter gene expression whilst preserving the integrity of the WNV open reading frame. Virus derived from WNV-GFP ic stably infected, replicated, disseminated and was transmitted by *Culex pipiens quinquefasciatus* mosquitoes, with subsequent replication in three-week-old mice fed upon by infected mosquitoes. Focal GFP expression in mosquito midguts was observed at three days post-infection (dpi) with a WNV-GFP infectious bloodmeal, with the majority of posterior midgut epithelia cells being positive by seven dpi. Additionally GFP foci were observed in salivary glands dissected 14 dpi. WNV-GFP viremia and lymph node infection were also detected in mice fed upon by intrathoracically infected mosquitoes. These data demonstrate the effectiveness of our strategy to generate a stable, replication competent flavivirus construct which expresses a reporter gene to study inter- and intra- host WNV transmission.

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DELIVERY OF WEST NILE VIRUS BY MOSQUITO BITE RESULTS IN HIGHER VIREMIA, EARLIER NEUROINVASION, AND FASTER SPREAD TO PERIPHERAL TISSUES

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Although most animals in nature are infected with West Nile virus (WNV) through the bite of infected mosquitoes, most laboratory animals are infected with WNV by needle inoculation. Our goal was to determine if mice infected with WNV by these two methods differ in their response to infection. A previous study showed that *Cx. tarsalis* females inoculate ~10^5 PFU of WNV extravascularly and ~200 PFU intravascularly (IV) while probing and feeding on a host. In this study, 6 wk old mice were infected with WNV by SC inoculation of 10^5 PFU in the left rear foot pad or through the bite of a WNV-infected *Cx. tarsalis* at the same location. Viremia peaked higher and earlier in mosquito-infected mice; peak viremia was $10^{4.3}$ PFU/ml at 48h for mosquito-infected and $10^{3.6}$ PFU/ml at 72h for needle-infected mice. WNV invaded central nervous system tissues earlier in mosquito-infected mice. At 48h, more mosquito-infected mice

had WNV in brain and spinal cord (42% and 75%) than did needle-infected mice (6% and 39%). WNV spread to peripheral tissues faster in mosquito-infected mice. WNV titers in the spleen were 8-23 fold higher in mosquito-infected mice at 24 and 48h ($p<0.01$). Additionally, WNV titers in the foot pad and draining lymph node opposite the inoculation site were 4-7 fold higher in mosquito infected mice at 48h ($p<0.05$). Higher WNV titer in sera and tissues did not increase morbidity and mortality in mosquito-infected mice. To determine if the small amount of virus inoculated IV by mosquitoes could account for the differences between mice infected by mosquito bite or needle, we compared serum and tissue titers of mice inoculated with 10^5 PFU SC ± 200 PFU IV. We found no significant differences between the two groups of mice. In conclusion, mice infected with WNV by mosquito bite exhibited higher viremia, earlier neuroinvasion, and faster spread of the virus to peripheral tissues. Enhanced infection in mice infected by mosquito bite was not due to direct inoculation of virus into the blood. Future studies will determine if mosquito bite enhancement is due to mosquito saliva.

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STRUCTURAL MUTATIONS WITHIN THE PRM AND E GENES OF A WEST NILE VIRUS FROM MEXICO CONFER AN ATTENUATED REPLICATION PHENOTYPE IN AVIANS

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In contrast to explosive epidemic/epiornitic West Nile virus (WNV) transmission in the United States and Canada, circulation of the virus in Mexico and Central America has been characterized by little disease in humans, equines or avian hosts. A WNV isolate (TM171-03) made from a raven in Tabasco state, Mexico in 2003 exhibited reduced replication and lower mortality as compared to other North American isolates following experimental infection of American crows (AMCR). To identify the genetic determinants responsible for a 5 $\log_{10}$ PFU/mL reduction in viremia observed for the Mexican strain, an infectious cDNA clone of the TM171-03 virus was engineered and used to generate recombinant WNVs for *in vivo* phenotypic comparisons with clone-derived, virulent NY99 virus in the AMCR virulence model. Four amino acid substitutions [prM-I141T, E-S156P, NS4B-I245V and NS5-T898I, with the envelope mutation (E-S156P; N-Y-P) encoding an ablation of the N-linked glycosylation site (N-Y-S)] and three 5'NCR and four 3'NCR mutations were identified between the avian virulent NY99 and the lesser virulent TM171-03 strain. Only chimeric viruses with altered prM and/or E mutations modulated viremia and virulence potential in AMCRs. The E-S156P mutant exhibited reduced virulence (75% with average survival times extended by approximately 2 days; about 800-fold reduction in mean peak viremia titer) as compared to the NY99 parental strain in which 100% of AMCRs died. The prM-I141T mutant resulted in 80% mortality and a 40-fold reduction in mean peak viremia titer. The prM-I141T + E-S156P combination mutant resulted in a virus that elicited a 38,000-fold drop in peak viremia titer and a 50% survivorship, not significantly different from the parental Mexican strain. These data indicate that the combined effects of the prM-I141T and E-S156P mutations encode the reduced avian replication phenotype of the TM171-03 virus, and perhaps of related strains, in Latin America, and that these genetic determinants might contribute to diminished WNV transmission south of the United States-Mexican border.

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ATTENUATING MUTATIONS IN THE WEST NILE VIRUS NS3 PROTEIN

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An attenuated strain of West Nile virus (WNV) that failed to induce mortality in mice was recovered after 20 passages of clone-derived virus in *Culex pipiens* mosquitoes. To determine the viral genetic changes associated with this attenuation, the complete genome was sequenced, and two putative attenuating mutations were identified. The attenuated strain had an aspartic acid deletion in the NS3 protein and a methionine to threonine substitution in NS4a protein. We evaluated the relative contribution of these mutations to the attenuated phenotype by engineering them, individually and in combination, into an infectious cDNA clone of the NY99 strain of WNV. Plaque size, virus production and mouse morbidity and mortality of the resulting viruses were then evaluated. The NS3 deletion was associated with small plaque size. Both the NS3 and NS4a single mutations suppressed virus production by BHK cells relative to wild-type WNV, and the double mutant was additionally deficient early during the course of infection. In C3H mice, the LD50 for both the double mutant and the single NS3 mutant were greater than 10^5 PFU/mouse and the LD50 for the single NS4a mutant and wild type WNV were less than 10 PFU/mouse. Ongoing studies are characterizing the ability of these mutants to produce viremia in mice, and determining their tissue tropism. These results suggest that deletion of an NS3 Asp residue that is relatively conserved among encephalitic flaviviruses leads to attenuation of WNV.

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IN VIVO PHENOTYPIC DIFFERENCES BETWEEN WEST NILE VIRUS GENOTYPES MAY CONTRIBUTE TO GENOTYPE DISPLACEMENT

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Studies examining the evolution of West Nile virus since its introduction into North America have identified the emergence of a new dominant genotype (WN02) that has displaced the introduced genotype (NY99). Our current work involved examination of potential mechanisms leading to this displacement. Previously, we found that the extrinsic incubation period (EIP) of viruses belonging to the WN02 genotype in *Culex (Cx.) pipiens* and *Cx. tarsalis* was two to four days shorter than the EIP of NY99 genotype viruses; however, there were no differences in either replication efficiency or fitness between the genotypes *in vitro*. To determine if replicative differences were present *in vivo* that could not be detected in our *in vitro* system, we inoculated *Cx. pipiens* intrathoracically with 10 pfu of a WN02 or NY99 virus and determined the virus titers in the mosquitoes from 1 to 14 days post-inoculation. Titers of the NY99 genotype were significantly lower than those of the WN02 genotype from 2 to 14 days post-inoculation, indicating that WN02 genotype viruses replicate more efficiently in mosquitoes than NY99 genotype viruses. We also examined whether the difference in EIP was observed following inoculation as well as following feeding, which would suggest that WN02 genotype viruses were more easily able to enter and replicate in the salivary glands. Both NY99 and WN02 viruses were transmitted beginning at 2 days post-inoculation, suggesting that the difference in EIP following feeding is due to WN02 genotype viruses more easily overcoming the midgut barrier. These data combined suggest that the WN02 genotype is more efficient at some aspect of the viral replication cycle, such as binding and entry, intracellular replication, packaging, and/or egress, allowing it to more

quickly disseminate from the midgut. Studies using mixed genotype infections in mosquitoes are providing further information regarding the potential mechanisms of genotype displacement observed in nature.

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LONG TERM IGM AND IGG INDEX VALUES TO WEST NILE VIRUS IN A MULTI-VARIANT SAMPLE SET FROM NEW MEXICO WNV SURVIVORS

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New Mexico experienced an epidemic of West Nile virus beginning in 2003, with a lesser number of cases recorded yearly since. Of the original 209 patients identified by serology and clinical symptoms, there were 67 cases of neuroinvasive disease (WND) and 142 West Nile Fever (WNF) cases. We identified all living patients (205). Additional patients were added as they were identified. All patients had positive CSF or serum WNV IgM. A comprehensive statewide followup study was undertaken over the next year, with data collected beginning 9 months post illness. A total of 137 patients completed initial followup interviews and phone interviews. Convalescent serum samples were obtained from 76 subjects. Paired serologies were run using archived samples from the acute illness whenever possible (n=22). Serial samples were obtained up to day 700 in 18 patients identified as persistently IgM positive. Samples were tested using Focus Technology's IgM capture enzyme-linked immunosorbent assay (MAC-ELISA) and WNV IgG ELISA. Results of these assays were interpreted as positive, equivocal or negative depending on the respective published index ranges. By day 200, a reversal of the IgM:IgG ratio was noted and by one year post illness, West Nile IgG levels exceeded IgM ratios in all subjects. By day 700, IgG levels were maintained significantly higher than IgM in 16 serofast patients. Using the IgM:IgG index ratio, a paired two sample test comparing IgM positive 0-60 day samples (95% CI, 2.7-6.8) to IgM positive convalescent samples ((95% CI, 0.45-0.64) are significantly different ($P < .0005$). The IgG to IgM ratio may be an important tool in determining acute vs. convalescent disease in patients who remain IgM serofast.

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IL-4 MEDIATES HUMAN B CELL RESPONSIVENESS TO SCHISTOSOMAL ANTIGENS

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Many studies have demonstrated an association of elevated serum IgE and the ability to produce Th2-cytokines with protective immunity to schistosomes. However, the mechanism(s) by which IgE might afford protection remain elusive. Furthermore, B cells, the producers of IgE, are greatly understudied in human schistosomiasis. We have begun to examine the interplay between IgE and B cells as well as potential additional roles B cells may have in mediating protective immunity. We recently observed that elevated expression of CD23 (Fc-epsilon RII, the low affinity IgE receptor), on circulating B cells correlates with resistance to reinfection to schistosomes in an occupationally exposed cohort of Kenyan adult men. Experimentally, IL-4 upregulates CD23 and CD23-bound IgE promotes nascent production of IgE in response to specific antigen. This suggests that CD23 might help to maintain existing levels of parasite-specific IgE in infected individuals but does not answer how the production of is IgE initiated. Because the ability to produce high levels of IL-4 is associated with resistance, we sought to determine whether IL-4 might stimulate naïve B cells to react with schistosome antigens. Human naïve B cells from unexposed, uninfected donors did not respond to crude schistosome antigens, demonstrating that naturally occurring B cell receptors do not recognize schistosomes. In contrast, naïve B cells that had been exposed to IL-4 developed the ability to respond to crude schistosome antigens and display characteristics of activation (upregulation of CD69) and differentiation (upregulation of CD77, CD38). We identified two pattern-recognition receptors induced by IL-4 which have not been traditionally considered to be expressed by human B cells. Biochemical studies on the receptor promoter sites show how IL-4 might mediate its effect on the expression of these receptors by human B cells. We propose that local IL-4 production allows B cells to react either directly with schistosome antigens via innate receptors in naïve people or indirectly via CD23-bound IgE in immune individuals, whereby the internalization of antigen would ultimately lead to nascent or elevated parasite-specific IgE.

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ABUNDANCE OF IMMUNOMODULATORY PROTEINS REVEALED BY ANALYSIS OF THE EXCRETORY-SECRETORY (E/S) PROTEOME OF *BRUGIA MALAYI*

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Relatively very little is known about the filarial proteins that interact with the human host's immune system. Although the filarial genome has recently been completed, the extent of our knowledge of their proteomes is limited to a few recombinant or purified proteins of interest. Here we describe a large-scale proteomic analysis of the excretory-secretory products of the filarial parasites. Proteomes of both the somatic and excretory secretory products of multiple life stages were analyzed using nanocapillary reverse-phase liquid chromatography-tandem mass spectrometry (nanoRPLC-MS/MS). The MS/MS spectra were searched against the *Brugia malayi* (Bm) database at TIGR (The Institute of Genomic Research). Methionine oxidation and phosphorylations on serine, threonine and tyrosine were included as dynamic modifications in the database search. To account for the contribution of Wolbachia, the spectra was also searched against a *Brugia malayi* Wolbachia database. The analysis revealed a total of 6131 proteins from all the stages (live adults, microfilariae, and L3 larvae) with 5911 somatic proteins being identified (of which 567 proteins were found in the E/S products of live adult parasites and microfilariae). 220 proteins were found to be exclusively E/S in origin. Annotation suggests that the functional and compartmental distribution is similar across each of the stages studied, but that Mf were more likely to secrete or excrete proteins compared to the other stages. Of the 787 proteins identified as excretory-secretory, only 164 proteins had confirmatory expressed sequence tag (EST) evidence; moreover, this analysis was able to confirm the presence of 291 "hypothetical" proteins inferred from gene predictions from the Bm genome . Not surprisingly, the majority (174 of 291) were predicted to be secreted by Signal IP and SecretomeP 2.0. Of major interest is the abundance of characterized immunomodulatory proteins such as ES-62 (leucyl aminopeptidase), MIF-1, SERPIN, glutathione peroxidase, and galectin in the ES of microfilariae (and mf-containing adult females) compared to the adult males. In addition, the ES protein spectra scanned against the Wolbachia database

resulted in the identification of 91 Wolbachia specific proteins, most of which were enzymes that have not been shown to be immunogenic. This proteomic profiling, thus, provides a direct (and non-theoretical) basis for understanding host-parasite interaction.

(ACMCIP Abstract)

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COEXISTENT FILARIAL INFECTIONS DOWNREGULATE ANTIGEN-SPECIFIC TH1 AND TH17 RESPONSES IN LATENT TUBERCULOSIS: ASSOCIATION WITH ENHANCED EXPRESSION OF CTLA-4 AND PD-1

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Filarial infections and tuberculosis are co-endemic in many parts of the world and co-infections occur often in the same individual. Consequently, one infection may influence the immune response to a second one. We examined the effect of filarial infection on the immune responses of PPD+ individuals to PPD, *Mycobacterium tuberculosis* culture filtrate antigen (Mtb CFA) or anti-CD3. We found that filarial infected individuals (n=15) have significantly lower PPD- and CFA- specific Th1 cytokine - IFN-γ (Geometric Mean (GM) of 1 vs. 49 [for PPD] and 1 vs. 48 ng/ml [for Mtb CFA], p=0.0397 for PPD and 0.0295 for Mtb CFA) and IL-12 p70 (GM of 4 vs. 68 and 8 vs 156 pg/ml, p=0.0095 for PPD and 0.0067 for Mtb CFA) - production compared to filarial uninfected individuals (n=12). In contrast, PPD and Mtb CFA specific IL-4 (GM of 32 vs. 1.2 and 32 vs. 1.3 pg/ml, p=0.0295 for PPD and 0.0253 for Mtb CFA) was significantly increased in filarial infected individuals. Filarial infected individuals also exhibited significantly diminished production of IL-17 to PPD (GM of 15 vs. 246 pg/ml, p=0.0012) and Mtb CFA (GM of 39 vs. 748 pg/ml, p=0.0004); and IL-23 to PPD (GM of 69 vs. 632 pg/ml, p=0.0012) and Mtb CFA (GM of 67 vs. 501 pg/ml, p=0.0026). This cytokine modulation is associated with diminished expression of T-bet (GM fold change of 0.57 vs. 1.4 and 0.73 vs. 1.36, p=0.0090 and 0.0163 respectively) - but not by alteration of GATA-3, Foxp3 (transcription factor for regulatory T cells), SOCS-1 and SOCS-3 (suppressors of cytokine signaling) in filarial infected individuals nor with differential expression of IL-10 and TGF-beta. However, filarial infected individuals exhibited significantly increased expression of the negative costimulatory molecules - CTLA-4 (GM fold change of 2.01 vs. 1.13 and 2.68 vs. 1.34, p=0.0374 and 0.0174) and PD-1 (Gm fold change of 1.83 vs. 0.94 and 2.04 vs. 1.22, p=0.0250 and 0.0374) in response to PPD and Mtb CFA respectively and - BTLA (p=0.0472) and GITR-L (p=0.0250) at baseline. No significant difference in the anti-CD3 induced immune response was noted between filarial infected and uninfected individuals. Thus, the presence of filarial infections has a profound downregulatory effect on protective Th1 and Th17 responses in latent tuberculosis and could potentially influence the development of active tuberculosis.

(ACMCIP Abstract)

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BRUGIA MALAYI MICROFILARIAE INHIBIT IFNγ AND TNF-α IN RESPONSE TO *MYCOBACTERIUM TUBERCULOSIS* INFECTION IN A MURINE COINFECTION MODEL

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Control of *Mycobacterium tuberculosis* (Mtb) infection requires a strong Th1 response, in which IL-12 and IFNγ predominate. Helminth infection may influence the response to a concomitant Mtb infection by inhibiting these critical Th1 responses. Moreover, filaria/Mtb coinfection is highly prevalent in human populations as these two infections have overlapping areas of endemicity. Previously, an *in vitro* coinfection model demonstrated that *Brugia malayi,* microfilariae (mf) rendered human monocyte-derived dendritic cells less capable of driving CD4+ T cell production of IFNγ in response to Mtb. We have now utilized C57BL/6 mice injected intravenously with *B. malayi* mf prior to an aerosolized infection with Mtb. We found first that the coinfected mice were not more susceptible to Mtb infection in the lung or spleen than Mtb-singly infected mice (GM lung: mf/Mtb 35 x 10^6 colony forming units (cfu) vs Mtb 52 x 10^6 cfu, p=0.55; GM spleen: mf/mtb 41 x 10^4 cfu vs Mtb 53 x10^4 cfu; p=0.12). Secondly, the mf/Mtb-exposed mice have significantly more cells in the spleens (GM 3.8 x 10^8) than either of the singly infected groups of mice (Mtb GM 1.8 x 10^8, mf GM 1.6 x 10^8 cells; p<0.005), a finding that suggests increased cellular recruitment and activation in the coinfected mice. Despite this, quantitative RT-PCR showed that the Mf/Mtb mice expressed significantly less IFNγ (GM ΔCT 1.5 vs 1.0; p=0.02) and TNF-α (GM ΔCT 2.6 vs 1.9: p=0.003) expression, but more IL-5 (GM ΔCT 12.23 vs 14.02 p=0.006) in their lungs than the Mtb-only infected mice. These data suggest that the IFNγ and TNF-α responses, known to be critical in controlling Mtb growth, is blunted in Mf/Mtb coinfected mice when compared to Mtb only infected mice and provides insight into the varied mechanisms by which pre-existing helminth infections may alter the outcome of mycobacterial infections.

(ACMCIP Abstract)

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EFFECTS OF CXCL10 ON DENDRITIC CELLS AND CD4+ T CELL FUNCTION DURING *LEISHMANIA AMAZONENSIS* INFECTION

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Leishmania amazonensis (*La*) can cause progressive disease in most inbred strains of mice. We have previously reported that treatment with CXCL10 activates Mɛs effector function in parasite killing and significantly delays lesion development in susceptible C57BL/6 mice via enhanced IFN-γ and IL-12 secretion; however, the mechanism underlying this enhance immunity against *La* infection remains largely unresolved. In this study, we utilized stationary promastigotes of *La* to infect bone marrow-derived DCs of C57BL/6 mice and assessed the activation of DC subsets and the capacity of these DC subsets in priming $CD4^+$ T cells *in vitro*. We found that CXCL10 induced IL-12p40, but reduced IL-10 production in DCs. Yet, *La*-infected DCs produced elevated levels of IL-10, despite CXCL10 treatment. Elimination of endogenous IL-10 led to increased responsiveness to CXCL10 treatment, as judged by increased IL-12 production in DCs, as well as increased proliferation and IFN-γ production by $CD4^+$ T cells. In addition, CXCL10-treated $CD4^+$ T cells became more responsive to IL-12 via increased expression of the IL-12Rβ_2 chain and produced elevated IFN-γ. This study indicates the interplay between CXCL10 and IL-10 in the generation of Th1-favored, pro-inflammatory responses and further highlights the utility of CXCL10 as a potential therapeutic for the control of non-healing cutaneous leishmaniasis.

(ACMCIP Abstract)

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EFFECT OF DRUG TREATMENT ON THE DEVELOPMENT OF $CD8^+$ T CELL MEMORY SUBSETS IN *TRYPANOSOMA CRUZI* INFECTION

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Trypanosoma cruzi is a protozoan parasite and the etiological agent of Chagas disease. The main drug available for the treatment of Chagas disease is benznidazole (BZ). However, the efficacy of BZ treatment is reported to vary, depending on the infective strain, host species, etc. Despite the large number of studies focusing on the immune response to *T. cruzi*, there are no reports regarding the immunological response of the host when complete cure is achieved. Here, we used a mouse model of *T. cruzi* infection in order to test the effectiveness of this drug to clear parasites and the effect of parasite clearance on the development of the CD8+ T cell response. C57Bl/6J mice infected with *T. cruzi* exhibited no evidence of infection or disease when treated with BZ in the acute (days 15-35) and chronic phases (days 150-170) of infection. Cure in BZ treated mice was documented by clearance of parasites from skeletal muscle, lack of exacerbation of infection following immunosuppression, failure to transfer infection to interferon-gamma KO mice and failure to detect parasites or disease by histological examination. Cure resulted in a marked change in the parasite-specific CD8+ T cell population. The majority of the *T. cruzi* specific-CD8+ T cells from treated/cured mice expressed CD62L, CD127 (IL-7Rα), CD122 (IL-15Rβ), consistent with the development of a central memory CD8+ T cell population. Moreover, treated/cured mice did not express the activation markers CD69 and CD25 and exhibited a significantly reduced *in vivo* CTL activity. CD8+ T cells from treated/cured mice responded faster and provided greater protective capacity following challenge than those from chronic/untreated mice. These results document that BZ can effectively clear *T. cruzi* infection and that in the absence of parasite persistence, a T_{CM} CD8+ T cell population emerges.

(ACMCIP Abstract)

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LEISHMANIA MAJOR INDUCED INTERLEUKIN-12 EXPRESSION IN HUMAN DENDRITIC CELLS IS MEDIATED BY NFκB, IRF-1 AND IRF-8

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The salient feature of dendritic cells (DC) is initiation of appropriate adaptive immune responses by discriminating between pathogens. Using a prototypic model of intracellular infection, we previously have shown that *Leishmania major* parasites prime human DC for efficient interleukin-12 (IL-12) secretion. *L. major* infection is associated with self-limiting cutaneous disease and powerful immunity. In stark contrast, the systemic, non-healing disease agent, *L. donovani*, does not prime human DC for IL-12 secretion. Here we report DC priming by *L. major* infection results in the early activation of NFκB transcription factors leading to subsequent up regulation and nuclear translocation of IRF-1 and IRF-8. Inhibition of NFκB activation by pretreatment with the NFκB inhibitor CAPE blocks *L. major* induced IRF-1 and IRF-8 activation and IL-12 expression. We further demonstrate IRF-1 and IRF-8 obtained from *L. major* infected human DC specifically bind to their consensus binding sites on IL-12p35 promoter, indicating that *L. major* infection stimulates a signaling cascade that activates IRF1 and IRF8 resulting in IL-12 transcription.

(ACMCIP Abstract)

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MATHEMATICAL MODELING OF MALARIA EPIDEMIOLOGY AND CONTROL

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Malaria interventions are usually prioritized using efficacy estimates from intervention trials, without considering the context of existing intervention packages or long term dynamics. There is a need for robust quantitative predictions of effectiveness of different strategies in reducing transmission, morbidity and mortality. We use numerical simulation of mathematical models of malaria in humans and mosquitoes to achieve this. We link individual-based stochastic simulation models for malaria in humans with deterministic models for mosquito infection and survival, incorporating variations in host exposure to infectious bites, naturally acquired immunity to infection and disease, effects of co-infection, and variations in human infectiousness. We use a volunteer computing platform to fit models to a set of 61 field scenarios from sub-Saharan Africa, to carry out probabilistic sensitivity analysis, and to analyze ensembles of different models. This provides a common platform for comparing likely impacts of different mixes of vector control, vaccines and chemotherapeutic interventions. We can reproduce reasonably well malariological patterns in endemic areas, including non-monotonic relationships between parasite prevalence and disease incidence with host age and force of infection; and quantitative relationships between increasing coverage of insecticide-treated nets (ITN) and a range of entomological outcomes. For example, assuming negligible impact on human infectiousness, ITN coverage of 60% reduces the entomological inoculation rate by 92% for ITN users and 80% for non-users. Integration of the entomological and human models enables us to predict the effects of all interventions on morbidity and mortality, including the nonlinear dynamics of changes in human infectiousness. In conclusion, we can simultaneously capture the dynamics of malaria epidemiology, interventions, and human demography. Combining the human and entomological models leads to plausible quantitative predictions and comparisons of effects of a comprehensive set of different interventions, individually, and in packages.

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COVERAGE, TARGETING AND IMPACT OF DIVERSE MALARIA CONTROL STRATEGIES: A PRAGMATIC APPROACH TO TRANSLATING THEORY INTO PRACTICE

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Theory suggests that targeting of malaria control measures to those most vulnerable or most exposed to transmission will maximize impact. However, theory also confirms that high coverage of entire populations is typically required to achieve large reductions of disease burden in areas of stable, intense transmission. Here I compare and contrast the theoretical and practical determinants of success for indoor residual insecticide spraying, insecticide treated nets, larviciding, environmental management, drugs and vaccines for malaria control. I conclude that in most realistic scenarios, comprehensive coverage of target populations or mosquito habitats is an optimal strategy for operational programmes, even when resources are limiting. I also identify niche areas where limited, more targeted intervention may have great potential and rationalize intervention choice according to ecological setting.

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MEASURING BURDEN OF MALARIA FROM PRIMARY MORBIDITY AND MORTALITY ESTIMATES IN JHARKHAND STATE OF INDIA-LESSONS LEARNED AND FUTURE PROSPECTS

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A measure of true burden of malaria is imperative from the view point of policy for allocation of necessary resources to combat the disease. Often, available secondary data is used to build estimates of disability adjusted life years (DALYs) lost and economic loss due to malaria. This could lead to underestimates, as often, there are lacunae in malaria surveillance, quality of diagnosis, and reporting of data to the National Control Programme. With the support of WHO/United States Agency for International

Development, we initiated malaria burden estimation studies covering 6 districts of Jharkhand, one of the high malaria risk states of India. 47 surveillance sites in 8 paradigms have been selected for conducting surveillance for estimating malaria morbidity and mortality incidence in 2007. Besides, malaria data from 2004 to 2006 has been collected from the state malaria control programme and tertiary care hospitals for comparison. Simultaneously health state valuation and anaemia surveys are being conducted and economic burden of malaria at household level is being estimated. The lessons learned and future prospects of estimation of malaria burden at the country level in India and the current findings would be presented in detail.

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COMMUNITY-LEVEL INTERVENTION COVERAGE AND THE BURDEN OF MALARIA IN ZAMBIA: RESULTS OF A NATIONAL MALARIA INDICATOR SURVEY

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Zambia is aggressively scaling up malaria interventions to meet national coverage targets. During this rapid scale up interval, we examined malaria parasite and anemia prevalence among children under 6 years of age in Zambia by household measures of intervention coverage including insecticide-treated nets (ITNs) and indoor residual spraying (IRS). Data from the 2006 Zambia National Malaria Indicator Survey (MIS), a nationally-representative household survey based on a two-stage cluster design, were used in the analysis. Households were surveyed on the availability and use of malaria interventions and children under 6 were tested for malaria parasites and hemoglobin levels. Adjusted probabilities were used to assess the risk of malaria parasite prevalence and mild and severe anemia in relation to age, gender, urban/rural and asset-based wealth status as well as levels of intervention coverage for children under 6 years of age. The adjusted probability of malaria parasite prevalence was lower among children under 6 years of age who slept under an ITN (14.5% vs. 20.0%; p<0.01) or mosquito net (14.7% vs. 20.3%; p<0.01) the previous night than children who did not sleep under nets, after controlling for age, gender, rural/urban residence, and asset-based wealth status. Children in households with at least two mosquito nets, regardless of whether they were slept under, had less parasitemia (13.9% vs 20.1%; p<0.01), mild anemia (Hb<11.0 g/dl; 59.6% vs 69.9%; p<0.01) and severe anemia (Hb<8.0 g/dl; 7.2% vs 11.8%; p<0.01) than children living in households with no ITNs. Actual ITN use among children under 6 years of age maintained its positive effect on severe anemia (7.8% vs. 11.6%; p<0.01). Children under 6 years of age living in households that were reported sprayed within the previous 12 months also had less parasitemia (5.4 vs 19.5%; p<0.01) and severe anemia (3.6% vs 11.1%; p<0.01) than children in households that were not sprayed. In conclusion, current national efforts to scale-up malaria control preventive interventions are showing coincident rapid improvement in child malaria and anemia in Zambia and these results are similar in magnitude to those described in controlled clinical trials.

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GEOGRAPHIC AND TEMPORAL CLUSTERING OF MALARIA IN AN URBAN COHORT OF UGANDAN CHILDREN

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Malaria risk may be heterogeneous over small distances, especially in urban areas. A better understanding of environmental risk factors and patterns of disease transmission on a local scale will enable more targeted implementation of interventions aimed at decreasing transmission. 601 children were randomly selected from a GPS mapped census area, approximately 2 km by 0.5 km, in Kampala, Uganda, and followed prospectively for cases of malaria as part of a longitudinal antimalarial efficacy trial. Recrudescent episodes of malaria after therapy, as determined by 6-locus genotyping, were removed from analysis. To identify clustering of malaria cases after 18 months of follow up, we performed spatial and space-time scans using SaTScan software. A spatial scan using circular scanning windows identified two statistically significant clusters with radii of 130 and 110 meters, containing 3% and 5% of the study population, where the risks of malaria were 3.7 (p<0.001) and 3.1 (p<0.001) times higher than for the remainder of the population, respectively. Using an elliptical scanning window, a single, large elliptical cluster of high malaria risk was identified, measuring 2.0 x 0.4 km, and encompassing 33% of the study population, where the relative risk of malaria was 3.0 (p<0.001) compared to the remainder of the population. The circular and elliptical clusters were located in and around a swamp that borders one side of the study area. After adjusting for pure temporal variation, a space-time scan identified 3 statistically significant clusters. The first was centered at one end of the swamp, had a radius of 50 meters, and lasted 34 days (RR=9.3, p<0.001). The second space-time cluster occurred 44 days later at the other end of the swamp, had a radius of 180 meters and lasted 55 days (RR=5.7, p=0.007), and was followed 16 days later by the immediately adjacent third cluster, which had a radius of 60 meters and lasted 52 days (RR=6.8, p=0.006). Unbiased spatial scanning revealed that residence near or within a swamp was a primary risk factor for malaria in an urban cohort of children. Allowing for an elliptical scanning window had a significant impact on the shape and size of the cluster, consistent with the elongated shape of the swamp. Identification of space-time clusters suggests that mini-epidemics of malaria may exist at small spatial and temporal scales, and that these may originate in areas of optimal transmission and then spread.

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MALARIA IN PREGNANCY IN AN AREA WITH INCREASED BEDNET COVERAGE: A TEN-YEAR HISTORY

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Tanzania's Kilombero Valley has been the site of an intensive effort for social marketing insecticide treated bednets (ITNs). KINET, the Kilombero Valley Insecticide-Treated Net Project successfully raised coverage of ITNs from 37% in 1996 to over 85% in 2006. During this period, recommendations for the prevention of malaria in pregnancy evolved from chloroquine prophylaxis to intermittent preventive treatment (IPTp) with sulfadoxine-pyrimethamine (SP). This study examines the impact of malaria in pregnancy for women not receiving IPTp in an area of high ITN coverage. A hospital-based study was completed in Ifakara, Tanzania, where bednet coverage is high. We enrolled 413 primi- and secundigravid women presenting at the labor ward who reported no IPTp at the time of delivery. Historical data were collected by questionnaire and neonatal birth weight and placenta parasitemia were objectively measured. Of the women enrolled, 62% were primigravid. 91% of these women reported sleeping under a bed net the previous night, 43% reported fever during their pregnancy, and 8% had placental malaria at delivery. Secundigravid women had lower risk of fever than primigravidae (OR 0.49; 95% CI: 0.32

- 0.74). Mean birth weight of the 416 newborns was 2881g with 15% weighing <2500g. Reported use of treated and untreated bed-nets were both protective against low birth weight (OR 0.22, CI: 0.08 - 0.59 and OR 0.34, CI: 0.16 - 0.74, respectively). In conclusion, since the inception of the KINET project, ITN coverage in the Kilombero Valley has increased from 37% in 1996 to over 85% in 2005. Previous studies conducted in the same facility in 1994-95 revealed placental parasitemia rates of 35.2%. Even for women who fail to receive IPTp, the prevalence of placental malaria has dropped by more than 75%. Reasons for this drop may include the substantial increase in ITN coverage over the past 10 years. The use of IPTp in this population could further decrease the rate of placental parasitemia and resulting sequellae.

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CHANGES IN PEDIATRIC BLOOD TRANSFUSION STATISTICS AS A POSSIBLE INDICATOR OF MALARIA CONTROL

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As Zambia continues to expand its Roll Back Malaria program, the Malaria Institute at Macha (MIAM) has analyzed hospital data for its usefulness in predicting changes in community levels of malaria. We have found that the monthly number of all pediatric blood transfusions given at our district-level hospital in rural Zambia correlates highly with the number of cases of uncomplicated pediatric malaria. This correlation exists for both hospitalized inpatient cases as well as outpatient cases diagnosed in the referral community. Data will be presented showing this close correlation, providing further evidence that in sub-Saharan Africa, tracking the number of pediatric blood transfusions may be a valid and easily obtained indicator for documenting malaria control at the district as well as the country level.

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A RANDOMIZED TRIAL OF AMBULATORY SHORT COURSE HIGH DOSE ORAL AMOXICILLIN THERAPY IN THE TREATMENT OF SEVERE PNEUMONIA IN CHILDREN

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Currently, WHO case management guidelines for severe pneumonia (lower chest indrawing) involve referral to hospital for treatment with parenteral antibiotics (benzylpenicillin or ampicillin). If equally effective, home-based oral antibiotic therapy could reduce referral, admission and treatment costs. We aimed to determine whether home treatment with high dose oral amoxicillin (80-90 mg/kg/day in two doses) and inpatient treatment with parenteral ampicillin were equivalent in the treatment of severe pneumonia in children aged 3-59 months. This randomized, open-label equivalency trial was undertaken at tertiary-care centers at seven study sites in Pakistan. Children aged 3-59 months with severe pneumonia were either admitted for 48 h and treated with parenteral ampicillin or were sent home to receive 5 days of oral amoxicillin. 2037 children were randomly allocated to hospitalization (n=1012) or home based therapy (n=1025). Follow-up assessments were performed at 1, 3, 6 and 14 days after enrolment. Primary outcome was treatment failure (clinical deterioration) by day 6. Analyses were per protocol and by intention-to-treat. Treatment failure was 8.6% in the hospitalized group and 7.5% in the home treatment group (risk difference 1.1%; 95% CI -1.3 to 3.5) at day 6. Young infancy (age 3-5 months; odds ratio 3.30, 95% CI 1.91 to 5.69), a weight-for-age Z score of < -2 (1.77, 1.22 to 2.58), and very fast breathing (1.67, 1.08 to 2.60) predicted treatment failure by multivariate analysis. In conclusion, home therapy with high-dose oral amoxicillin is equivalent to initial hospitalization and parenteral ampicillin for severe pneumonia treatment. These data suggest that WHO recommendation for severe pneumonia treatment need to be revised.

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EVALUATION OF A MICROCOLONY DETECTION METHOD AND PHAGE ASSAY FOR RAPID DETECTION OF *MYCOBACTERIUM TUBERCULOSIS* IN SPUTUM SAMPLES

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Early and rapid diagnosis of tuberculosis is necessary for both treatment and control of the disease. This study evaluated two microcolony observation techniques based on liquid and solid media and a mycobacteriophage assay, to evaluate their effectiveness in the diagnosis of pulmonary TB compared with a standard culture (BACTEC 460 and LJ medium). Middlebrook7H9 (M7H9) broth based on microcolony determination detected 57/61 positives cultures (n=200) with a sensitivity of 93.4% and a specificity of 87.1%. M7H11 agar detected 57/62 positive cultures (n=198) with a sensitivity of 91.9% and a specificity of 89.7%. The mycobacteriophage assay detected 98/143(68.5%) positive samples. The time to positivity was 48 hours in the mycobacteriophage assay versus 7 days in both the M7H9 broth and M7H11 agar. The costs in comparison with the culture (BACTEC 460 and LJ) were 33% and 48% for the microcolony and mycobacteriophage methods, respectively. Microcolony methods were rapid and cost effective compared to standard cultures. The mycobacteriophage assay, despite its lower sensitivity, has a short turn around time, and may be recommended as a screening test in countries with a low prevalence of tuberculosis.

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TUBERCULIN SKIN TESTING HAS LIMITED DIAGNOSTIC UTILITY FOR ADULT PULMONARY TUBERCULOSIS IN ENDEMIC REGIONS

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The tuberculin skin test (TST) is often used to assess the likelihood that symptoms are caused by TB disease. However, reliability may be limited because advanced TB causes false-negative TST and in TB-endemic countries many healthy people have positive TST for reasons including latent asymptomatic TB infection. TB is a wasting disease and we hypothesized that low BMI may be more reliable for diagnosing TB disease than the TST. The objective of this study was to compare the diagnostic utility of the TST vs. BMI for TB disease in adults in Peru. In an initial prospective case-control study we found 46% (298/647) of healthy individuals were TST-positive vs. 65% (261/355) of newly diagnosed pulmonary TB patients (odds ratio [OR] 3.4 95% confidence intervals [CI] 2.6-4.6). The size of the TST reaction was not associated with the likelihood of TB nor with prognosis. We therefore did a prospective cohort study of 428 symptomatic patients with suspected TB disease after TB contact. 376 of these symptomatic contacts had both TST and diagnostic

sputum culture. The TST was positive in 63% (215/339) of the contacts who were found not to have TB disease compared with 59% (22/37) of contacts whose sputum grew *Mycobacterium TB*, confirming TB disease (OR 0.95 95%CI 0.48-1.9). Low BMI was associated with TB disease and with false-negative TST. Consequently, BMI<20 predicted TB disease more reliably than the TST result (OR of TB if BMI<20 was 9.2 in the case-control study and 4.8 in the contact cohort study, both P<0.001). Low BMI was a better diagnostic indicator of active TB than a positive TST. Therefore, in adults from endemic regions, the likelihood that an illness is caused by TB is influenced little by the TST but greatly by the BMI. Performing a TST is not indicated for diagnosing TB disease in symptomatic contacts and BMI should be given greater emphasis in assessing the likelihood that a patient's illness is TB.

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FATAL INFLUENZA A/H5N1 INFECTION IN A 14-YEAR-OLD MALE PRESENTING WITH FEVER AND DIARRHEA

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We report a 14-year-old male from Indonesia whose presentation with influenza A/H5N1 infection was manifest by profuse diarrhea and fever. Pulmonary involvement was not prominent until the second day after hospitalization, when tachypnea developed and chest radiograph revealed a right lower lobe infiltrate. Influenza A/H5N1 infection was established by detection of virus genome with RT-PCR from nasal, throat and rectal samples, as well as virus isolation from throat and rectal samples. No enteric pathogens were found in stool to account for the diarrhea. Sequence analysis of viral genomic material from throat and rectal isolates demonstrated homology with contemporaneous influenza A/H5N1 isolates from humans in Indonesia, including the V27A mutation in M2 protein suggestive of amantadine resistance. This case corroborates earlier reports of influenza A/H5N1 isolation from intestinal tract samples and highlights the importance of considering extra-pulmonary presentations for H5N1 infections in humans.

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H5N1 OUTBREAK IN BURKINA FASO

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Since February 2006, nine African countries including Nigeria, Niger, Egypt, Cameroon, Burkina Faso, Ivory Coast, the Sudan, Djibouti and Ghana have officially reported H5N1 outbreaks to the World Organization for Animal Health (OIE). Here we analysed the epidemiological situation in suspect sources of H5N1 in Burkina Faso and On the basis of HA1 sequences we compared H5N1 detected strains to existing clusters. We collected paired tracheal and cloacal swabs from 283 birds including 271 chickens, 2 ducks, 5 turkeys, 3 vultures, 1 sparrow hawk and 1 grey tourraco from March to June 2006, around the towns of Bobo-Dioulasso and Ouagadougou. We have used Generic influenza A M-RT-PCR and specific H5 PCR for viral strains detection. Complementary sequencing has been performed to determined specific N1 and the related cluster. We detected nine cases of influenza A virus including five cases of H5N1 virus giving respectively the prevalence of 3.2% (95% CI: 0-6.6) and 1.77%.The positives cases of H5N1 virus were found in 2 vultures at Ouagadougou, 3 local chickens Bobo-Dioulasso and Sokoroni. The five detected H5N1 strains from Burkina Faso as strains from Northern Nigeria, the Sudan, and Côte d'Ivoire constitute the putative African cluster C. Our results confirmed the presence of influenza A H5N1 virus in domestic and wild birds in Burkina Faso and for the first time, the cluster of detected H5N1strains have been identified to belong to cluster C.

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PREPAREDNESS FOR PANDEMIC INFLUENZA IN A DEVELOPING COUNTRY: KNOWLEDGE, ATTITUDES AND PRACTICES CONCERNING INFLUENZA CONTROL IN PERUVIAN NAVY HEALTH CARE FACILITIES

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Influenza is one of the agents most likely to cause the next pandemic threat. It is unclear whether the current avian strain (H5N1) will fill this role, but preparedness for this inevitable event is imperative. Many of the current strains with pandemic potential have arisen in the developing world, at sites where preparedness for pandemic influenza is likely the poorest. A questionnaire to assess the knowledge, attitudes and practice of health care providers with regard to pandemic/avian influenza was developed in order to assess current levels of preparation and to target areas for education. The questionnaire was applied to personnel from various Naval health care facilities in Peru. 145 health care providers responded to the questionnaire, the mean age was 40.27 ± 9.6 yo and 30.1% were male. 37% were technicians, 43% nurses, and 13.3% physicians. 76.8% worked in a large hospital while 23.2% worked in small centers. Overall knowledge was self-rated as intermediate by 89.5%. The majority identified correctly the mode of transmission (87.2%) and the main symptoms (94.9%) regardless of their profession. However, only 35.8% could identify the proper treatment and 26.1% believed that the seasonal vaccine is useful for avian flu. Hand washing was more frequent in hospitals than small facilities (82.8% vs. 64.3%). Overall 75.2% reported use of masks, 77.9% gloves and 61.9% gowns. Physicians were less willing to perform these practices Only 38.5% from hospitals referred having been trained, whereas 58.6% at small centers; similarly, the small facilities more often had a contingency plan (48.3% vs. 36.0%). Only 42.9% perceived avian influenza as a high-risk threat to public health and themselves, without difference between professionals. Around 65% thought that hand washing, use of mask and patient isolation were effective countermeasures. Quarantine was considered useful by 50.4%; public buildings closure by 73.4% and travel restriction by 95.5%. Finally, 96.3% considered that the response plan should be a national effort. Peru is a developing country with no current circulation of influenza strains with pandemic potential. The knowledge concerning pandemic influenza among health care workers was generally low, and likely representative of other developing countries. Education concerning the control, diagnosis and treatment of influenza with pandemic potential should be provided to all health care workers.

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BURDEN AND EPIDEMIOLOGY OF INFLUENZA-LIKE ILLNESS IN A PEDIATRIC COHORT IN NICARAGUA

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Little is known about the burden and epidemiology of influenza in the tropics. Additional knowledge is essential not only from a public health standpoint in tropical countries, but also for pandemic influenza models and planning worldwide. To characterize the burden and epidemiology of influenza-like illness (ILI) in a tropical developing country, we performed a community-based cohort study examining the incidence of ILI in children in Managua, Nicaragua. Influenza-like illness was defined as fever ≥37.8°C with cough and/or sore throat at presentation. A total of 4,276 children between 2 and 12 years old were followed for every primary medical appointment for a two-year period between April 16, 2005, and April 15, 2007. The population was 49.4% female, 2.5% had asthma, and 25.9% of person-time was contributed by children under 5 years old. Participants contributed a total of 7,586 person-years and experienced 2,596 episodes of ILI, yielding an incidence rate of 34 cases per 100 person-years. Seasonal variation of ILI activity was observed; a single peak occurred in June in the 2005-2006 period and a marked biphasic pattern was documented in 2006-2007, with one peak in July and another peak in November. Restricting the analysis to children 5 years and over and to an outcome of high-probability ILI (fever ≥38.5°C with cough) resulted in the same biphasic pattern. Risk factors were examined using multivariate random-effects logistic regression; asthma and age less than 5 years old were significant risk factors for ILI, with odds ratios of 1.68 (95% CI 1.31-2.17) and 2.25 (95% CI 2.05-2.46), respectively. Our results suggest that Nicaragua has a high influenza burden and experiences two seasonal influenza periods in some years. These peaks correspond in timing to those observed in the Southern and Northern hemispheres. To further characterize the epidemiology of influenza, we began a prospective study of laboratory-confirmed influenza in this pediatric cohort in May of 2007, in which RT-PCR and viral isolation is being used to detect influenza and other respiratory disease agents (e.g., respiratory syncytial virus, parainfluenza 1-3) in the cohort. This study should provide critical information about the transmission of influenza and other respiratory viruses in the American tropics useful for both national and international surveillance efforts.

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CONUNDRUMS IN EVOLUTION OF JAPANESE ENCEPHALITIS VIRUS

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Japanese encephalitis virus (JEV) is a flavivirus thought to have originated from an ancestral virus in the Indonesia-Malaysia region and to have evolved there into 4 or 5 different genotypes, of which genotypes I to III then spread across Asia. As part of ongoing investigations into the molecular epidemiology of JEV, we have determined the nucleotide sequences of the envelope (E) gene and 3′ untranslated region (3′-UTR) of a number of isolates from Indonesia, Thailand and the Philippines. We identified the contemporaneous co-circulation of strains belonging to different genotypes in the same local geographical region (Kamphaeng Phet in Thailand). Furthermore, for 2 isolates from Indonesia, there was conflict between the E gene and 3′-UTR sequence in the assignment of genotype suggesting that recombination may have occurred. To further evaluate the extent of recombination in JEV, we examined more than 100 E gene sequences. Initial exploratory tree analysis using a sliding window method and generating trees for the different regions identified 10 strains that clearly changed topological position over different regions of the gene, including 4 already identified as putative recombinants in the literature. For one strain identified as a putative recombinant using the published E gene sequence, when a diversity plot was generated with the same putative parents using the E gene sequence published as part of the complete genome sequence, there was no evidence of recombination, highlighting the need for independent confirmation of putative recombinant sequences. All of the putative recombinants identified had a genotype III parent, which may reflect the predominance of this genotype. Analysis of E gene sequences from strains for which the date of isolation is known suggested that genotypes IV and V are the most ancient, with genotype II most recently diverged, and also the least widespread. As no genotype IV or V strain has been isolated for over 25 years one may speculate that these genotypes have been superceded.

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DEFINITION OF THE MAJOR DETERMINANT RESPONSIBLE FOR NEUROVIULENCE OF JAPANESE ENCEPHALITIS VIRUS

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Japanese encephalitis virus (JEV), a major cause of acute viral encephalitis in Asia, is composed of three structural proteins (C: core, M: membrane, E: envelope) and seven nonstructural proteins. Genetically, JEV can be divided into five genotypes. The genotype changed in early 1990's from genotype 3(G3) to genotype 1(G1) in East Asia including Japan. Our recent study shows that Mie41 strain which belonging to G1 had significantly lower neurovirulence in mice than a G3 JEV Beijing1 strain. Conversely, Beijing1 shows slow-growth phenotype in Vero cells as compared to Mie41. In this study we tried to clarify the major genetic determinant responsible for neurovirulence and growth properties in JEV. We first constructed a full-length infectious cDNA clone based on the Mie41 which isolated from porcine serum in 2002 in Japan and obtained viral RNA by a reverse genetics method. Focusing on the structural proteins, two chimera viruses rJEV(CME-Beijing1/Mie41) and rJEV(E-Beijing1/Mie41), whose structural protein and E protein were replaced by those of Beijing1, were also produced. One-step growth analyses showed that not only Beijing1, but also rJEV (CME-Beijing1/Mie41) and rJEV(E-Beijing1/Mie41) replicated slower than Mie41 in Vero, porcine kidney PK15 and mosquito C6/36 cells. By contrast, Beijing1 and two chimera viruses had better growth properties than Mie41 in mouse neuroblastoma N18 cells.
The neurovirulence in mice showed that Beijing1 and two chimera viruses were significantly more virulent than Mie41. Interestingly, mice inoculated with Mie41 showed slightly flaccid paralysis on lower legs, whereas the other mice showed hyperexcitability. From these results, we concluded that E protein in JEV is a major determinant regulating the viral replication *in vitro* and neurovirulence in mice. Comparing genome sequences in E protein between the Mie41 and Beijing1, only eight amino acids were different. The key amino acids are now being analyzing.

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EFFECT OF PRE-EXISTING ANTI TICK BORNE ENCEPHALITIS VIRUS (TBE) IMMUNITY ON NEUTRALIZING ANTIBODY RESPONSE TO THE NOVEL, VERO CELL DERIVED INACTIVATED JAPANESE ENCEPHALITIS VIRUS (JEV) VACCINE IC51

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Japanese Encephalitis Virus is the leading cause of viral encephalitis in Asia with a case fatality rate up to 35% and long-term sequelae up to 75%. Preventive vaccination is of utmost importance for travelers into these regions. Preexisting immunity against other flaviviruses may influence successful JE vaccination. We report on immunogenicity results of the novel Vero cell-derived, inactivated, alum adjuvanted JE vaccine IC51 under late stage development by Intercell AG in a population with

pre-existing immunity against TBE. The aim of this investigation was to study the effect of pre-existing anti-TBE immunity on seroconversion rates in terms of neutralizing JEV specific antibodies after one and two JE vaccinations with IC51. In this multicenter, observer blinded, randomized controlled phase 3 study the new Vero cell-derived, SA_{14}-14-2 based vaccine IC51 was administered i.m. (0.5 mL, Days 0 and 28) to 430 healthy adults. To assess the immunogenicity of IC51 in subjects with pre-existing TBE immunity, anti flavivirus immune status was assessed at baseline with a TBE - ELISA tests in secondary analyses in the ITT population. All subjects were immunologically naïve to JE. JE specific immunity after one and two IC51 vaccinations was analyzed with the Plaque Reduction Neutralization Test ($PRNT_{50}$). 18.7 % of IC51 vaccinees (81/428) were TBE positive at baseline. Twenty-eight days after a single vaccination with IC51 the SCR was 76.5 % in TBE positives, whereas in TBE negatives the SCR was 49.0 % ($p < 0.0001$ Fisher exact test). After two IC51 vaccinations the SCR was not significantly different in TBE positives (96.3%) compared to TBE negatives (91.4%). This advantageous effect was even more obvious in vaccinees younger than 50 years of age 28 days after a single IC51 vaccination. In summary, pre-existing TBE immunity augmented neutralizing JEV specific antibody responses to a single IC51 vaccination. Three out of four vaccinees seroconverted 28 days after the first vaccination. After 2 vaccinations with IC51 the vast majority of vaccinees seroconverted irrespective of pre-existing TBE antibodies. The benefit of preexisting TBE immunity is predominant in vaccinees younger than 50 years. In conclusion, these data indicate that pre-existing anti-TBE immunity enhances the neutralizing antibody response to the novel, Vero-cell derived JE vaccine IC51, rather than leading to diminished JEV-specific responses.

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MIGRATION AND TRANSMISSION HISTORY OF ST. LOUIS ENCEPHALITIS VIRUS

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St. Louis encephalitis virus (SLEV) is a mosquito borne flavivirus found throughout the Americas. Previous phylogenetic analyses have demonstrated three major clades (two North American and one South American). Despite strong geographic clustering, there is evidence of movement between North and South America, as well as within each region. We used a parsimony method to assess the degree and direction of gene flow within the Americas. Our data set comprised of 67 previously published partial envelope gene sequences (1604 bp). Results suggest gene flow from South America to the Western USA and Central America, and from the Eastern USA to both South and Central America. Texas, Florida and Brazil, were identified as centres of high viral diversity and the most likely locations for founding populations in North and South America respectively. We then used a Bayesian coalescent approach to infer changes in genetic diversity in South America, and in one of the North American clades (the other having too few sequences for analysis). The latter was estimated to have arisen around 1905 (95% HPD 1832 - 1951) with an initial constant level of genetic diversity followed by a rapid increase in the number of lineages from 1943 -1948 and then a plateau. In contrast, the South American clade which is estimated to have arisen between 62 and 886 years ago (mean =331), demonstrated an exponential decline in diversity over the period represented (1972 - 2005). There was no significant difference in substitution rates between the two clades, so extrinsic factors may account for the differences in demographic histories. The observed changes in genetic diversity may not reflect actual changes in viral population size since positive selection was detected at amino acid position 58, 156 and 179 in the envelope genes of the North American isolates and amino acid positions 151, 156 and 344 of the South Americans.

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CHARACTERIZATION OF THE BARKEDJI VIRUS, A NEW MOSQUITO-BORNE FLAVIVIRUS ISOLATED IN SENEGAL

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The genus *Flavivirus* contains about 70 single-stranded positive RNA viruses including important emerging pathogens such as Dengue, Yellow fever or West Nile viruses. Flaviviruses are primarily transmitted by arthropod vectors (ticks or mosquitoes) and can been grouped according to their serological, phylogenetic or ecological characteristics. Here, we report the characterization of a new flavivirus, isolated in 1991 from a mosquito pool in Barkedji (Ferlo region of Senegal) and referred as « Barkedji virus » (BARKV). BARKV has been shown to i) infect both mosquito (AP61) and mammalian (PS) cells ii) induce neurological symptoms and kill suckling mice within 4-5 days when injected intracerebrally and iii) have no effect on adult mice when injected intra-peritoneally. Neutralization and complement fixation tests suggest that BARKV is closely related to Bagaza virus and its variant (ArD65239). However, phylogenetic analysis based on full length genome of BARKV and other flaviviruses revealed that it has a unique phylogenetic position within the the mosquito-borne flaviviruses group, clearly distinct from the major complexes of yellow fever, dengue and japanese encephalitis groups. Analysis of the amino acid sequence of BARKV shows conservation of cleaveage sites and enzymatic motifs common to all flaviviruses, except for the serine protease NS3 gene which exhibits mutations in the catalytic site suggesting that BARKV could have a new type of protease activity comparatively to other flaviviruses. In conclusion, the genomic characterization of BARKV as well as analysis on the NS3 protease activity should give new insights on the evolution and biology of flaviviruses. Additional data on the ecology and pathogenicity of BARKV should help to assess its potential impact on public health.

(ACMCIP Abstract)

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CHIKUNGUNYA FEVER IN MAURITIUS, 2006

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An epidemic of chikungunya (CHIK) fever initially struck islands in the Indian Ocean in 2005, drawing the attention of the international public health community to chikungunya virus (genus *Alphavirus*, family *Togaviridae*). In Mauritius, more than 10,000 cases were diagnosed and confirmed in 2006. The primary vector of CHIK virus in Mauritius is the highly abundant "Asian Tiger" mosquito, *Aedes albopictus*. This mosquito was previously considered an ineffective vector, since it bites other vertebrates besides humans. Although CHIK is considered a non-fatal disease, an excess number of deaths has been documented for the recent outbreak on the Indian Ocean islands. We report mortality data in Mauritius from 1995 to 2006, and show an increase in the total number of deaths during the CHIK epidemic in Mauritius (March - May 2006). We also present the distribution of clinically diagnosed cases of CHIK fever for 2006 along with seasonal abundance data for *Ae. albopictus.*

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PATHOGENESIS OF CHIKUNGUNYA VIRUS INFECTION IN MICE

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Chikungunya virus (CHIKV) is an *Alphavirus* that is endemic in tropical regions of Asia, Africa and the islands in the Indian Ocean. It is transmitted by the *Aedes* mosquitoes and has recently been added to the National Institute of Allergy and Infectious Diseases priority pathogen list. People infected by CHIKV have an illness characterized by high fever, rash and severe arthralgia. The pathology of CHIKV is still unclear and has not been well studied. This research describes the pathology of CHIKV infection in mice. When CHIKV is inoculated subcutaneously in outbred/ICR mice younger than one month old, there is a resulting viremic period lasting 2-8 days. CHIKV specific antibodies develop 5-10 days after inoculation. Infectious virus persists in the muscle and brain tissues after the viremic period has ended. CHIKV antigen can be observed in infected skeletal muscle cells as well as in the endothelium and cross-sections of the skin. Histological findings show CHIKV causes severe myositis with focal necrosis of the skeletal muscle. Plasma cells as well as eosinophils and macrophages can be observed to have infiltrated the skeletal muscle. Despite the severe muscle cell necrosis, the clinical signs in the infected mice are minor. Some mice develop transient hind limb dysfunction and recover within a few days. CHIKV infection also appears to cause damage to the hair follicles, resulting in bald patches on some mice. Although CHIKV can be found in multiple organ systems, very few pathological changes are seen outside of the skeletal muscle. These pathology findings in the skeletal muscle are similar to those reported in mice with Ross River virus infection. The goal of our work is a better understanding of the pathological changes that occur after CHIKV infection so that a more effective treatment may be developed for people.

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CYTOKINE-ASSOCIATED NEUTROPHIL EXTRACELLULAR TRAPS AND ANTINUCLEAR ANTIBODIES IN *PLASMODIUM FALCIPARUM* INFECTED CHILDREN UNDER THE AGE OF SIX

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In *Plasmodium falciparum*-infected children, the relationships between blood cell histopathology, blood plasma components, development of immunocompetence, and disease severity remain poorly understood. This investigation relates levels of the pro-inflammatory cytokines IFN-γ, IL-2, TNF-α, CRP, and IL-6, and select anti-inflammatory cytokines TGF-β and IL-10 to the formation of neutrophil extracellular traps (NETs), IgG antinuclear antibodies (ANA), and IgG antineutrophil cytoplasmic antibodies (ANCA) in blood collected before and seven days after initiation of Sulfadoxine-Pyrimethamine treatment from 21 Nigerian children under six years old presenting with uncomplicated malaria. The children exhibited a Th2 dominated cytokine profile and left-shifted leukocyte differential. Elevated TNF-α levels correlated with significant NET formation (DNA confirmation by DAPI staining) evident in the peripheral blood smears. ANA levels (inclusive of all subsets of ANA) were positive in 86% of the children pretreatment (remaining14% were equivocal) and in 100% of the children seven days after SP treatment but in only 33% of age-matched control samples collected during the season of low parasite transmission. IgG ANA subset levels to dsDNA were positive in 81% of both the pre- and post treatment samples (remaining 19% were equivocal), whereas ANCA levels were positive in only 14% of both the pre-and post-treatment samples. Our results suggest that an inverse relationship between TGF-β and CRP levels may contribute to homeostasis and that TNF-α-associated NET formation and ANA may induce pathology in falciparum-infected children or activate a protective mechanism against falciparum malaria in adults. The significance of *in vivo* circulating chromatin in NETs and ANA to dsDNA as a causative factor in the hyporesponsiveness of CpG olignucleotide-based malaria vaccines is discussed.

(ACMCIP Abstract)

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HIV INFECTION IMPAIRS OPSONIC PHAGOCYTOSIS OF *PLASMODIUM FALCIPARUM*-INFECTED ERYTHROCYTES

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Women acquire immunity against pregnancy-associated malaria (PAM) with increasing gravidity, but this protection is decreased by human immunodeficiency virus (HIV) infection. Immunity to PAM is associated with production of antibodies to pregnancy associated variant surface antigens (VSA-PAM) expressed by chondroitin sulfate A-binding infected erythrocytes (IE). We hypothesized that antibody function, rather than level, may be a better predictor of immune protection against PAM. We tested sera from 94 multigravid Malawian women (65 HIV-infected, 29 HIV-uninfected) with malaria. For total IgG to VSA-PAM and for ability of sera to block adhesion of IE to CSA, or to opsonise IE and promote phagocytosis by a macrophage cell line. Serum opsonic activity correlated better with total IgG to VSA-PAM (r=0.63) than did adhesion blocking activity (r=0.36). HIV infection was associated with significantly lower opsonic activity (p=0.02) which varied in a CD4 T-cell-dependent manner. In contrast, no effect of HIV infection on adhesion-blocking activity or total IgG could be demonstrated in these women. Women with anemia (Hb <11.0 g/dl) showed significantly lower opsonic activity than non-anemic women (t = 3.04, p=0.003), regardless of HIV serostatus. In separate experiments, we infected human monocyte-derived macrophages (MDM) with HIV *in vitro.* HIV inhibited phagocytosis of opsonised IE by 86.4 ± 15% (p=0.0078, n=8). HIV infection impairs opsonic but not adhesion-blocking antibody responses to PAM. Opsonic activity but not adhesion-blocking activity is strongly associated with protection from malaria-associated anemia in pregnancy. The mechanisms underlying this protection require further investigation. Impaired opsonic activity in combination with decreased macrophage phagocytic activity may contribute significantly to the increased susceptibility to malaria seen in HIV infected individuals. Opsonizing activity in serum may be an important parameter to incorporate into studies of malaria pathogenesis, and of the effectiveness of potential vaccine candidates.

(ACMCIP Abstract)

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ROLE OF IL-10 AND HEMOZOIN IN REGULATING IL-12 AND IL-17 PATHWAYS IN KENYAN CHILDREN WITH SEVERE MALARIAL ANEMIA

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Severe malarial anemia (SMA) in African children is characterized by an imbalance in pro- and anti-inflammatory cytokine production. Our recent studies demonstrated that hemozoin-induced interleukin (IL)-10 production mediates suppression of IL-12 and up-regulation of IL-23 in children with SMA. Previous studies showed downstream effector functions of IL-12 and IL-23 occur via CD4 T cell-induced IFN- γ and IL-17 production, respectively. Production of both IFN-γ and IL-17 is further modulated by IL-15, an important mediator of erythropoiesis. To further investigate the pathogenesis of SMA, the role of IL-10 and hemozoin in modulating IL-12, IFN- γ, IL-17, and IL-15 production was examined in children (n=198; aged <36 mos.) exposed to intense *Plasmodium falciparum* transmission in western Kenya. Measurement of inflammatory mediators with a multiplex antibody bead array revealed increased IL-10 and decreased IL-12 and IL-17 levels in children with SMA relative to aparasitemic healthy controls (AC). The pattern of cytokine production observed closely paralleled the levels of hemozoin deposition in monocytes. In addition, hemoglobin (Hb) concentrations showed a positive relationship with IL-12 (r=0.174, P<0.05), IL-15 (r=0.171, P<0.05), IL-17 (r=0.238, P<0.05) and IFN- γ (r=0.171, P<0.05), and an inverse correlation with IL-10 production (r=-0.119, P<0.05). Erythropoiesis, determined by measuring the reticulocyte production index (RPI), revealed a positive association between the RPI and IL-12 (r=0.154, P<0.05) and an inverse correlation between the RPI and IL-10 (r=-0.272, P<0.05), but no significant relationship with the other mediators examined (i.e., IFN- γ, IL-15 or IL-17). Taken together, results presented here suggest that hemozoin-induced over-production of IL-10 promotes decreased IL-12 and IL-17 levels in children with SMA. Furthermore, elevated levels of IL-10 and decreased IL-12 production appear to be important in conditioning a suppressed erythropoietic response that promotes the development of SMA.

(ACMCIP Abstract)

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TLR9 POLYMORPHISMS ARE ASSOCIATED WITH ALTERED IFN-γ LEVELS IN CHILDREN WITH CEREBRAL MALARIA

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Elevated IFN-γ and TNF-α levels have been implicated in the pathogenesis of cerebral malaria. Toll-like receptor (TLR) single nucleotide polymorphisms (SNPs) may be associated with altered signaling in response to *Plasmodium falciparum* infection, with downstream elevation of IFN-γ and TNF-α levels and more severe disease. To test the relationship between TLR SNPs, cytokine production and disease severity, TLR2, 4, and 9 SNP frequencies and serum IFN-γ and TNF-α levels were compared in Ugandan children aged 4-12 years with cerebral malaria (CM, n=69) and uncomplicated malaria (UM, n=53). A microbead suspension array system was used to determine post-PCR SNP genotypes and cytokine levels. Of the TLR2, 4 and 9 SNPs tested, only TLR9 SNPs (T-1237C, G1174A, T-1486C) were present in frequencies >15% in either group. Children with CM and the T-1237C SNP had higher levels of IFN-γ than those without this SNP (median level, pg/ml: C/C, 154.1; T/C, 127.2; T/T, 42; P=0.03). Homozygosity for the 1237 SNP (C/C) was more frequent in children with CM than UM (17.4% vs 5.7%, P=0.06). Children with CM and the G1174A SNP had lower levels of IFN-γ than those without this SNP (A/A, 32.8; G/A, 69.7; G/G, 161; P=0.002). Homozygosity for the 1174 SNP (A/A) was similar in children with CM and UM (18.8% vs. 26.4%, P=0.32). Children with UM did not have differing IFN-γ levels according to any TLR9 SNP, and neither children with CM nor UM had differing TNF-α levels according to any TLR9 SNP. In conclusion, in children with CM, 2 different TLR9 polymorphisms are associated with opposing effects on serum IFN-γ levels. Children with CM are more frequently homozygotes for the T-1237C SNP (C/C), which is associated with elevated IFN-γ levels. Thus, TLR9 polymorphisms may affect severity of disease through alterations in IFN-γ responses to *P. falciparum* infection. However, TLR9 - IFN-γ associations are not seen in children with UM, suggesting that additional factors in children with CM may be required in conjunction with TLR9 polymorphisms to alter IFN-γ levels.

(ACMCIP Abstract)

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THE ROLE OF IMMUNOREGULATORY CELLS IN NATURAL IMMUNITY TO *PLASMODIUM FALCIPARUM*

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An important aspect of clinical immunity to malaria is the ability to down-regulate inflammatory responses once parasitaemia is under control; this is crucial to avoid host-mediated pathology. Data from our group, derived from sporozoite-challenged volunteers, demonstrate that in a proportion of subjects, regulatory T cells can be induced and activated as early as 10 days following infection with *Plasmodium falciparum*. The cells were shown to down-regulate pro-inflammatory responses and to facilitate parasite growth *in vivo*. However, it is not yet known whether natural exposure to malaria activates/induces T regulatory cells. The aim of this study was to characterize the frequency, phenotype and functional importance of regulatory cells in healthy donors having different levels of previous malaria exposure. Sixty individuals from two sites in the Gambia were selected for the study: 30 subjects from Brefet, with high levels of antimalarial antibodies and 30 from Bakau, with low levels of antibodies. Seroprevalence of $MSP1_{19}$ was used to estimate the entomological inoculation rate (EIR) at each of the two locations. Using flow cytometry, we compared the number of circulating lymphocytes with a regulatory phenotype from these two sites. We used Foxp3 and CD127 (IL-7R) to define the regulatory cell subset. Cytokine production, as well as the type of immune response (Th1, Th2, Th17 and Treg), was studied using bead arrays and RT-PCR. The results show a significant difference in both the number and percentage of regulatory cells (defined here as $CD4^+Foxp3^+CD127^{-/low}$ cells) in individuals from the two sites. In areas with higher malaria exposure, donors had a higher number of circulating regulatory cells. This may reflect the importance of the regulatory environment in dampening host-mediated pathology and thus limiting severe disease. In order to confirm and complete our findings, future research will assess the levels of different Treg phenotypes and their degree of activation in both populations. A number of age groups will be studied to determine whether the regulation observed is population-wide or age-dependent.

(ACMCIP Abstract)

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RISK FACTORS FOR MALARIA IN A RURAL AMAZONIAN COHORT (GRANADA, ACRE, BRAZIL)

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