1

ALTERATIONS IN *PLASMODIUM FALCIPARUM* GENETIC STRUCTURE AFTER INCREASED MALARIA CONTROL EFFORTS IN TWO DISTRICTS OF WESTERN KENYA

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The collective collaborations of the Roll back malaria program, President's Malaria Initiative and the Global Fund to fight AIDS, Tuberculosis and Malaria have resulted in the implementation of large scale malaria control efforts. Increased insecticide-treated bed net usage (ITNs) and the use of a new antimalarial formulation, artemisinin-combination therapy (ACT), are the major tools currently being deployed for malaria control. However, most control programs lack a critical evaluation of the effectiveness of these tools. This study examined alterations in *Plasmodium falciparum* prevalence, genetic diversity and drug resistant mutations in two districts of Western Kenya with differing endemicities: an endemic lowland site (Kombewa), and an epidemic highland site (Kakamega). Comparisons were made for blood samples collected in 2005 (prior to new malaria control regime) and 2008 (2 years after implementation). The lowland site, Kombewa, showed an initial decrease in overall parasite prevalence (from >50% in 2005 to ~15% in 2007), but a sharp increase occurred in late 2007-2008, with a current parasite prevalence close to 40%. Kakamega also showed a decrease in parasite prevalence (~30% in 2005 vs. ~7% in 2008) with no noticeable rebound. Interestingly, the genetic diversity, as measured by expected heterozygosity (*He*), of both populations have not significantly changed, despite significant reductions in transmission intensity (*He*= 0.75 and 0.82 Kombewa and *He*=0.69 and 0.79 Kakamega, years 2005 and 2008 respectively). These preliminary results suggest that current malaria control efforts have not caused a major change in parasite genetic structure. We are currently examining the impact of this new malaria control regime on the frequency of mutations in genes conferring resistance to chloroquine, sulfadoxine-pyrimethamine and the multi-drug resistance genes.

2

MULTIPLE GENETIC BACKGROUNDS OF THE AMPLIFIED *PLASMODIUM FALCIPARUM* MULTIDRUG RESISTANCE (*PFMDR*1) GENE AND SELECTIVE SWEEP OF 184F MUTATION IN CAMBODIA

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The border between Thailand and Cambodia has remained as the epicenter of antimalarial drug resistance. The resistance against two widely used antimalarial drugs chloroquine and sulfadoxine-pyrimethamine originated in this region and later spread to other parts of the world. The recent evidence of decreasing efficacy of artesunate plus mefloquine (AS + MQ) combination therapy in this region is a major global concern for malaria control. Accumulating reports suggests that copy number increase and key alleles in the *Plasmodium falciparum* multi drug resistance (*pfmdr*1) gene are associated with AS + MQ resistance. In this study, we attempted to understand the genetic background of the amplified (multicopy) *pfmdr*1 gene in eastern and western Cambodia. For this purpose, we analyzed 13 microsatellite loci flanking *pfmdr*1 (± 99 kb) in a total of 93 isolates (singly infected), of which 31 had multiple copies and 62 had single copy of the *pfmdr*1 gene. Genetic analysis of these loci revealed no difference in the overall mean expected heterozygosity (H_e) at loci around single (H_e = 0.75±0.03) and multi copy (H_e = 0.76±0.04) *pfmdr*1. Importantly, amplification of *pfmdr*1 occurred on several different genetic backgrounds suggesting multiple origins of the multi copy *pfmdr*1. No evidence of genetic hitchhiking of any particular microsatellite haplotypes associated with multi copy *pfmdr*1 was found. However, genetic hitchhiking with the selective sweep of certain haplotypes was seen due to the spread of mutant (184F) *pfmdr*1 allele especially in western Cambodia, irrespective of the copy number. There was an overall reduction in H_e of 28% around the mutant *pfmdr*1 (H_e = 0.56±0.05) as compared to the ancestral type (184Y) allele (H_e = 0.84±0.02). Also, a significant linkage disequilibrium (LD) was observed between the loci flanking mutant *pfmdr*1 but not between the loci flanking ancestral type *pfmdr*1. In conclusion, the results suggest 184F mutant alleles to be under selection while amplification of *pfmdr*1 gene in this population occurs on multiple genetic backgrounds.

3

COPY NUMBER VARIATION AND POINT MUTATIONS IN *PFMDR1* IN *PLASMODIUM FALCIPARUM* ISOLATES FROM VENEZUELA

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Mefloquine is used in combination with artemisinin derivatives for the primary treatment of malaria in Southeast Asia and South America. Resistance to mefloquine has been associated with duplications of the multi-drug resistance-1 (*pfmdr1*) gene and is evolving in Southeast Asia. In South America, there has been no documented evidence to suggest that mefloquine pressure has altered parasite response, despite several years of artesunate plus mefloquine (ACT) as a primary treatment for *Plasmodium falciparum* malaria. In Venezuela in 2002, mefloquine alone or in combination with sulphadoxine-pyrimethamine was used occasionally during epidemics (1994-2000) in areas with recognized treatment failures to chloroquine and sulphadoxine-pyrimethamine. In 2004, artesunate plus mefloquine combination therapy was introduced and implemented as national policy. We investigated if there was evidence of copy number variation in *pfmdr1* in Venezuela. Ninety three *P. falciparum* isolates collected from a malaria surveillance study conducted in Bolivar state from 2003-2004 were analyzed to assess molecular changes related to *pfmdr1*. We found that 37% had the *pfmdr1* triple mutant genotype Y184F/S1034/N1042D/D1246Y and 63% had the *pfmdr1* quadruple mutant genotype Y184F/S1034C/N1042D/D1246Y; no ancestral genotypes were found. Importantly, using real time quantitative PCR, 12% of isolates had two or more copies of *pfmdr1*. The high prevalence of *P. falciparum* isolates with more than one copy of *pfmdr1* in this region may potentially be related to selection pressure from mefloquine monotherapy, suggesting that mefloquine resistance may be evolving in South America. It will be instructive to determine if the prevalence of *pfmdr1* duplications has changed after the introduction of ACT treatment. To our knowledge, this may be the first report of duplicated *pfmdr1* outside of Southeast Asia. Our data highlight the importance of surveillance for molecular markers of antimalarial drug resistance and the need to link these data with clinical studies to detect changes in the ACT efficacy in South America.

4

SULFADOXINE-PYRIMETHAMINE, SULFADOXINE-PYRIMETHAMINE + ARTESUNATE, AND AL FOR UNCOMPLICATED MALARIA INFECTION IN TANZANIA FROM 2004 TO 2006

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In 2001, the Tanzanian government adopted sulfadoxine-pyrimethamine (SP) as the first line antimalarial treatment. Continuous monitoring of antimalarial efficacy is crucial in light of increasing parasite resistance to antimalarials. We measured *in vivo* efficacy of SP alone versus SP+artesunate (SPAS) or artemether-lumefantrine (AL) five years after SP introduction and prior to widespread deployment of AL. Patients <5 years old with uncomplicated *P. falciparum* monoinfection were enrolled and randomized to receive either SP, SPAS, or AL using the standard WHO 28-day protocol. PCR genotyping was used to distinguish recrudescence from re-infection and characterize known molecular markers of antimalarial drug resistance. We enrolled 361 patients: 121 in the SP arm, 122 in the SPAS arm, and 118 in the AL arm. The uncorrected cure rates were 39%, 56%, and 77% in the SP, SPAS, and AL groups, respectively. PCR corrected cure rates are pending. This represents a significant decrease in efficacy for SPAS since 2004, when respective uncorrected cure rates were 58%, 78%, and 80%. In comparison to AL, treatment with both SP and SPAS resulted in a significant increase in the hazard ratio for infection (3.6, 95% CI: 2.3- 5.6 for SP and 2.2, 95% CI: 1.4- 3.5 for SPAS). Both SPAS and AL were significantly more efficacious for treatment of uncomplicated malaria than is SP, however, the efficacy of SPAS is rapidly decreasing. SP should no longer be used for treatment of malaria illness in Tanzania, either as monotherapy or as part of artemisinin combination therapy.

5

IN VITRO SELECTION OF PIPERAQUINE RESISTANT *PLASMODIUM FALCIPARUM*

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The combination of piperaquine and dihydroartemisinin has become the official first-line therapy in several Southeast Asian countries and is under clinical investigation in South America and Africa. The pharmacokinetic mismatching of these drugs, whose plasma half lives are ~35 days and ~1 hr respectively, implies that recrudescent parasites or new infections emerging shortly after the cessation of treatment will encounter piperaquine as a monotherapy agent. This creates a substantial selection pressure for the emergence of resistance. To elucidate potential molecular determinants of piperaquine resistance, we subjected a cloned *Plasmodium falciparum* Dd2 line to continuous drug pressure *in vitro* (46.7 nM, two-fold higher than the Dd2 IC_{50} value). The phenotype of outgrowth parasites was assayed in two independent clones, which were found to possess an IC_{50} value against piperaquine of 2.6 μM and 2.4 μM, over 100-fold greater than the parent, along with a loss of chloroquine resistance. To identify the genetic determinant of piperaquine resistance, we employed whole genome array analysis. Compared to the parent clone, this analysis found (in both clones) a novel single nucleotide polymorphism in *pfcrt*, deamplification of an 82 kb region of chromosome 5 (that includes *pfmdr1*), and amplification of a neighboring 63 kb region of chromosome 5. Using previously published genetically modified *pfmdr1* lines generated in FCB, we observed a statistically significant, 20% *decrease* in the piperaquine IC_{50} value in isogenic lines that carried a reduced *pfmdr1* copy number. Thus we believe that the *pfmdr1* deamplification is not causal for piperaquine resistance but may have been selected as a compensatory genetic event. We are currently performing allelic exchange experiments to evaluate the *pfcrt* single nucleotide polymorphism, and are using the Bxb1 integrase system to assess candidate determinants on chromosome 5. Once elucidated, the genetic basis of piperaquine resistance can be leveraged to screen for the emergence of resistance in malaria-endemic areas.

6

FREQUENT CHROMOSOMAL REARRANGEMENT IN *PLASMODIUM FALCIPARUM* RESISTANT TO ARTELINIC ACID *IN VITRO*

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Gene amplification of *pfmdr1* in *Plasmodium falciparum* correlates with resistance to various antimalarial drugs. Previously, we reported that *pfmdr1* copy number increased when parasites developed resistance to artelinic acid (AL) *in vitro* and decreased when the drug pressure was withdrawn indicating that the higher copy number of *pfmdr1* was not a favourable state for the parasite. This reduction could result from de-amplification of *pfmdr1* and the *pfmdr1* containing amplicon, or from loss of fitness in parasites with higher *pfmdr1* copy number. Recently we characterised the dynamics of the amplicon containing *pfmdr1* in 3 clones that all have 3 copies of *pfmdr1* at the start of the study. After 3 months *in vitro* culture without drug pressure, sub-clones of one of the 3 clones contained 1, 2, and 3 copies of *pfmdr1*, while sub-clones of the other two clones contained 2 and 3 copies of *pfmdr1*. The results demonstrate that the de-amplification of *pfmdr1* is a frequent event and the rate of de-amplification varies. Further characterisation demonstrated that an chromosomal section containing 19 genes including *pfmdr1* was amplified following AL selection and that the entire amplicon of 19 genes was lost in all clones that demonstrated a reduction of *pfmdr1* copy number following the withdraw of drug pressure. A moderate increase in susceptibility to artelinic acid and mefloquine was observed when the parasites lost their extra copies of the amplicon. The result suggests that the *pfmdr1*-containing amplicon in AL resistant parasites is unstable when the drug selection pressure is withdrawn and that the entire amplicon is deleted during de-amplification. This has practical implications for the maintenance and spread of parasites resistant to artemisinin derivatives.

7

LARGE GENETIC POLYMORPHISM OF THE *PLASMODIUM FALCIPARUM* NA+/H+ EXCHANGER (PFNHE-1) AND ITS ASSOCIATION WITH QUININE RESISTANCE

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Quinine remains the first-line therapy for pregnant women and severe malaria. However, new data highlight the appearance of *Plasmodium falciparum* isolates less susceptible to quinine (Q). The mechanisms involved in this resistance are not yet clearly understood, but, as for chloroquine (CQ) and mefloquine (MQ), resistance was expected to arise from mutations in the *Pfcrt* and *Pfmdr1* genes. However, recent studies point towards a role for the Na/H exchanger PfNHE in this resistance. Polymorphism in a coding microsatellite (ms4760) was suggested to be

linked to a higher Q IC_{50} phenotype. We studied a large set of isolates collected from different part of the world, and analyzed their ms4760 polymorphism, together with their *in vitro* quinoline sensitivity and *Pfcrt* (codon 76) and *Pfmdr1* polymorphisms (codons 86, 184, 1034, 1042, and 1246). For 113 isolates, *Pfcrt* K76T and *Pfmdr1* N86Y were associated with high CQ and MQ IC50 (p<0.01). *Pfmdr1* Y184F was associated with higher MQ IC_{50} (p=0.01). No *Pfcrt* or *Pfmdr1* polymorphism could be significantly linked to Q susceptibility. Sequences of the pfnhe-1 ms4760 were obtained for 69 additional isolates, totalizing 182 sequences. Out of the 36 different profiles 24 had not been previously described. This highlights the large diversity of this gene as a result of i) variations in the number of repeats in the 6 blocks that constitute the microsatellite ii) and SNPs generated in these blocks. As previously described, our data support the use of DNNND repeats in block 2 as a marker for Q-resistance. Parasite isolates carrying two or three DNNND repeats had significantly higher Q IC50, as compared to those carrying only one repeat (p<0.01). Moreover, this number of repeats was also associated with increase in CQ IC_{50} (p<0.01). Presence of a block 3 was also associated with Q (p=0.017) or CQ (p<0.01) decreased sensitivity. All these results, taken together, raise the question of the role of NHE and of microsatellite polymorphisms in drug sensitivity. There are contradictory data pointing towards a role of PfNHE in pH or Na+ control, but structure analyses support a closer relationship between the plasmodial transporter and plant SOS genes, rather than to the human NHE-1 H+ extruder. As Q is a known inhibitor of the Na/K ATPase this last point suggests a complex link between Na, K and H+ metabolisms which should be taken into account in future studies on Q sensitivity.

8

ROLE OF ELEVATED TRANSCRIPTION OF GLUTATHIONE S-TRANSFERASES (GSTS) IN PYRETHROID RESISTANT SCABIES MITES

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In northern Australia, 5% permethrin is widely used in community-based treatment programs for control of endemic scabies. Increased in-vitro survival of human mites exposed to permethrin suggests emerging acaricide resistance. In this study, we used biochemical and molecular approaches to investigate the role of GSTs in mediating permethrin resistance, and evaluated the contribution of GST mediated detoxification as a mechanism of permethrin tolerance in human scabies mites. Comparison of GST activity in permethrin tolerant and sensitive mites showed a two-fold increase in enzymatic activity ($p < 0.0001$). Quantitative real time PCR was then used to evaluate contribution of different scabies mite GST isoenzymes in permethrin resistant *Sarcoptes scabiei* var. *canis*, tolerant var. *hominis* and sensitive var. *suis* mites. Up regulation of three different GST transcripts was observed in resistant mites: $\mu 1$ ($p < 0.0001$), $\delta 1$ ($p < 0.0001$) and $\delta 2$ ($p < 0.001$). In recent bioassays, a significantly increased *in vitro* survival was observed in *S. scabiei* var. *hominis* exposed to permethrin compared to naïve *S. scabiei* var. *suis* ($p < 0.0001$). The addition of the GST inhibitor diethyl maleate restored permethrin susceptibility, supporting a role for GST mediated detoxification in permethrin resistance. Altogether, these findings validate metabolic mechanisms as mediators of pyrethroid resistance in scabies and highlight the threat of emerging permethrin resistance to the treatment of this disease.

9

UNIQUE POPULATION STRUCTURE OF *BORRELIA BURGDORFERI* IN THE WESTERN BLACK-LEGGED TICK (*IXODES PACIFICUS*) IN NORTHERN CALIFORNIA

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Factors potentially contributing to lower incidence of Lyme disease (LD) in the far western U.S. compared to the northeast include lower densities of *Borrelia burgdorferi*-infected vector ticks in peridomestic environments, tick host-seeking behavior resulting in fewer human bites, and genetic variation in the spirochetes to which humans are exposed. We determined the population structure of *B. burgdorferi* in over 200 infected host-seeking nymphs of the primary bridging vector to humans, *Ixodes pacificus*, collected in Mendocino County, CA, by sequence typing regions of the spirochete lipoprotein *ospC* and the 16S-23S rRNA intergenic spacer (IGS). The predominant *ospC*/IGS biallelic profile in the population, found in about 22% of ticks, represented a novel *B. burgdorferi* clone. Eight of the most common northeastern *ospC* major groups, including I and K, which are strongly associated with disseminated human infections, were absent in *I. pacificus*. The two most abundant *ospC* major groups in California ticks, H3 and E3, have not been reported from the northeast. These two *ospC* major groups also were found commonly in western gray squirrels (*Sciurus griseus*) in oak-woodlands in northern CA. The differences observed between Californian and northeastern U.S. populations of *B. burgdorferi* suggest that pathogen genetics may contribute to lower rates of reported human LD cases in the far west.

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PREDICTED EFFECTS OF HOST RESERVOIR-TARGETED VACCINATION ON LYME DISEASE RISK

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Transmission-blocking vaccines against the bacterial agent of Lyme disease, *Borrelia burgdorferi*, are currently under consideration for targeting wildlife reservoir hosts. In endemic regions, multiple host species are involved in transmission and maintenance of this bacterium, but only a few host species may be vaccinated. To predict how disease risk is affected by vaccinating a subset of hosts in this multi-host system, we constructed a model of susceptible-infected-vaccinated transmission dynamics. The infection prevalence of B. burgdorferi among nymphal tick vectors largely depends on the percentage of blood meals taken from the highly reservoir-competent white-footed mouse. We generated and compared prediction surfaces for the probability of human Lyme disease exposure at various levels of vaccine effectiveness among mice. If 60% of tick blood meals are provided by mice, implementing a vaccine that is 100% effective would lower the probability of human exposure by 52%, whereas 50% vaccine effectiveness would only result in a 15% decrease in exposure probability. Targeting other reservoir-competent hosts for vaccination should be strongly considered to further reduce Lyme disease risk. Increasing overall vaccine effectiveness by widening the range of targeted hosts may allow this approach to compare favorably with other intervention measures.

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TICK BORNE RELAPSING FEVER IN MALI

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Detailed evaluation of the presence and impact of tick borne relapsing fever (TBRF) in West Africa has been limited primarily to Senegal. The high prevalence of TBRF in Senegal led our group to evaluate the potential of this disease in Mali, neighboring Senegal to the east. In West Africa, TBRF is caused by the spirochete *Borrelia crocidurae* which is vectored by a soft tick, *Ornithodoros sonrai*. We conducted two cross-sectional studies during December 2007 and January 2009 to investigate the presence of TBRF in Mali. During the two studies we excavated burrows and collected rodents from 11 locations along the Sahelian band of Mali from Kita to the west to Douentza to the east. Approximately 200 rodents were collected representing 13 different species. The dominate rodent species collected was *Mastomys natalensis* whereas the rodents most often found to be seropositive for *Borrelia* sp., based on anti-GlpQ IgG antibody recognition (ELISA and immunoblotting) were *Paromys derooi* and *P. daltoni* (40% and 17% seropositivity, respectively). *Crocidura* species (shrews) were also consistently collected and frequently infected (18% seropositive). All but one location surveyed contained seropositive animals with an overall seroprevalence of 9.6%. Out of 125 Giemsa-stained rodent blood smears 2 were positive for spirochetes, indicating active infection. Nymphal *O. sonrai* were collected from one location. Finally, 129 anonymous human serum samples, collected from various locations in Mali, were tested by ELISA. Eight samples had positive titers indicating exposure to relapsing fever *Borrelia* spirochetes. Combined, our work supports the presence of an established enzootic foci of TBRF in Mali with the potential to cause infection in humans. In light of possible mis-diagnosis of relapsing fever as Malaria and an ever-growing increase in drug resistance, particularly of antimalarials, it is important to recognize the prevalence of TBRF in West Africa and promote proper differential diagnosis.

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REDUCED GENE FLOW BETWEEN DOG TICK DEMES ON MARTHA'S VINEYARD AS A BASIS FOR INSULAR METAPOPULATION STRUCTURE OF *FRANCISELLA TULARENSIS*

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Tularemia has long been characterized as an agent of natural nidality, stably persisting in characteristic sites of transmission. We have identified stable natural foci on Martha's Vineyard, Massachusetts and using VNTR typing of *Francisella tularensis tularensis* DNA in *Dermacentor variabilis* ticks, we characterized the bacterial haplotypes circulating in two such sites on the island. Our analysis suggests no overlap in the diversity and range of bacterial haplotypes from these sites, consistent with our hypothesis that *F. tularensis* is maintained in a metapopulation comprising geographically isolated transmission cycles even on a small island. To determine whether this bacterial metapopulation structure could be a result of the subdivision of the vector tick population, we sought to determine whether dog ticks from these two sites showed evidence of population differentiation. Specifically, we asked whether these two populations showed evidence against the hypothesis that ticks from the two sites, SQ and K, are characterized by equivalent distributions of alleles and genotypes, as would be expected for a single interbreeding population. Accordingly, we amplified 5 microsatellite loci from 83 ticks from SQ and 48 from K. Evidence for population differentiation was obtained by estimating Fst, the reduction in heterozygosity attributable to population subdivision, using Genepop. The frequency of alleles and genotypes were significantly different across all loci (p=0.0000 across all alleles, and p=0.0000 across all genotypes) between the two sites. We conclude that there is evidence of genetic differentiation of dog ticks between our two field sites and this may, in part, be responsible for the metapopulation structure of *F. tularensis* there.

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RICKETTSIA RICKETTSII IN LONE STAR TICKS FROM KANSAS

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The role of Lone Star ticks as vectors for Rocky Mountain spotted fever (RMSF) remains poorly described. We estimated the entomological inoculation rates (EIR) for *Rickettsia* spp. for representative sites in Missouri and Kansas**,** states that historically report greater numbers of RMSF cases than the national average. Host seeking ticks were collected during 2006 and pooled tick homogenates analyzed by PCR targeting a portion of the rOmpA gene, with confirmation for multiple gene targets (a second *rompA* target as well as *rompB*, and *gltA*) and amplicon sequencing performed on individual ticks from pools that screened positive. Of 271 adult Lone Star ticks collected from a single site in Kansas in spring 2006, 1.5% (n=4) contained DNA of *Rickettsia rickettsii*. Interestingly, 2 of these positive ticks were concurrently infected by *R. amblyommi*. Dog ticks were not found to be infected by either *Rickettsia* spp. EIR for *R. rickettsii* and *R. amblyommii* in Lone Star ticks (1.91 and 20.63 infected ticks per minute, respectively) from this field site suggests that exposure to infected Lone Star ticks greatly exceeds that to infected dog ticks, which had an EIR of 0.48 ticks per minute (for *R. montanensis)*. We conclude that Lone Star ticks are epidemiologically significant vectors for Rocky Mountain spotted fever.

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IMPROVEMENT OF SEROLOGICAL ASSAYS FOR RICKETTSIAL AND RICKETTSIAL RELATED DISEASES BY RECOMBINANT ANTIGENS

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The diagnosis of rickettsial diseases, although can be isolated from or detected in clinical specimens, still relies mainly on serological methods. In general whole cell antigens have been used in serological assays such as the old Weil-Felix test, immuno-fluorescence assay (IFA), indirect immuno peroxidase assay (IIP), Dip-S-Ticks (DS), complement fixation (CT), and ELISA. The sensitivity and specificity of these tests heavily depend on the quality of whole cell antigen preparation. Only a few specialized laboratories have the BSL-3 facilities necessary to propagate, culture, and purify rickettsiae. The biosafety hazards, refrigerated storage requirements, and reproducibility problems associated with production of native rickettsial antigens are among those factors that hinder the development of inexpensive but reliable assays. Our laboratory has developed more than 10 recombinant proteins of immuno-dominant antigens from various rickettsia and rickettsia related pathogens including *Orientia tsutsugamishi*, SFG group rickettsiae and Typhus Group rickettsiae, *Coxiella burnetii*, and *Bartonella baciliformis*. Various r56 for proto type strains of Orientia and chimeric r56s generated based on the sequences of 56 kDa antigen have been shown to provide high sensitivity and specificity in ELISA. We produced two recombinant fragments (fragment K and AN) of OmpB, the major outer membrane antigen of typhus group rickettsia, which encompass the intact OmpB from *R. typhi*. Fragments R, X, and Y of the immunodominant antigen OmpA of SFG group rickettsiae were also prepared. The performance of Com-1 recombinant antigens has been shown to be very similar to the whole cell phase II *C. burnetii*. In addition, Recombinant Pap 31 also performed similarly as B. bacilliformis

whole cell antigen did. These recombinant antigens can be produced in large quantities much easily and cheaply without the need of BSL-3 laboratory. Lot to lot consistency also ensures the reproducibility of data analysis. Availability of these reagents will enable the epidemiology study in endemic areas where the infrastructure support is very limited.

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MOLECULAR PHYSIOLOGY OF THE DIAPAUSE PROGRAM IN THE ASIAN TIGER MOSQUITO, *AEDES ALBOPICTUS*

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The Asian tiger mosquito, *Aedes albopictus*, has recently been described as the most invasive mosquito in the world. This species is also of particular public health concern due to its ability to efficiently transmit Dengue fever, Chikungunya, West Nile and a variety of other arthropod-borne viruses. The photoperiodic diapause response of *Ae. albopictus* is a critical ecological adaptation that has facilitated its rapid spread throughout temperate habitats. Suppressive subtractive hybridization (SSH) and quantitative reverse transcriptase PCR (qRT-PCR) were performed in order to identify transcriptional elements of the diapause response of Ae. albopictus. Three transcripts putatively identified as: 1) epithelial membrane protein, 2) F-box and WD40 domain protein 7 (FBW7) and 3) fatty acyl coA elongase were significantly up-regulated under diapause-inducing conditions in temperate but not tropical populations of *Ae. albopictus*. Physiological experiments were performed in order to probe the potential functional significance of the diapause-associated upregulation of these transcripts.

16

EVIDENCE FOR COMPETITIVE REDUCTION OF NATIVE MOSQUITOES IN THE NORTHEASTERN UNITED STATES BY THE INVASIVE EXOTIC SPECIES, *OCHLEROTATUS JAPONICUS JAPONICUS* (DIPTERA: CULICIDAE)

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Ochlerotatus japonicus japonicus is an invasive mosquito native to Japan, Korea and eastern China. The species was first detected in the northeastern United States in 1998 and has rapidly spread throughout much of eastern North America. In addition to used tire casings, *Oc. j. japonicus* develops in a wide variety of man-made and natural container habitats, especially rock pools along stream beds. In an effort to evaluate the invasion success and impact of *Oc. j. japonicus* on native container dwelling species, waste tire disposal sites and natural rock pool habitats were sampled for mosquito larvae throughout Connecticut in 2005, and data were compared with results from prior surveys of similar sites made in 1987 and 1999. *Oc. j. japonicus* was the predominant species collected at the waste tire disposal sites regardless of surrounding landscape features (developed or forested), accounting for 56% of all larvae. A comparison with collections from prior surveys revealed a 90% reduction in larval populations of *Ochlerotatus triseriatus* (5% from 45%) and significant reductions among larval populations of *Ochlerotatus atropalpus* (3% from 19%) and *Culex restuans* (19% from 33%). Among the major species inhabiting used tire casings, only *Cx. pipiens* (15%), itself a non-native invasive species, appeared to be unaffected by the expansion of *Oc. j. japonicus*, retaining abundance levels that were comparable to those observed prior to invasion. *Oc. j. japonicus* was also the most abundant mosquito collected in rock pool habitats, accounting for nearly 80% of all collected larvae, except where water temperatures exceeded 30°C. This was concomitant with significant declines in the abundance of *Oc. atropalpus* (15% from 18%) and *Cx. restuans* (8% from 25%). Other minor cohabitating species included *Anopheles punctipennis* (4%), *Culex territans* (2%), and *Cx. pipiens* (1%). We conclude that *Oc. j. japonicus* is a superior competitor in rock pool and tire environments, is expanding its range, and is most likely responsible for reducing populations of native species occupying these habitats through interspecific competition for limited resources. The exclusion of *Oc. j. japonicus* from warm water pools further suggests that a temperature barrier may exist for *Oc. j. japonicus* and that populations may not be able to effectively colonize regions of the United States with relatively high summer temperatures.

17

VARIATIONS IN RESTING PATTERNS OF *AEDES AEGYPTI* IN RESPONSE TO MATERIAL TEXTURE AND COLOR USING EXPERIMENTAL HUTS

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The identification of preferred indoor resting sites of disease vectors will help drive the development of innovative control strategies that reduce host-vector contact inside homes. Targeting chemical treatment at resting sites is a cost-effective approach to initiating house exiting and thereby reducing indoor densities of vector populations. The success of such a strategy however involves a thorough understanding of the changes in resting pattern trends that occur in response to varying interior wall surface conditions. We report on the resting behavior patterns of *Aedes aegypti* females upon exposure to different types of textile materials within experimental huts in Thailand. Materials and colors were selected based on data from focus group studies that were conducted within the study locale as part of a larger research program developing a push-pull strategy for dengue control. Material strips were placed along the interior hut walls in three different surface area coverage ratios: 75, 50 and 25% under both horizontal and vertical configurations. Results from this study will serve as baseline guidance for the placement of chemically-treated material during mosquito hut entry and exit behavioral studies.

18

UNEXPECTED ANTHROPOPHILY IN THE POTENTIAL MALARIA VECTORS *ANOPHELES COUSTANI* AND *AN. SQUAMOSUS* IN MACHA, ZAMBIA

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Anopheles coustani and *An. squamosus* are sub-Saharan mosquito species that are competent for *Plasmodium falciparum* transmission. However, these species are believed to be unimportant in malaria transmission, due to their overwhelmingly zoophilic behavior. During the 2007-08 and 2008-09 malaria seasons in Macha, Zambia, a large proportion of *An. coustani* and *An. squamosus* were caught in human landing catches, rather than cattle-baited catches. PCR-based blood meal species identification showed that the majority of blood meals from mosquitoes caught in human landing catches and human-baited CDC light traps were from human hosts. A phylogenetic tree of anophelines with Zambian and southern African distributions was constructed based on sequences from the NADPH dehydrogenase 4, internal transcribed spacer 2, and cytochrome oxidase I genes. This tree places the two species closest to the *An. gambiae* species complex. However, *Plasmodium* was not detected in any of these mosquitoes, suggesting that they have little to no role in malaria transmission in southern Zambia.

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ANALYSIS OF *ANOPHELES ARABIENSIS* BLOOD FEEDING BEHAVIOR IN SOUTHERN ZAMBIA DURING THE TWO YEARS FOLLOWING THE INTRODUCTION OF INSECTICIDE TREATED BED NETS

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Anopheles arabiensis is the primary vector responsible for *Plasmodium falciparum* transmission in Macha, Zambia. Since insecticide treated bed nets (ITNs) have the potential to alter host feeding behavior, the extent of the vector's zoophilic and exophagic tendencies was evaluated during the two rainy seasons following ITN introduction. Paired indoor/outdoor human landing catches (HLCs) and outdoor cattle-baited collections were used to assess potential changes in host preference. Results support the hypothesis that *An. arabiensis* in Macha remains highly anthropophilic despite high ITN use. Additionally, HLCs and Centers for Disease Control light traps were employed to determine if ITNs were having an effect on foraging behavior. Similar numbers of mosquitoes were caught in light traps hung next to treated and untreated bed nets, suggesting that ITNs have little effect on entering behavior. Although no repellant effect was observed, *An. arabiensis* in Macha appears to be relatively exophagic and has been caught biting outdoors both right after sunset and right before sunrise, potentially circumventing the protective effects of ITNs.

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ANTHROPOPHILY OF *SERGENTOMYIA* SPECIES IN A LEISHMANIASIS OUTBREAK AREA IN THE HO DISTRICT OF GHANA

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A leishmaniasis outbreak in the Volta Region of Ghana was reported in 2004. Since then studies have been made to determine the causative organism(s), reservoir host(s) and the potential vectors. *Leishmania major* and a yet to be identified species have been found in human lesions but the reservoir not known. Entomological studies carried out in outbreak communities have shown that 99.5% of the sand fly species belong to the genus *Sergentomyia* and that the density of the two *Phlebotomus* species identified (*P. duboscqi* and *P. rodhaini*) in endemic communities is so low that their vectorial role is uncertain. We therefore aimed at determining the potential vector(s) in the outbreak area among the various species in the area. Indoor resting sandflies from human habitations were collected, those found blood-fed were identified morphologically and the blood-meal sources determined using direct enzyme-linked immunosorbent assay (ELISA) against human, chicken, goat and bovine anti-sera. A total of 1,845 sand flies were collected of which 275 were found blood-fed. The blood-fed flies *Sergentomyia africana africana*, (26.9% n=74); *S. antennata*, (0.4% n=1); *S. ingrami* (34.5% n=95) and *S. simillima* (38.2% n=105). *Sergentomyai africana africana*, *S. ingrami,* and *S. simillima* were found with mixed blood-meals including human blood. The highest proportion of domestic animal blood source taken was chicken blood (73%; n=93)) and goat blood (27%; n=35). The human blood index (HBI) for *S. simillima* was the highest (31%) followed by *S. ingrami* (23%), *S. africana africana* (14%) and *S. antennata* (0%). The results indicate that these three *Sergentomyia* species occur in large numbers in the outbreak area, are anthropophilic and show eclectic behaviour. It is not known whether the reactivity to chicken blood anti-sera is indicative of feeding on birds and neither is it known whether chickens and goats could serve as reservoir hosts. Follow-up studies on natural infections with *Leishmania* species in these *Sergentomyia* species are planned.

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IDENTIFICATION OF A NATURAL POPULATION OF HYBRIDS BETWEEN TAXONOMIC GROUPS OF *TRIATOMA DIMIDIATA* IN THE YUCATAN PENINSULA, MEXICO, AND ITS EPIDEMIOLOGICAL IMPORTANCE

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Triatoma dimidiata is one of the major Chagas disease vectors, with an extensive geographic distribution as well as considerable diversity in its morphology, habitat and level of domestication. Molecular studies based on the internal transcribed spacer 2 (ITS-2) have subdivided this species in four taxonomic groups distributed in different regions. Using both ITS-2 and cytochrome B markers, we confirmed the presence of taxonomic Groups 2 and 3 in the Yucatan peninsula, Mexico, but found them in apparent sympatry. Here we examine in detail the distribution of *T. dimidiata* ITS-2 genotypes in this region and compared their fertility and prevalence of *Trypanosoma cruzi* infection. PCR genotyping of large natural populations showed extensive sympatry of Groups 2 and 3 in most of the peninsula, often within the same house. We also detected a large hybrid zone with individuals displaying ITS-2 sequences from both Groups 2 and 3. Linkage disequilibrium between the ITS-2 and cytochrome B types was detected. Females from Groups 2 and 3 as well as hybrids produced identical numbers of eggs, suggesting normal fertility of hybrids. However, analysis of genotype frequencies indicated a strong departure from Hardy-Weinberg equilibrium in females only (not males) due to a large hybrid deficit. These results suggest random mating between Groups 2 and 3 combined with reduced viability and/or survival in females. This and other factors may allow for the maintenance of distinct groups 2 and 3 populations despite high hybrid frequencies. Importantly, *T. cruzi* infection was much higher in hybrids compared to Groups 2 and 3 bugs, but all three genotypes appeared to seasonally infest houses in a similar manner in the region. These finding warrant further studies on *T. dimidiata* taxonomy and its epidemiologic implications.

22

EFFECTS OF INCREASING ARTESUNATE DOSE IN SEVEN-DAY MONOTHERAPY REGIMENS ON TREATMENT RESPONSE IN CAMBODIAN PATIENTS WITH UNCOMPLICATED *FALCIPARUM* MALARIA

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Reports of declining sensitivity to artemisinin combination therapies (ACT) along both sides of the Thai-Cambodian border have prompted a series of studies investigating the possibility that resistance to the artemisinin derivatives is emerging. In this on-going, prospective, open-label, randomized comparison adult patients with uncomplicated falciparum malaria receive one of three oral artesunate (AS) monotherapy regimens (2, 4 or 6 mg/kg/day for 7 days). Subjects remain on-site for the duration of treatment, then have weekly follow-up to 42 days. Parasitemia is measured 2 to 6-hourly until negative on 2 consecutive readings, IC_{50} for dihydroartemisinin (DHA) is assessed at baseline and at time of failure,

and all patients undergo full pharmacokinetic profiles for AS and DHA on the first and final days of treatment. Outcome measures include parasite clearance times and reduction ratios, fever clearance, presence of parasitemia at 72 hours, and re-emergence of parasites within the follow-up period. At the time of writing 102 of the planned 150 evaluable patients have completed the study, 52 AS2, 25 AS4 and 25 AS6. Using WHO (2003) classification of treatment outcome, 5 (10%) patients in AS2, 1 (4%) in AS4 and 1 (4%) in AS6 met criteria for early treatment failure at 3 days, though all subsequently cleared their parasites within the dosing period. Preliminary data (without PCR correction) indicates 5 late treatment failures (AS2 2, AS4 1 and AS6 2), occurring 21 to 35 days after the original infection. PCTs were prolonged in these 5 cases, and were 108 hours in both the patients who failed after high-dose treatment. Overall approximately 50% of patients had PCTs longer than 72 hours. Preliminary findings suggest that individual parasite isolates from this location in western Cambodia can resist high doses of AS given for 7 days, and underscore the importance of current elimination strategies.

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EFFICACY OF THREE DIFFERENT REGIMENS OF PRIMAQUINE FOR THE PREVENTION OF RELAPSES OF *PLASMODIUM VIVAX* MALARIA IN THE AMAZON BASIN OF PERU

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Because of problems with patient compliance, most national malaria control programs in the Americas no longer use the standard 14-day course of primaquine (PQ) together with cloroquine (CQ) for infections by *Plasmodium vivax.* For this reason, it was decided to do an efficacy study for shorter regimes of primaquine for the prevention of relapses of *P. vivax*. Patients were treated under direct supervision in three health centers in Iquitos, the largest city of the Peruvian Amazonian, with chloroquine, 25 mg/kg over three days, plus primaquine in one of three different randomly-assigned regimens: 0.25 mg/kg daily for 14 days, 0.5 mg/kg daily for 7 days or 0.5 mg/kg for 5 days. After the treatment the patients were followed up for 6 months with temperature recordings twice per week and malaria smears twice per month. Polymerase chain reaction (PCR) was used to differentiate between relapse and reinfection between D0 and DF blood samples. In total, 540 patients were enrolled in the study with 180 patients in each arm (5, 7 and 14 days). There was no significant difference among three groups for age and sex. Forty five patients were lost to follow-up, and four were withdrawn from the study. From the remaining 491 patients, 86 presented with reappearance of parasitemia after 35 days of initiating the therapy (possible failure of the primaquine to prevent relapse): 48 (28.4%) in the 5-day arm, 16 (10.2%) in the 7-day arm and 22 (13.5%) in the 14-day arm. Four patients presented with reappearance of parasitemia before 35 days, only one of which was probably resistant to chloroquine. Molecular analysis of the 86 paired (D0/DF) blood samples indicated 46 were relapses of the same strain (26, 8 and 12 in arms 5, 7 and 14) and 40 were different strains indicating a probable reinfection (22, 8 and 10 in arms 5, 7 and 14). Thus, the 7 and 14 day PQ regimens are similar to each other in efficacy and superior to the shorter 5 day regimen in preventing replapse of *P. vivax* malaria. There is also new evidence of the transmission of CQ-resistant *P. vivax* in the Peruvian Amazon basin.

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MEASURING PREVENTATIVE DRUG EFFICACY OF SULFADOXINE/PYRIMETHAMINE (SP) PLUS ARTESUNATE (ART) THREE DAYS IN THE CONTEXT OF AN INTERMITTENT PREVENTATIVE TREATMENT IN INFANT (IPTI) STUDY IN PAPUA NEW GUINEA

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Intermittent preventive treatment (IPT) has emerged as one of the most promising new intervention to reduce the burden of malaria in infants. To-date most studies of IPT have used Sulphadoxine-Pyrimethamine (SP) alone or in combination with other antimalarial drugs. Even in areas with high levels SP resistance (when used for treatment of uncomplicated malaria cases), IPTi with SP has proved to be still effective, indicating the curative and preventative efficacy of SP are not necessarily tightly linked. In order to monitor the efficacy of SP or other drugs for IPT it is necessary to investigate their efficacy for preventing rather than curing malarial infections. We conducted an in-vivo preventative efficacy study in a cohort of children 6-60 months in Madang Province, an area of PNG that is a highly endemic country for malaria with similar prevalence for *Plasmodium vivax* (Pv) and *P. falciparum* (Pf). Children were allocated randomly to either SP + 3 days Artesunate (Art) or aparasitemic control group (pre-treated with 7 days of Art). All were followed up weekly for 2 months as well as passively when presenting acute febrile illnesses. 423 children from 8 villages were enrolled in the study (SP: 213, controls: 210) from April to July 2008. Sex ratio (F/M) is 220/202. Mean age is 33.3 months (range [5; 60]). Parasite prevalence (%) over time (days) in SP vs controls was respectively: D7: 2 vs 4 (p=0.18), D14: 2 vs 10 (p=0.001), D21: 4 vs 6 (p=0.3), D28: 4 vs 7 (p=0.17), D35: 7 vs 13 (p=0.03) and D42: 12 vs 12. Results of on going PCR diagnosis of infections are showing similar trends. Complete results including difference in efficacy for prevention of *P. falciparum* and *P. vivax* infections as well as time-to reinfection data will be presented. In conclusion, a preventative dose with SP + Art significantly delayed the occurrence of parasitema indicating the benefits of using SP (+/- Art) for prevention of malarial even in an area of significant levels of resistance to SP.

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THE BEST APPROACH TO RETREATING PATIENTS WITH RECURRENT MALARIA IN THE ERA OF ARTEMISININ BASED COMBINATION THERAPY

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Several African countries having adopted artemisinin combination therapy (ACT) as first line treatment for uncomplicated falciparum malaria use also quinine monotherapy as second line. This policy goes against the World Health Organisation (WHO) recommendations for combination therapy. The adherence to a seven-day quinine treatment schedule is probably poor. The study aims at assessing the best approach for retreating patients with recurrent malaria within 28 days of initial therapy with ACT's. The study was conducted in Tororo, Uganda, and is a nested, randomized, single blinded, multi-arm clinical trial of rescue therapy. Children aged 6 - 59 months with recurrent uncomplicated *Plasmodium falciparum* malaria after an ACT treatment were recruited and randomised to either the standard 7-day quinine or to a 3-day ACT (Artemether Lumefantrine (AL) to quinine or Dihyroartemisinin Piperaquine (DP), DP to quinine or AL). All doses were directly observed. The main outcome measure was the risks of recurrent parasitemia at 28 days, unadjusted and adjusted by PCR genotyping. Recruitment is ongoing with a planned sample size of 260 patients. 221 of 222 (99.5%) participants enrolled completed

follow-up. The risk of recurrent *P. falciparum* parasitemia unadjusted by genotyping for all study participants is 57.2% after 28 days of follow up. Full corrected results shall be presented. We shall discuss whether quinine monotherapy or an alternative ACT is the most appropriate treatment for recurrent malaria.

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EFFICACY AND SAFETY OF QUININE VS. ARTEMETHER-LUMEFANTRINE IN UNCOMPLICATED MALARIA DURING PREGNANCY, MBARARA, UGANDA

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Malaria during pregnancy is associated maternal and fetal morbidity and mortality. Quinine was the only recommended drug until 2006, when WHO recommended ACTs during the 2nd or 3rd trimesters. However, data on efficacy and safety of ACTs during pregnancy in Africa is scarce. This study aimed to examine the efficacy and safety of artemether-lumefantrine (AL) compared to oral quinine (SQ7) for treating uncomplicated falciparum malaria during 2nd and 3rd trimesters pregnancy in Mbarara, Uganda. An open label, randomized, prospective, non-inferiority trial in which pregnant women were followed weekly until delivery (α 5%, power 80% to detect a -5% margin). The cure rate at day 42 (primary outcome) and at delivery were confirmed by PCR genotyping. Adverse events, pregnancy outcome and newborn growth and development at 1 year of age were assessed. Overall 304 women, 152 in each arm, were enrolled. In the Per Protocol analysis, AL efficacy was high: 99.2 [95.7-99.9]% at D42 and 98.1 [93.3-99.8]% at delivery. SQ7 efficacy was 97.4 [92.1-99.3]% at D42 and 95.7 [88.7-98.6]% at delivery. In the PP analysis AL efficacy was non inferior to SQ7 at D42 (+1.8% difference with 95%CI lower limit at -0.9%), and at delivery (+2.4% difference with 95%CI lower limit at -1.7%).The trends and significance in the Intention to Treat Analysis were similar. There were no Serious Adverse events related to the treatment. Intolerable side effects were significantly higher with SQ7, resulting in 2.6% interrupted treatment in this arm (none in AL). Birth outcomes were similar between treatment arms. In conclusion, high efficacy and better tolerability of a 3 day regimen of AL compared to SQ7 adds further reassuring information to the data published on malaria in pregnancy treated with artemisinin derivatives. The lumefantrine pharmacokinetic data currently being analyzed are important to fully interpret these results. More information on the safety of ACTs in the first trimester is needed urgently.

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EPOIETIN β-QUININE DRUG COMBINATION IN CHILDREN WITH CEREBRAL MALARIA

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Cerebral malaria carries an unacceptable case fatality rate in children despite timely and adequate chemotherapy. To improve the survival rate, adjunctive therapies previously tested mainly focused on the modulation of the inflammatory response, without definitive effect in humans. In this context, we proposed a new adjunctive strategy using a neuroprotective drug: erythropoietin (epoietin-β). An open-labelled study including cerebral malaria children (Blantyre coma score below 3) was conducted in Mali (Clinical Trials.gov ID: NCT00697164). The objective was to assess the short term safety (7 days) of erythropoietin high doses (1500 U/kg/day during 3 days) combined to quinine. 35 patients with unrousable coma were included in the study. None of expected side effects of erythropoietin were observed during the 7 days follow-up. No significant increase in the case fatality rate (7 / 35 patients) was observed compared to other studies with mortality rates ranging from 16 to 22% in similar endemic areas. These data provide the first evidence of the short term safety of erythropoietin high doses combined to quinine. A multicenter study is needed to assess the potential of Epo as an adjunctive therapy to increase the survival during cerebral malaria.

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MASS DRUG ADMINISTRATION: IS IT NECESSARY FOR MALARIA ELIMINATION?

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As countries work toward improved malaria control and eventual elimination there is much debate about the role of mass drug administration (MDA). Advocates for MDA cite past examples of successful elimination and rapid impact, while opponents highlight failed past campaigns that stimulated the spread of drug resistance and potential drug side-effects. Currently there is little information available about the likely success of elimination efforts without the use of MDA, and therefore little data on which to determine whether MDA forms a necessary component of malaria elimination strategies. We use a computer simulation model to explore the impact of various elimination strategies with and without MDA to highlight if, and in what transmission settings, MDA may be needed to help achieve elimination. We also consider the likelihood that parasite reintroductions re-establish transmission. Where MDA was indicated as an important tool in successful elimination, we were able to estimate the minimal coverage required to achieve the goal. This study provides valuable information about the necessity of MDA to assist public health officials plan and optimise elimination strategies.

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THE LENGTH AND NON-HYDROPHOBIC RESIDUES IN THE TRANSMEMBRANE DOMAIN OF DENGUE VIRUS ENVELOPE PROTEIN CRITICAL FOR RETENTION AND ASSEMBLY IN ENDOPLASMIC RETICULUM

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Increasing evidence suggested a coupling between replication and morphogenesis of flavivirus, in which assembly of precursor membrane (PrM) and envelope (E) proteins into virus particles occurs in the membranous structures derived from endoplasmic reticulum (ER). We reported previously that the stem-transmembrane (TM) domains of dengue virus (DENV), the leading cause of arboviral diseases worldwide, contain an ER retention signal, as reported previously. In this study, we examined the mechanisms involved. A series of chimeric constructs between PrM/E genes of DENV2 and CD4 were generated. Site-directed mutagenesis was also carried out to generate mutants in TM domain of these constructs. After transfection to 293T cells, cells or lysates were subjected to immunofluorescence analysis, flow cytometry, endoglycosidase H digestion and Western blot analysis. Replacing the TM and cytoplasmic (CY) domains of CD4 with the TM domain of DENV2 E protein retained the chimeric CD4 predominantly in ER, whereas reciprocal DENV2 PrM/E construct containing the TM and CY domains of CD4 expressed on surface, suggesting the TM domain of DENV2 E protein contains an ER retention signal. Substitutions of three non-hydrophobic residues at the N-terminus of the first helix (T1) and at either N- or C-terminus of the second helix (T2) of TM domain of E protein with three hydrophobic residues, and an increase in the

length of T1 released the chimeric CD4 from ER. Moreover, introducing similar mutations to the PrM/E construct led to the release of E protein from ER and increased production of virus-like particles. In conclusion, these findings suggest that the relatively short length and certain non-hydrophobic residues of TM domain of DENV2 E protein are critical for its localization and assembly in ER. This was further supported by the trend of changes in length and number of non-hydrophobic residues in the TM domain based on the analysis of different enveloped viruses assembled at various sites along the secretory pathway.

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IMMUNODOMINANCE IN DENGUE VIRUS INFECTION

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Epidemiological observations suggest that severe dengue disease occurs more frequently in the setting of secondary infection with heterologous serotypes, and a role for DV serotype cross-reactive T cells in the pathogenesis of severe disease has been proposed.We aim to determine the contribution of serotype cross-reactive T cells to immunopathogensis, hypothesizing that skewing of the T cell repertoire hierarchy in primary and sequential DV infections can modify T cell effector functions such that they may be suboptimal and ultimately fail to adequately control virus replication. In order to determine how immunodominance within the DV-specific memory T cell population impacts memory and recall responses in secondary infection, we assessed TCR usage in subjects with an immunodominant DV1 CD8+B*5502+NS5329+ KPWDVIPMV (KP9) memory T cell response using TCR CDR3 length analysis, or spectratyping, up to 14 years after infection. PBMC were stimulated with the cognate DV1 epitope peptide, as well as variant peptides representing heterologous serotypes. In addition, the TCR CDR3 of T cell clones specific for B*5502+NS5329+ KPWDVIPMV, derived by limiting dilution cell culture, was sequenced. Stimulation of PBMC from DV1-exposed B*5502 subjects with KP9 cognate epitope peptide dramatically reduced diversity of the TCR repertoire as measured by CDR3 length spectratyping. The Vβ1 and Vβ7 families were clonally expanded in 9 of 9 subjects, and therefore represent a conserved immunodominance hierarchy or 'public specificity' of epitope-specific T cell responses that are similar between individuals. This skewing of the TCR repertoire towards Vβ1 and Vβ7 was also seen in DV1-specific T cell clones generated by limiting dilution cell culture: CDR3 sequencing showed a dominance of Vβ1 and Vβ7 TCR sequences. Interestingly, a 12 aa Vβ7 Jβ1-5 CDR3 sequence was identified in 2 of 2 subjects, indicating that a public or shared clonotype may exit in DV-specific memory T cell responses. We conclude that a conserved memory T cell response persists for many years following DV infection, and stimulation with variant epitope peptides representing heterologous DV serotypes can dramatically alter the memory T cell receptor hierarchy. Our studies are directly relevant to dengue vaccine design by assessing cross-reactivities that may impact safety.

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DISSECTING THE POLYCLONAL HUMAN ANTIBODY RESPONSE TO DENGUE VIRUS

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The goal of the present study was to characterize the binding specificity and functional properties of envelope (E) protein reactive antibodies in dengue virus (DENV) immune human sera. Individual subunits of E consist of three β-barrel domains designated E domains I (EDI), II (EDII) and III (EDIII). Almost all B-cell epitopes mapped on DENV E protein are based on mouse monoclonal antibodies (Mabs). The main epitopes engaged by human antibody remain to be defined. Six dengue immune human sera and a library of 64 overlapping peptides covering the entire length of E protein were used to map linear epitopes recognized by polyclonal human antibody. Antibodies in immune sera bound to serotype cross reactive, peptide epitopes located on EDI, and at the lateral ridge and fusion peptide of EDII. As several strongly neutralizing mouse Mabs bind to a type specific, conformational epitopes on the lateral ridge of EDIII, we used recombinant proteins to measure EDIII reactive antibody in primary and secondary dengue immune human sera. Human antibodies bound to a serotype specific epitope on EDIII after primary infection and a serotype cross reactive epitope on EDIII after secondary infection. A panel of recombinant EDIII proteins with mutations in lateral ridge amino acids was used to map human antibody epitopes on EDIII. Primary sera bound to a serotype specific epitope located outside the lateral ridge of EDIII. In contrast, secondary sera bound to a serotype cross reactive epitope that included residues on the A strand and BC loop of the lateral ridge of EDIII. Overall, EDIII-binding antibodies constituted only a small fraction of the total antibody in human sera binding to DENV. Studies with complete and EDIII antibody depleted immune sera demonstrated that EDIII binding antibodies make only a minor contribution (~10%) to the total neutralizing capacity of human immune serum. We propose that human antibodies directed to inter-domain epitopes and/or epitopes on EDI and II of E protein are mainly responsible for DENV neutralization. Our results have implications for understanding protective immunity following natural DENV infection, and for developing and evaluating DENV vaccines.

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ASSESSING THE ACCURACY OF INFERRING THE SEROTYPE OF DENGUE VIRUS INFECTIONS BASED ON PRE- AND POST-INFECTION NEUTRALIZING ANTIBODY TITERS

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Dengue is one of the most important emerging infectious diseases and an increasing threat to public health worldwide. Most hope for dengue control is currently based on the development of a safe, tetravalent vaccine. The most widely used test to determine serotype specific immunity against dengue is plaque reduction neutralization but little has been done to quantify the validity of this test as indicator of past exposure to dengue serotypes. We used a multinomial logistic regression model to predict the serotype of recent dengue infections based on pre- and post-infection PRNT50 values from children with a confirmed dengue infection in Kamphaeng Phet, Thailand. The validity of model predictions was determined by comparison to PCR outcomes. A total of 208 children were included. Low pre-infection and high post-infection PRNT50 values against a dengue serotype were associated with a higher probability for that serotype to be the infecting serotype. Overall, 67% positive and 89% negative agreement was obtained between model predictions and PCR outcomes (κ: 0.463). Better predictions were obtained for 36 cases that had no neutralizing antibodies before infection (89% positive and 96% negative agreement). High levels of cross-reactivity were found even when PRNT50 patterns seemed very serotype-specific. This is the first study that applied a statistical model to quantify the validity of inference based on PRNT50 values. It is believed that due to high levels of cross-reactivity, PRNT50 values are not indicative of the serotype of dengue exposure, especially in case of a heterotypic antibody response. We found statistically significant patterns in serotype specific PRNT50 responses to dengue infection and could predict the serotype of infection with high rates of agreement in cases without pre-existing neutralizing antibodies, even when antibody responses were heterotypic. This is an important step in quantifying the validity of neutralization assays that will be widely used in the assessment of dengue vaccine efficacy.

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INVESTIGATION OF ANTIBODY DEPENDENT ENHANCEMENT IN HUMAN PRIMARY TARGET CELLS

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Antibody Dependent Enhancement (ADE) is implicated in complicated, usually secondary, dengue virus (DV) infection. Pre-existing, heterologous antibodies, via their Fc-γ receptors, likely predispose to severe disease through enhancement. Both high levels of viremia and certain cytokines (proinflammatory and immunomodulatory) are associated with severe disease. Monocytes, macrophages, immature and mature dendritic cell are regarded as major cellular targets for DV. We previously reported antibody dependent enhancement (ADE) of dengue virus infection in primary human mature dendritic cells. Currently we will report on studies to evaluate ADE in all four types of primary human myeloid target cells: monocytes, macrophages, immature and mature DC. All cells types were compared directly and were experimentally controlled by using autologous donors in the high throughput ADE assay. All cell types studied, except monocytes, express DC-SIGN (CD209) and unique patterns of Fc-γ receptors; which in the case of dendritic cells changed with maturation. All cells undergo ADE with the exception of immature DC. Monocytes express high FcγRI and FcγRIIa and low level of FcγRIIb. Dendritic cells mainly express FcγIIa and FcγRIIb; notably the inhibitory FcγRIIb is down regulated upon maturation likely influencing this cells' susceptibility to ADE. Interestingly, macrophages uniquely express all three forms of FcγRs. We compared direct DV infection of these cells, as well as ADE infection, and explored the downstream consequences in all cell types including cytokine response patterns, viral output and productivity of infection. We will detail our current understanding of ADE in all primary myeloid human target cells.

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INHIBITION OF DOWNSTREAM MEDIATORS OF THE TYPE I INTERFERON RESPONSE IN DENGUE VIRUS INFECTED MONOCYTE-DERIVED DENDRITIC CELLS AND BYSTANDER T CELL ACTIVATION

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Dengue virus (DENV) stimulates a strong innate immune response in patients. Type I interferon α/β (IFN) genes are highly up-regulated in patients with dengue fever; but less activated in patients with hemorrhagic disease. DENV has been shown to antagonize IFN signaling, a mechanism that could potentially facilitate infection. However, the downstream consequences of the IFN signaling blockade on the immune response to DENV infection have not been fully elucidated. Because DENV naturally infects and replicates in dendritic cells (DCs) which produce IFN, we have established a biologically relevant in-vitro assay using DCs in order to ascertain the downstream effects of IFN blockade on the immune response to DENV infection. We infected primary monocyte-derived DCs with DENV field strains and quantitatively recorded changes in gene expression patterns of IFN-induced and IFN-sensitive genes, revealing characteristic features of these pathways during DENV infections. To measure activation of T cells during DENV infection, primary naïve T cells were exposed to DENV-infected DCs and their activation responses characterized by flow cytometry. In addition, individual DENV non-structural proteins that are believed to be the principle mediators of IFN signaling blockade were expressed in RNA-transfected DCs. These experiments have allowed us to establish in-vitro assays using DENV strains in which we have characterized how the virus modulates the IFN response. Our findings will help us establish specific markers to better characterize naturally occurring infections.

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ANTIVIRAL ACTIVITY OF ANTI-DENGUE INHIBITORS IN HUMAN PRIMARY DENDRITIC CELLS

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Dengue virus can infect multiple human cell types; however, dendritic cells, macrophages and monocytes are the major cells in which this virus replicates. Nucleoside inhibitors of the viral polymerase depend on the phosphorylation by host enzymes whose activity is influenced by the metabolic activity of the target cells. It is therefore necessary to evaluate the potency of potential antiviral inhibitors in the target cells. In this study, we used a quantitative real time PCR method to quantitate dengue viral RNA in infected cells in the presence of inhibitors. Viral replication of iDC infected with DENV1-4 at different multiplicities of infection (MOI) was monitored for up to 96 hrs post infection. We found that viral RNA copy number peaked at 24 hrs post infection, followed by a rapid decline to nearly undetectable level at 96 hrs. Even though the pattern of viral RNA replication was similar for all four dengue serotypes, the absolute copy number of viral RNA varied among the serotypes indicating replicative fitness differences among the viral strains. Furthermore, we infected iDC at varying MOIs by using dilutions of viral stock (0.2-2.0). While the magnitude of replicating viral RNA copy numbers corresponded to the virus MOI input, the peaks of viral replication remained unchanged at 24 hrs post infection. These results suggested that kinetics of dengue replication in iDC is independent of the infection rate. We next determined the antiviral potency of known dengue inhibitors such as 7-Deaza-2 C-methyl-adenosine (7-DMA) at the peak of viral replication in iDC. 7-DMA's 50% inhibitory concentrations (IC_{50}) values ranged from 1.2 to 6.0 uM for DENV1-4. Pre-incubation of iDC with nucleoside inhibitors to increase compound triphosphate levels did not change the IC50 significantly. In summary we established and characterized a reproducible dengue infection assay of iDC with a quantitation of genome copies (viral RNA) read out. Dengue viral RNA replication peaked at 24 hrs post infection. The potency of nucleoside analogue polymerase inhibitors was determined 24 hrs post infection against all four dengue serotypes. Given the fact that nucleosides rely on host metabolism for phosphorylation to the active moiety, the above assay is an excellent tool for evaluation of dengue antiviral inhibitors that require host metabolism for activation in a biologically relevant *in vitro* system.

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IDENTIFICATION OF POTENTIAL BIOMARKERS FOR ANTIMONY SUSCEPTIBILITY/RESISTANCE IN *LEISHMANIA DONOVANI*

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Leishmania donovani, a flagellated protozoan parasite, is the causative agent of visceral leishmaniasis (VL). Post Kala-Azar Dermal Leismaniasis (PKDL) is a complication of visceral leishmaniasis (VL), characterized by macular, maculopapular and nodular leisons. The interval at which PKDL follows VL ranges from 1-10 years in India and is considered as a reservoir for *Leishmania* parasites. Pentavalent antimonial drugs are the standard first-line treatment for leishmaniasis, although resistance is a growing problem. In the present study an attempt has been made to understand the mechanism of antimony resistance in both VL and PKDL isolates. Anti-leishmanial effect of conventional drugs was evaluated in an *in vitro* macrophage-amastigote model using a colorimetric β-lactamase assay. A clear correlation was observed between resistance to antimony and the time lapsed between transition from VL to PKDL in these isolates. The uptake of trivalent antimony (SbIII) and involvement of aquaglyceroporin (AQP1, influx pump) in developing antimony resistant phenotype was

investigated. SbIII accumulation, copy number of AQP1 gene and transcript levels were compared in antimony sensitive versus resistant isolates.The ABC tranporter gene MRPA, was amplified in resistant VL field isolates as part of an extrachromosomal circle. MRPA gene was observed on two different chromosomes in PKDL isolates.There was no amplification of MRPA gene as observed by Southern blot analysis in PKDL isolates. However, incresed expression of MRPA by real-time PCR was observed in resistant isolates. We also report existence of genetic heterogeneity between PKDL and VL isolates. Molecular karyotyping by pulse field gel electrophoresis (PFGE) analysis further confirmed the chromosomal variation. Taken together, our study suggests that variation of parameters reported in the present study may be responsible for varying pathologies of Indian kala azar and PKDL. However, additional studies including comparative proteomics needs to be further investigated.

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CD4+ T CELLS SUBSETS IN HUMAN CUTANEOUS LEISHMANIASIS HAVE A DISTINCT RECEPTOR REPERTOIRES AND CYTOKINE EXPRESSION

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The establishment of an effective immune response directed against *Leishmania* is critical for the disease control. Different immune responses elicited by *Leishmania* infection can be directly related to distinct effectors T cell subpopulations expressing specific TCR. Thus, subpopulations of $CD4^+$ T cells expressing distinct Vβ may have distinct role in the human immune response against *L. braziliensis*. To address this question, a detailed study defining the activation/memory state and the cytokine profile was performed with a group of well-defined cutaneous leishmaniasis patients and a group of normal individuals. We evaluated the frequency of T $CD4^+$ cells *in vitro* expressing the following Vβ, using flow citometry: Vβ 2, 3, 5.1, 5.2, 8, 11, 12, 17 and 24 in the different groups. The frequency of Vβ positive, $CD4^+$ within $CD4^+$ T cells was calculated without stimulus, as well as after culture with soluble *Leishmania* antigen (SLA). Our results show that: (1) $CD4^+$ T cells expressing distinct Vβ, increased expression of Vβ 5.2 and Vβ 24 in cutaneous leishmaniasis as compared with normal individuals; (2) cutaneous leishmanisis patients demonstrate an increase of $CD4^+$ T cells expressing Vβ 5.2, 11, 12 and 17, after stimulus with SLA; (3) Mainly sub-populations of $CD4^+$ T cells expressing Vβ 5.2, 11 and 24, are found expressing activation markers (HLA-DR), memory markers (CD45RO), as well as pro-inflammatory (IFN-γ, TNF-α), and anti-inflammatory (IL-10), cytokines; (4) Positive correlations between $CD4^+$ T cells expressing Vβ 5.2 and 24, producing as IFN-γ and IL-10 as TNF-α and IL-10 cytokines are seen; (5) a higher frequency of $CD4^+$ T cells expressing Vβ 5.2 were positively correlated with a larger lesion area, stimuli independent. Given that the activation of specific subpopulations during this disease could allow the formation of an effective cellular response, this study might lead us to the discovery of immunodominant *Leishmania* antigens important for triggering efficient host responses against the parasite.

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MOLECULAR DIAGNOSIS, GENOTYPING AND FOLLOW-UP OF *TRYPANOSOMA CRUZI* LINEAGES IN CARDIAC SAMPLES FROM PATIENTS WITH CHAGAS HEART DISEASE AND BLOODSTREAM AND REACTIVATION LESIONS AFTER HEART TRANSPLANTATION

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The pathogenesis of Chagas Heart disease (ChD) is not understood. Heart transplantation (Tx) represents a valid treatment option for end-stage heart failure but reactivation of infection (RA) may result as the main complication. *Trypanosoma cruzi* polymorphism has supported distinction of lineages *T. cruzi* I and II and hybrid strains. Among Tc II, five discrete typing units were described (IIa/e). *T. cruzi* I is highly heterogeneous though discrete miniexon genes- based haplotypes $I_{1/4}$ were described. We followed-up *T.cruzi* populations directly in clinical specimens from ChD argentinean pts who underwent heart transplantation to characterize lineage and infra-lineage complexity linked to ChD, post-Tx recrudescence and RA. 11 ChD pts and 5 seropositive pts with cardiopathies of non-chagasic etiology who underwent Tx were monitored. 6 ChD pts suffered RA within a mean period of 71,6 days post-Tx, non-ChD pts did not reactivate. Lineages were characterized from kDNA-PCR positive samples by PCRs targeted to nuclear markers (Lg-PCR) and infra-lineage polymorphism was profiled by RFLP-PCR from kDNA amplicons. Tc I haplotypes were obtained after sequencing the intergenic region of miniexon genes. Parasitic loads after Tx were monitored in 3 RA pts by Q-PCR of satellite DNA. 7 cardiac explants from 11 ChD pts were Lg-PCR positive (3 Tc I_4 and 4 Tc II b/d/e DTUs). RFLP-PCR profiles were polymorphic among ChD hearts and among sections of a same heart. Tc I was found in post/Tx blood in 5 ChD (3 Tc I_1, 1 Tc I_4, 1 ukn) and Tc II in other 5 (3 Tc IId, 1Tc IIe and 1 IIb/e). In non CHD, Lg-PCR was positive in two pts (Tc IId). *T. cruzi* I was detected in skin chagomas from 2 RA pts (1 Tc I_1, 1 ukn) whereas *T. cruzi* II was found in EMB from 3 cases and skin biopsies from 2 RA. Single lineage infections were detected at different locations in 5 cases (2 Tc I and 3 Tc II) and mixed infections in the remainder. One Tc I infected case showed TC I_4 in heart and TC I_1 in blood. In conclusion, both TcI and Tc II DTUs are linked to myocarditis causing ChD. The occurrence of mixed infections with different tissue localizations showed differential histotropism. Our findings also demonstrate that Tc I exists more frequently in the human infection at the southern cone of America, than it was previously assumed from studies with cultured isolates from patients' blood samples of the same endemic region.

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SERUM LIPID PROFILE, APOLIPOPROTEIN E GENOTYPE AND VISCERAL LEISHMANIASIS INFECTION IN A NORTHEASTERN BRAZILIAN POPULATION

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Visceral leishmaniasis (VL) is a disease of the developing world caused by the protozoa *Leishmania chagasi (infantum)* and *L. donavani* and transmitted by the bite of an infected phlebotomine sand fly. Inoculation usually results in asymptomatic infection, with subsequent development of protective T-cell mediated immunity. Due to unclear host, environmental, and genetic factors, some inoculated hosts (often children) will develop symptomatic VL with fever, hepatosplenomegaly and immunocompromise.

There exists a growing body of literature which suggests a vital immunomodulatory role for cellular elements of lipid metabolism and regulation. *In vitro*, cholesterol is necessary for full leishmania infectivity and studies have shown *in vitro* and murine immunosuppression secondary to dyslipidemia. This immunosuppression has been found to be due in part to apolipoprotein E (ApoE), a polymorphic protein encoded in a region of chromosome 19 associated with VL resistance in a recent genome-wide scan of 1254 Brazilians. In the endemic region surrounding Natal, Brazil, we tested fasting lipid levels in patients with acute VL (N=10), recovered patients (N=20) and environmentally matched controls within patient's households (N=78). In preliminary results, acutely ill patients demonstrated significant depression of total cholesterol (TC) ($P<0.001$) and high-density lipoprotein (HDL) ($P<0.001$) with significant elevation of triglycerides (TG) ($P=0.001$) and C reactive protein (CRP) ($P<0.001$) as compared with controls. Recovered patients demonstrated similar trends in HDL and TG despite normalized CRP (a marker of infection), although these trends did not meet statistical significance. ApoE genotyping showed E2 allele frequency of 4.2%, E3 allele frequency 80.2% and E4 frequency of 15.6% without significant differences between VL patients and controls. This study confirms characteristic dyslipidemia in acute VL, which may contribute to patient immunosuppression. Furthermore, results are suggestive of a baseline dyslipidemia in former patients, which may contribute to disease susceptibility and warrants further study.

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CARBOHYDRATES DERIVED FROM PATHOGENS PROMOTE DIFFERENTIAL IL-12 PRODUCTION IN MACROPHAGES

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Leishmania and *Mycobacterium tuberculosis* are pathogens that cause disease in billions of people around the world. *Leishmania* and *M. tb* both primarily infect and reproduce in macrophages. Lipophosphoglycan (LPG) and mannosylated lipoarabinomannan (Man-LAM) are glycolipid molecules found on the surface of these two pathogens and are involved in a number of important events during human infection from evasion from complement, macrophage adhesion, and alteration of macrophage signaling. Many studies have confirmed LPG and Man-LAM as a virulence factors but specific mechanisms of molecular interaction with the immune system have yet to be identified. We have developed a model system that is able to test which carbohydrate components are involved in alteration of macrophage uptake and cytokine production. Our repertoire consists of synthetically-produced different carbohydrates found on the surface of these pathogens. These sugars have natural conformation and appropriate biological affinity, and promote differential IL-12p40 production from both mouse and human activated macrophages. As clearance of infection with either of these pathogens relies on a productive of Th-1 immune response to heal, decreases in IL-12p40 production related to cap sugars may prompt chronic disease.

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IMMUNOGENICIY OF TUBULIN-BASED SUBUNIT VACCINE CANDIDATES WHICH PROTECT ANIMALS AGAINST CHALLENGE WITH *TRYPANOSOMA BRUCEI BRUCEI*

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African trypanosomiasis is fatal to humans and animals if left untreated. The disease poses a serious threat to public health and causes enormous economic losses in sub-Saharan Africa. No effective vaccine has been developed against this disease. The trypanosome continuously changes the dominant variable surface glycoprotein (VSG) antigens that cover nearly the entire surface of the parasite, making it unavailable for vaccine development. To overcome this obstacle, we identified non-variable antigens of the parasite that can generate protective immunity. Tubulin, one such candidate, was shown to confer protection in mice when animals were challenged with homologous or heterologous strains of *Trypanosoma*. We have engineered regions of α and β tubulin of *Trypanosoma brucei* as fusions with the coat protein of a plant virus, *Alfalfa mosaic virus* (AlMV) and produced them as virus particles. Some of the plant-produced recombinant AlMV particles displaying target peptides from α or β tubulin stimulated high level protective immune responses in animals.

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QUANTIFICATION OF PARASITEMIA IN *LEISHMANIA DONOVANI*-INFECTED HAMSTERS BY REAL-TIME PCR

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Visceral leishmaniasis affects over a half million individuals in 88 countries each year and is caused by the intracellular parasite *Leishmania donovani*. New drugs are urgently required to treat this devastating disease. Preclinical evaluation of new drugs is modeled in hamsters infected with *L. donovani,* although measurement of parasite loads is difficult and time consuming. In the current study a real-time PCR assay has been optimized and validated against two other methods to estimate the parasite load within the liver of infected hamsters. Reverse and forward primers were used that amplify a 120-bp target template of the kinetoplast DNA. Serial ten-fold dilutions of parasites were either assayed directly or spiked into un-infected liver homogenates. Quantification of the parasite load in infected-hamster livers was analyzed by a SYBR Green-based PCR assay and compared to microscopic and growth dilution assays. Standard curves were generated from parasite cultures and parasite-spiked tissue. *L. donovani* DNA was readily detected in spiked homogenates and no products were observed with un-infected tissue and non-template control samples. The real time PCR assay was able to detect as few as 2 parasites in 2 mg of liver. In addition quantitative differences in parasite load were detected at low levels where microscopic confirmation was not possible. In conclusion, the real time PCR method is sensitive and specific for the quantification of the parasite load within *L. donovani*-infected hamsters, which will accelerate the evaluation of new drugs in the definitive preclinical model of disease.

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MAPPING SANDFLY-PLANT INTERACTION IN RELATION TO THEIR BREEDING SITES IN PERKERRA IRRIGATION SCHEME, KENYA

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Phlebotomine sandfly prevalence among vegetation may be implicative of survival dependency of the Sandflies on vegetation while impacting their status as vectors of Visceral Leishmaniasis. Previous studies have shown Closed-canopy Acacia vegetation units as favored by these Sandflies and their breeding sites. Mapping plant taxa that are directly or indirectly associated with vector and non-vector Sandflies has been done to reveal significant plant associations that are possibly preferred. The plant species Salvadora persica and Balanites aegyptica are shown as most prominent species found in association with the Sandflies and their breeding sites in Perkerra Irrigation Scheme. The proportion of non-vector Sandflies, *Sergentomyia antennatus*, *S. bedfordi* and *S. schwetzi* is the highest among these vegetation units while known vectors in the area including *Phlebotomus martini* and *Ph. duboscqi* show negligible numbers in occurrence among the vegetation. The ratio of male to female Sandflies

captured is also shown. The gonotrophic status of females captured which is possibly indicative of their vectorial activity is also mapped. Implications of these findings are elucidated.

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SUPPORT OF FAR-FORWARD DISEASE SURVEILLANCE OPERATIONS WITH DEPLOYABLE, REAL-TIME VECTOR-BORNE DISEASE AGENT ANALYTIC CAPABILITY: ENHANCED AND EXPANDED APPLICATIONS

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The Vector Surveillance Analytic System (VSAS) program is currently focused on three objectives: First, research and development of deployable assays to detect causative agents of vector-borne, zoonotic and infectious diseases. Second, support of forward deployed disease surveillance operations. Third, assay validation and approval for use in environmental surveillance and human diagnostics. The core component of the VSAS is a polymerase chain reaction (PCR) instrument. The PCR instrument, assay chemistry and format, sample preparation technology, operational capability and field utility provide a far-forward, real-time analytic capability. Research and development associated with the VSAS and operational applications provide unique training opportunities for preventive medicine and infectious disease specialists. A primary objective of this program is to immerse Graduate Medical Education (GME) residents, fellows and/or staff in laboratory and field work to provide scholarly and challenging opportunities in basic research and real-world experience in disease surveillance, prevention, and control.

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PREVALENCE AND FINE-SCALE DISTRIBUTION OF RIFT VALLEY FEVER VIRUS AND WEST NILE VIRUS IN MOSQUITOES DURING A RIFT VALLEY FEVER OUTBREAK IN NORTHEASTERN PROVINCE, KENYA

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During the 2006-7 Rift Valley Fever outbreak in Northeastern Province, Kenya, mosquitoes were collected from households within two affected areas; Gumarey (rural) and Sogan-Godud (village). Mosquitoes (N=920) were pooled by trapping location and tested for Rift Valley fever virus (RVFV) and West Nile virus (WNV) by two-step RT-PCR analysis (reverse transcription followed by real-time quantitative PCR). The most common species trapped was *Culex quinquefasciatus* (75%). Of 105 pools, 18% were RVFV positive and 18% were WNV positive. When analyzed by community, Sogan-Godud had 25% WNV and 23% RVFV positive pools, while Gumarey had 14% WNV and 15% RVFV positive pools. Estimated mosquito minimum infection rates did not differ between villages. Three percent of mosquito pools were positive for both viruses. Our data demonstrates the local abundance and capacity of mosquito vectors that propagate arboviral infections in Kenya and the high prevalence of vector arbovirus positivity during an outbreak of RVF.

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SPECIFICITY OF HOST CELLULAR IMMUNE RESPONSE AGAINST SAND FLY SALIVA

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Repeated exposure to sand fly saliva leads to protection against *Leishmania* infection. The cell-mediated immunity is most likely responsible for this protective effect, thus we tested species-specificity of adaptive immune response in repeatedly bitten mice. After the fifth exposure to *Phlebotomus sergenti*, BALB/c mice were sampled for spleen and blood. Spleen cells were incubated in the presence of *P. sergenti, P. papatasi*, or *P. arabicus* salivary gland homogenates. While *P. sergenti* antigen suppressed spleen cell proliferation in naive mice, it had no significant effect in repeatedly exposed mice. Partial cross-reactivity was found between *P. sergenti* and *P. papatasi* salivary antigens, but not between *P. sergenti* and *P. arabicus*. As concerned antibody response, ELISA test revealed no cross-reactivity between anti-*P. sergenti* antibodies and *P. papatasi* or *P. arabicus* antigens. In immunoblot, anti-*P. sergenti* antibodies did not recognize any of *P. arabicus* salivary antigens, however cross-reactived antigenic bands were observed only with *P. papatasi* saliva. Similar specificity of host immune response was observed in a model of outbred Crl:SKH1-hr mice bitten by *P. perniciosus* and tested against *P. perniciosus*, *P. papatasi,* and *P. sergenti* salivary antigens. Species-specificity of antibody and cellular immune response against sand fly saliva may have important implications for the development of vaccine based on sand fly salivary antigens - a unique *Leishmania* transmission-blocking vaccine would be required for each sand fly vector. Our data indicate that species-specificity/cross-reactivity might be similar for both arms of adaptive immune response, thus reaction of anti-saliva antibodies might be used to predict specificity of cell-mediated immune response.

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DYNAMICS OF HOST ANTIBODY RESPONSE TO SAND FLY SALIVA

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Sand flies (*Diptera: Phlebotominae*) are vectors of leishmaniases and immune response against sand fly saliva plays an important role in the epidemiology disease of the disease. Repeated exposure to sand fly bites elicits antibody response in bitten hosts; those anti-saliva antibodies could be used as a marker of exposure and consequently as a risk marker for *Leishmania* transmission. However, in many leishmaniases foci, sand flies show seasonal fluctuation, which may influence the level of host antibodies. Therefore, in experiments lasting up to seven month, we studied dynamics and persistence of anti-saliva antibodies in mice and dogs bitten five times by *Phlebotomus papatasi* and *Lutzomyia longipalpis*, respectively. Specific IgE, IgG and its subclasses were measured in host sera using ELISA. Specific IgE response did not correlate with sand fly exposure. Mice bitten by *P. papatasi* showed higher levels of IgG, IgG1, and IgG2b; difference between unexposed and exposed mice was detectable from the week 4 onward. The antibody response of dogs exposed to *L. longipalpis* reflected the intensity of exposure. The difference in IgG production between high- and low-exposed dogs was detectable throughout the study, i.e. more than 6 months after the last exposure. In conclusion, IgG are the principal anti-saliva antibodies that reflect the intensity of host exposure to sand flies and persist for several month in host sera.

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DISCOVERY, RESEARCH, DEVELOPMENT AND EPA REGISTRATION OF A NEW INSECT AND TICK REPELLENT: COMPARATIVE STUDIES TO OTHER COMMERCIAL REPELLENTS

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A new, EPA registered insect repellent, BioUD, was developed. The registered active compound is undecanone, originally isolated from the wild tomato plant, Lycopersicon hirsutum Dunal f. glabratum C. H. Mull. BioUD is classified by the EPA as a biopesticide. In human trials under practical field conditions, BioUD with 7.75% undecanone 6 h after application to skin had equivalent activity to 25% Deet in studies in North Carolina (USA) and was more effective than 30% Deet in studies in Canada. BioUD with 7.75% undecanone in two choice (treated versus untreated skin) laboratory studies was repellent after 2.5 h (longer time periods were not tested). In two choice filter paper assays between BioUD and 98% Deet, a 50% dilution of BioUD was more repellent than undiluted Deet for three species of ticks examined. In two choice assays on cotton cloth (treated versus untreated cloth), BioUD demonstrated equivalent activity to that of 98% DEET, 19.6% IR3535 and 30% oil of lemon eucalyptus. Products containing 5 and 15% picaridin and 0.5% permethrin were less active than BioUD as a repellent. In two choice repellent to repellent comparisons on cotton cloth, BioUD was more repellent than IR3535 against two tick species tested, more repellent or the same depending on the species for oil of lemon eucalyptus, or showed no difference with Deet. BioUD on cotton cloth provided greater than 90% tick repellency after five weeks when held at room temperature. Additional studies will be discussed in an ongoing effort to evaluate the practical value of this new technology compared to current EPA registered repellents available for personal protection from mosquitoes and ticks.

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GENOME ORGANIZATION OF TANDEMLY-REPETITIVE DNA IN *IXODES SCAPULARIS*, THE LYME DISEASE TICK

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Genome research is revealing the molecular basis underlying diverse aspects of tick biology that may contribute to novel methods for eliminating tick-borne diseases on a global scale. Progress in tick genome research lags behind that in other arthropod vectors such as mosquitoes, however the anticipated publication of the Lyme disease tick (*Ixodes scapularis*: subphylum Chelicerata; class Arachnida; family Ixodidae) genome sequence will provide significant advancement in this regard. To date, physical mapping studies to localize DNA sequences to *I. scapularis* chromosomes (2n=28) have not been reported; however, this research is warranted to improve genome assembly and to address questions about the evolution of tick genomes. In general, Ixodid genomes are relatively large compared to those studied in other arthropods and host large amounts of repetitive DNA. Here, we developed the first fluorescence *in situ* hybridization (FISH)-based chromosome markers in *I. scapularis* that target prevalent tandem repeats in the genome. Using a shotgun sequencing approach, we identified six tandem repeat families (TRFs) estimated to contribute ~159 Mbp of the 2.1 Gbp *I. scapularis* genome (8%), which exceeds the entire genome sizes reported for two other chelicerate species, *Tetranychus urticae* and *Metaseiulus occidentalis*. TRFs were localized to significant blocks of heterochromatin in *I. scapularis* chromosomes prepared from the mitotic cell line ISE18. Probes for the nucleolar organizing regions, telomere, and centromere were used with TRF probes to characterize chromosome architecture and to develop a preliminary FISH-based karyotype. FISH experiments using bacterial artificial chromosome (BAC) clone probes typically resulted in non-specific hybridizations, illustrating that repetitive DNA is prevalent and widely-distributed throughout the genome. Our identification and localization of significant TRFs spawns new questions about their role in chromosome structure and influence on cellular processes such as gene expression and chromosome pairing. This research lays the foundation for developing a high-density FISH-based physical map in *I. scapularis* useful for the international vector biology community.

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MOLECULAR TYPING OF *TRYPANOSOMA CRUZI* STRAINS FROM VECTORS IN YUCATAN, MEXICO

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Trypanosoma cruzi, the agent of Chagas disease, presents extensive genetic variability. Current classification divides into two lineages, I and II. Lineage II is further subdivided into 5 sublineages, from IIa to IIe. Molecular characterization of strains from Mexico indicate a strong predominante of lineage I in the country. Nonetheless, it is necessary to expand these studies to have a better descriptions of strains circulating in the different regions. In this work we used a strategy based on the PCR amplification of the variable region of the kinetoplast minicircle to characterize the strains of *T. cruzi* present in *Triatoma dimidiata* in the state of Yucatan. We used abdominal DNA samples to detect insects positive for *T. cruzi* by PCR with the primers TcZF and TcZR. In a second PCR of the positive samples, we used primers TC1, TC2 and TCC to differentiate between lineages I, IIb, IId and IIe. Overall *T. cruzi* infection rate was 24%, as reported previously in the region. A total of 34 samples were typified, resulting in 33/34 (97%) of the strains belonging to lineage I and 1/34 that presented an abnormal amplicon size from lineage I. These results confirm the predominance of lineage I in Yucatan, Mexico. Additional strain typing from reservoirs and patients will provide a detailed understanding of parasite transmission cycles in the region.

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BREEDING HABITATS FOR MALARIA VECTORS ASSOCIATED WITH A LAKE

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Intense malaria transmission is often observed in areas around lakes in Africa. It has been assumed that stagnant water pools on lakeshore become suitable habitats for malaria vectors, and vector populations and malaria transmission are high in the areas adjacent to lakes. On the other hand, it has been believed that vectors do not breed within lakes. However the habitats associated with lakes have been little studied for malaria vectors. This study examined various types of aquatic habitats on the shore of Lake Victoria and reveled the extent of which malaria vectors breed in the habitats. *Anopheles funestus* inhabited in short grass and water hyacinth habitats within the lake. However, its habitats were limited in the areas that were not exposed to waves. *Anopheles arabiensis* were also found in a few calm areas in the lake. The vectors inhabited in stagnant water pools on the lakeshore. *An. funestus* mainly inhabited in vegetated habitats except tall vegetation, while *A. arabiensis* inhabited mainly in open areas. In particular, *An/ funestus* was abundant in the large water pools isolated by sand bars. A lake may provide various types of breeding habitats for malaria vectors.

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IDENTIFICATION OF DAMAGED ADULT FEMALE CONTAINER-INHABITING *AEDES* MOSQUITOES IN LA CROSSE VIRUS ENDEMIC AREAS

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The purpose of this study was to identify novel and useful morphological characters to aid in the identification of container-inhabiting *Aedes* mosquitoes found in La Crosse virus endemic areas. La Crosse virus is an emerging and/or underreported vector-borne disease that is the number one cause of arboviral encephalitis in North Carolina. Commonly used collection methods, such as CDC light traps, are often damaging to the adult specimens when they are captured. Likewise, the morphological characters used in the current keys to mosquitoes are often destroyed or damaged by these methods of collection. In this study, five *Aedes* species (i.e., *Ae. triseriatus, Ae. albopictus, Ae. japonicus, Ae. atropalpus*, and *Ae. aegypti*) were examined. An exhaustive literature search and the examination of preserved specimens were used to create a character data matrix to aid in the selection of characters. Using the matrix, a single distinguishing morphological character or a combination of two characters was found for each of the studied species. The results reported here should immediately benefit public health professionals in La Crosse virus endemic areas who are responsible for the collection, identification, and processing of mosquitoes.

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A REMEDY FOR CHRONIC, NON-HEALING WOUNDS UNRESPONSIVE TO CONVENTIONAL THERAPY: MAGGOT DEBRIDEMENT THERAPY

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Therapeutic maggots used most commonly today are those of the greenbottle fly (*Lucilia sericata*) only attacks necrotic tissue. Maggot debridement therapy has been used for treating non-healing wounds with conventional therapy since ancient ages. Maggot debridement therapy has the following three benefical effects on a wound: debridement of non-healing necrotic and soft-tissue wounds such as pressure ulcers, neuropathic ulcers and non-healing traumatic or post-operative wounds, promotion of tissue granulation and wound antisepsis due to antibacterial secretions. The medical literatures have shown that maggot debridement therapy is very effective in the treatment of gram-positive bacterial infections. We aimed to assess the potential efficacy of maggot debridement therapy in eight patients (mean ± SD age, 65 ± 7 years) with neuroischemic diabetic foot wounds (n=7) and pressure ulcers (n=1). Microbiological samples were collected by using deep tissue biopsy from wounds of patients. Bacterial cultures showed a wide variety of organisms, but *Enterococcus fecalis* and *Staphylococcus saprophyticus* were causative agent in up to 65% of all cases. The mean bacterial density on infected or colonized chronic wounds was $6x10^4$-10^6 germs/g. Bags containing 20-30 sterile maggots were placed on the wounds. The mean therapy was 4.8 ± 2.2 times for patients. After therapy with maggots in bags, treated wounds showed significant improvement. Wounds were completely debribed, granulating tissue begun to grow (at least 50% of wound surface area) and necrotic tissue decreased by an average of 5.4 cm^2. Also, bacterial density is reduced to10^3 germs/g bacteria after the application of maggot secretions onto wounds. Thus, we observed that maggot debridement therapy was effective and efficient in patients with non-healing foot and leg ulcers. Maggots may prevent these patients to undergo partial amputation of a limb.

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MULTIPLEX PCR ASSAY FOR THE DETECTION AND SIMULTANEOUS DIFFERENTIATION OF CONTAINER-INHABITING *AEDES* MOSQUITOES IN LA CROSSE VIRUS ENDEMIC AREAS

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Mosquito surveillance requires the rapid and accurate identification of medically important mosquitoes in a "real-time" fashion. Species identification of these mosquitoes is especially critical in areas where vector-borne diseases are endemic or have epidemic potential. Because adult container-inhabiting *Aedes* mosquitoes are poorly represented in CO_2-baited CDC light trap collections, their eggs are typically collected with oviposition traps. Identification of these eggs in situ is impractical at best. Therefore, identification is often determined by adult emergence or rearing to the 4th instar larval stage. This process routinely takes 7-10 days and is not ideal during outbreak investigations. Here we report the development of a multi-plex PCR assay to identify five (5) container-inhabiting *Aedes* mosquito species (i.e., *Ae. albopictus, Ae. triseriatus, Ae. hendersoni, Ae. japonicus*, and *Ae. atropalpus*). Most of these species are capable of vectoring La Crosse virus, an emerging and underreported cause of arboviral encephalitis. Species diagnostic differences based on ribosomal DNA internal transcribed spacer sequences and size polymorphisms were employed to develop and validate species specific primers. This assay allows the rapid identification of these species from any life stage (e.g., egg, larva or adult).

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A RODENT SPECIES (*SPERMOPHILUS DAURICUS*) INFECTED WITH *ECHINOCOCCUS GRANULOSUS* IN NINGXIA, CHINA: A POTENTIALLY NEW MODE OF HYDATID TRANSMISSION

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Transmission of *Echinococcus granulosus* is primarily maintained in a cycle involving dogs and domestic livestock worldwide, although sylvatic cycles have also been reported in some areas, involving, for example, wolves and moose in North America, and dingoes and marsupials in Australia. Despite a few of reports indicating the involvement of small mammals in *E. granulosus* transmission, such as the European hare in Argentina and rabbits in Australia, there had been no reports of rodent species naturally infected with larval *E. granulosus.* As part of extensive eco-environmental investigations for monitoring transmission of both *E. granulosus and E. multilocularis* in Ningxia Hui Autonomous Region in China, a well known co-hyperendemic area for human alveolar and cystic echinococcosis (AE and CE), we undertook a pilot rodent survey in 2007. As a result, approximately ten percent of 500 captured small mammals were found with liver cystic lesions which were preserved in ethanol for parasitic examination and molecular genotyping. A partial cox1 gene sequence (789 bp) was obtained from one liver lesion from a rodent (*Spermophilus dauricus/alashanicus*), and was unambiguously identified as

E. granulosus (G1 genotype; common sheep-dog strain) which was shown microscopically to be a viable cyst with numerous fertile protoscoleces present. This is the first report that a rodent species can act as a reservoir host for CE. This species of rodent may play a critical role in the transmission of *E. granulosus* in this Chinese setting since dog/fox-rodent predator-prey relationships are commonly found there. Further extensive investigations are now required to determine the overall importance of this new mode of transmission because knowledge of the existence of sylvatic cycles is important when control programs are proposed.

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MESOCESTOIDIASIS: A NEW U.S. CASE AND THE IMPORTANCE OF DIFFERENTIAL DIAGNOSIS IN CESTODE INFECTIONS

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Mesocestoidiasis is a disease caused by cestodes in the genus, *Mesocestoides*. *Mesocestoides* spp. have a complex, three-host life cycle involving an arthropod intermediate host, a vertebrate paratenic host, and a mammalian definitive host. The normal definitive hosts in nature for *Mesocestoides* spp. are primarily carnivores, including canids, felids, and mustelids. The definitive host is infected by the ingestion of undercooked meat or viscera containing the infective third-stage larva (tetrathyridium); humans can also be infected by this route. Diagnosis is typically made by identifying proglottids or eggs shed in feces, as there are no serologic tests available for this disease. To date there have been 28 cases reported worldwide in humans, with seven previously from North America; the eighth U.S. case is reported here, from a one-year-old girl in Ohio. The rarity of this disease in humans, coupled with the morphologic similarity to other cestodes in humans, can make diagnosis difficult to the laboratorian and treatment options challenging for the medical provider. In the newest case presented here, the initial presumptive diagnosis of a cestode (*Hymenolepis* vs. *Diphyllobothrium*) was made based on images of craspedote proglottids, originally sent as a telediagnosis inquiry to the CDC DPDx Project in December, 2008. The definitive diagnosis was made based on a more detailed analysis of the actual proglottids, sent to the CDC as follow-up to the telediagnosis. Despite the low public health impact of this disease in humans, this case raises the importance of differential diagnosis in cestode infections, so that appropriate prevention messages can be delivered.

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AN UNUSUAL CASE OF MYOCUTANEOUS CYSTICERCOSIS MASQUERADING LUDWIG'S ANGINA

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Ludwig's angina is a cellulitis frequently occurring as a result of infections of the second and third lower molar. The cellulitis involves sublingual and submaxillary spaces and clinically patient presents with pain, trismus, brawny swelling of submmental and submandibular region. Floor of the mouth is edematous, displaced due to sublingual space involvement. The potential for rapid respiratory obstruction is the greatest concern. Familiarity with the anatomy of the neck and recognition of symptoms are essential for effective treatment. Treatment focuses on maintenance of an airway, antibiotic therapy, and surgery. Asphyxia, aspiration, mediastinitis, pneumonia, empyema, and septicemia are possible complications. Despite a decrease in mortality from 50% to less than 10% since the introduction of antibiotics, it remains a rare but life-threatening illness. Cysticercosis is a condition in which a human acts as the intermediate host of Taenia solium, a pork tapeworm. The larvae infestation sites frequently include cerebral tissue, ocular organs, and muscles.. Although subcutaneous cysticerci are inconsequential, their verification is important in the diagnosis of more severe CNS involvement. They may be confused with other painless swellings such as lymphadenopathies, neurofibromas, and epidermoid cysts. The present case report describes an unusual and rare incidence of Ludwig's angina caused by cysticercosis of submandibular region and also highlights utility of FNAC in the diagnosis.

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HELMINTH GLYCANS INDUCE REFRACTORINESS AGAINST IFN-Γ VIA TLR-2 LEADING TO MYELOID DERIVED SUPPRESSOR CELL (MDSC) MEDIATED IMMUNE REGULATION

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Incidence of Type 1 diabetes (T1D) has shown a significant increase, particularly among children, the world over. Reports show an inverse correlation between helminth infections and T1D. Helminth induced Th-2 immunomodulation in the host is said to be responsible for this protection. Previously we have shown that glycans from *Taenia crassiceps* up-regulate IFN- γ production in naïve spleen cells, but prolonged stimulation induced long term suppression of IFN-γ responses. The initial recognition of the glycans was by macrophage TLR-2. Infection with *T. crassiceps* abolished the IFN-γ response in a manner similar to LPS tolerance, while IL-6 responses were either unaffected or up-regulated. This phenomenon was seen in infection susceptible BALB/c mice but not in resistant C57BL/6 mice. The refractoriness to glycan induced IFN-g was functional. *T. crassiceps* infected mice responded differently to Streptozotocin induced hyperglycemia compared to uninfected mice, where *T. crassiceps* infection prevented STZ induced hyperglycemia. *Taenia* infected Balb/c mice were more susceptible to *Leishmania mexicana* (Th-1 protective) induced footpad lesions than uninfected mice. The responsible glycans have been isolated. Oral feeding or intraperitoneal injection of naïve BALB/c mice with the purified glycans also induced suppression of IFN-γ responses to further stimulation. Glycan stimulated mice were relatively resistant to streptozotozin induced diabetes. We propose an IFN-γ mediated myeloid derived suppressor cell (MDSC) mechanism in helminth glycan induced immune regulation and protection against Th1 diseases.

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THE RELATIVE UTILITY OF RECOMBINANT PROTEINS AND ASSAY FORMATS FOR DETECTION OF CYSTICERCOSIS AND TAENIASIS

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Laboratory detection of cysticercosis relies heavily on the enzyme-linked immunoelectrotransfer blot (EITB) developed at CDC. The EITB uses lentil-lectin purified glycoproteins (LLGP) extracted from *Taenia solium* cysticerci; but, purification of the LLGP antigens has been difficult to standardize and the polyacrylamide gel system used for the EITB is not easily transferred

to other laboratories. Over the last 10 years we purified and cloned the diagnostic glycoproteins in the LLGP fraction. We also identified and generated recombinant proteins for serologic detection of taeniasis cases. These antigens were used in various formats for serologic diagnosis of neurocysticercosis and taeniasis cases. In this report we summarize the relative utility of these different antigen-test format combinations to identify the preferred methods for case detection in epidemiological studies and immunodiagnosis of neurocysticercosis. In multiple assay formats, rT24H was the most sensitive and specific protein antigen for detection of cysticercosis cases. An T24 EITB had a sensitivity (99%) and specificity (100%) that was comparable to the classical EITB using native proteins. For large epidemiological surveys we recommend the use of rT24H in a standard ELISA format (sensitivity = 96%, specificity = 98%) because the ELISA method is easy to perform and transfer to regional reference laboratories. For simultaneous detection of taeniasis and cysticercosis cases we propose a single method utilizing the multi-print antigen immunoassay using rT24H (sensitivity = 97%, specificity = 99%) in combination with either of the 2 taeniasis proteins, rES33 or rES38 (sensitivity = 99%, specificity = 94%). We also demonstrated proof-of-principle that both rT24H and rES33 can be used in rapid immunochromatographic test formats. In summary, depending on the specific need, different methods are preferred for detection of cysticercosis and taeniasis; all methods utilize sustainable supplies of antigens, are simple to perform, and easily transferred to laboratories in cysticercosis endemic areas of the world.

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CYSTICERCOSIS IN AN ISLAMIC STATE: MAKING THE CONNECTION IN A GLOBAL ENVIRONMENT!

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We have previously reported neurocysticercosis (NC) and the transmission of the cestode in an Islamic country where porcine products are prohibited. We presented an autochthonous case where transmission was probably due to the contamination of food by a cook with detectable antibodies to adult *Taenia solium* and a series of Kuwaiti children with NC and positive by the immunoblot (IB). We now present three patients illustrating the diversity of clinical presentations, the diagnostic methodology and experience with a commercially-available IB test kit in this geographic area. In the first case, we show direct evidence that a Kuwaiti child presenting with NC was infected by a maid excreting ova of *T. solium* confirmed by staining negative with the Ziehl-Neelsen stain. The serum of the child however did not show any precipitin bands in the IB. We further describe a child presenting with the typical symptoms of NC and confirmed by the IB test. We then discuss a patient with cystic hydatid disease where the IB test showed precipitin bands other than those associated with NC. We elaborate on the interpretation of such findings. We then discuss our experience with the performance of the IB test kit; of the 135 requests for cysticercosis over a two-year period the test was positive in 24 (17.8%), the majority 19 (80%) were imported infections. All of them showed the expected variable precipitin bands as in the positive control. Five patients (20%) with autochthonous infections were all children; 4 Kuwaitis and one Syrian. They were all cared for by maids from endemic areas. We also show the limitations of the test, especially in this area, where hydatid disease is endemic. Our data illustrates and reconfirms the continuing transmission of cysticercosis in an Islamic country. The increased prosperity in households in oil-rich countries, leads to the employment of domestic help from developing countries endemic for *T.solium.* Thus, the presentation of the typical manifestation of NC does not preclude the diagnosis in Islamic countries as at least one of the risk factors for transmission exists in this global environment.

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DENGUE INFECTION IN DIFFERENT AGE GROUPS

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Dengue is the most common mosquito-borne virus causing disease in many countries worldwide. The diseases vary from asymptomatic infection, undifferentiated fever, dengue fever (DF), dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). The clinical manifestations and laboratory results may vary in different age groups. This study was undertaken to study and understand the variety of these will help clinicians to achieve early diagnosis, proper management and reduce mortality. Clinical data in children with dengue infection, confirmed by serologically and/or virologically, admitted to King Chulalongkorn Memorial Hospital during 1987-2007 were analyzed. The patients were classified into 3 different age groups: 0-1 year, 2-12 years and 13-15 years. For all three age groups, fever, hepatomegaly and bleeding were the most common clinical manifestations of dengue infection. The respiratory tract and neurological symptoms were prominent in the 0-1 year age group, however, less common in older children. DHF was common in every age groups, but DSS was more common in 2-12 years age group. In conclusion, clinical manifestations and severity of dengue infection varied with age. Central nervous system manifestations were common in infants while DSS was less prominent. The study emphasizes that there is significant variation of clinical manifestations in different age groups, suggesting that proper treatment must take into account the different age-specific clinical manifestations.

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OUTPATIENT PNEUMOCOCCAL BACTEREMIA: RISK FACTORS AND OUTCOMES IN BAMAKO, MALI

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In industrialized countries, pneumococcal bacteremia (PB) occurs in ~2-3% of highly febrile outpatients 3-36 months of age. Untreated, 10 to 25% of PB cases have complications, including 3-6% who develop meningitis. To better define the course of outpatient PB in developing countries, we conducted a nested case-control among febrile outpatients < 36 months of age under surveillance for invasive bacterial infection at the Emergency Department (ED) of Hôpital Gabriel Touré in Bamako. Cases were defined as children with *Streptococcus pneumoniae* isolated from blood. Three age-matched controls with negative cultures were enrolled for each case. Participants underwent HIV antibody testing and hemoglobin electrophoresis, and had a follow-up visit 30 (+/- 7) days after initial consultation at the ED. From September 2006 to September 2008, 118 cases and 353 controls were included. Forty-five cases were <12 months old, 51 were 12- to 23- months old and 22 were 24- to 35-months old. Cases were more likely to have refused hospitalization after initial consultation ($p < 0.01$). Of the 108 cases and 331 controls who underwent HIV testing, 11 (10.2%) and 6 (1.8%) were HIV positive ($p < 0.001$); all had hemoglobin electrophoresis and 10 cases (8.5%) were diagnosed with sickle cell disease (SCD; HgbSS or HgbSC) versus 9 controls (2.5%) ($p < 0.01$). At follow up, 5 cases (4.2%) and 3 controls (0.8%) had died ($p = 0.02$). Two cases died following hospitalization for meningitis, one following hospitalization for pneumonia, and the remaining 2 cases died at home having never been hospitalized, one with pneumonia and the other with HIV. Two controls died after hospitalization, one of HIV infection, the other with anemia; the third control died at home of pneumonia.in conclusion, outpatient PB in Bamako is associated with

frequent complications and a high case fatality. SCD and HIV infection were shown to be predisposing conditions. Control strategies such as improved case management and introduction of pneumococcal vaccine are needed.

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CONCOMITANT MALARIA PARASITEMIA AND BACTEREMIA IN HOSPITALIZED CHILDREN IN BAMAKO, MALI

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In malaria-endemic countries, the treatment of children with fever can be a dilemma as the importance of a positive malaria smear may obfuscate the presence of a concomitant bacteremia. To study the importance of malaria and bacterial co-infection, systematic blood smears for malaria parasites were integrated into ongoing hospital-based surveillance for invasive bacterial infection at a Hôpital Gabriel Touré (HGT) in Bamako, Mali. Children aged 0-15 years with fever ≥ 39° C or suspicion of invasive bacterial infection (SIBI) admitted to HGT were eligible. Blood and relevant body fluids were collected and cultured after obtaining informed consent. Blood smears for malaria were performed. Co-infection was defined as the presence of a positive malaria smear and bacterial pathogen on culture. From January 2007 to December 2008, 3725 children were included. Of these, 17 (0.5%) were co-infected. Children with a positive smear were less likely to have a positive culture (17/454 vs 520/3271, $p < 0.01$). Co-infected children were equally likely to be admitted to the intensive care unit as well as to another service (7/1621 vs 10/2033; $p = 1.00$). Co-infection was more commonly noted among children presenting with isolated fever (8/916) rather than SIBI (9/2809, $p = 0.04$). Outcomes were known for 3585 participants (96%), including 16 co-infected children, among whom 4 deaths (25%) were observed. Mortality rates were similar among children who were co-infected (4/16), culture positive alone (129/506), or smear positive alone (52/410), $p > 0.05$. Among those with positive culture, non-Typhoidal *Salmonella* (NTS) was isolated more commonly from the blood of patients with a positive malaria smear than with a negative smear (6/17 vs 56/520, $p < 0.01$). Mortality rates among those co-infected with NTS and those with NTS alone were similar (1/5 vs 9/55, $p > 0.05$). In conclusion, concomitant malaria and bacterial infection is relatively uncommon and afflicted children do not appear to be more adversely affected. Nonetheless, each condition alone carries a high case fatality. NTS is more commonly associated with a positive malaria smear.

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AN OUTBREAK OF PUFFER FISH EGG POISONING IN SOUTHERN, COASTAL BANGLADESH

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In late October 2008, the Institute for Epidemiology, Disease Control and Research (IEDCR) learned about an outbreak of suspected puffer fish poisoning in Maiskhal, a remote island in southern Bangladesh. A team from IEDCR and International Centre for Diarrheal Diseases Research, Bangladesh (ICDDR, B) investigated the outbreak to determine the cause and to identify local uses of puffer fish. We used a structured questionnaire to collect exposure history and symptoms experienced by case-patients. Physicians from the local health complex verified this clinical presentation during a group discussion. The team conducted in-depth interviews with community members, fish wholesalers, and workers at the local fish market to understand different uses of puffer fish in the area. The outbreak affected one poor family struggling to provide three meals a day. On the day of the outbreak, the grandmother collected discarded puffer fish eggs from outside a shack in the local fish market where puffer fish were cleaned to make shrimp food and fish bait. She used them to make a curry lunch, despite being warned by an acquaintance that the puffer fish eggs were poisonous. Nine persons from the family ate the egg curry; five of them became ill within four hours of consumption. Tingling sensation, vomiting, watery stool, paresis of the limbs, stomach ache and drowsiness were some common symptoms of the case-patients. Two (40%) of the 5 people who developed symptoms died. It is unclear why this woman fed her family puffer fish eggs since she understood that they were poisonous; she may not have believed that they were puffer eggs or she may not have been able to secure other food, but foul play cannot be ruled out. Outbreaks of puffer fish poisoning seem to be increasing; this was the fourth outbreak in Bangladesh reported in 2008. We need to explore why and how puffer fish are sold and processed in local industries and understand how we can raise awareness about puffer fish toxcitity to prevent future outbreaks.

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IDENTIFICATION OF MICROORGANISMS RELATES WITH SEPSIS IN ACUTE INFECTIONS IN THE INTENSIVE CARE UNIT OF AN INSTITUTION OF HEALTH ATENTION IN MONTERIA-CORDOBA, COLOMBIA

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This study was undertaken to estimate the prevalence of microorganisms producing sepsis in patients of the Intensive Care Unit (ICU) of an health attention institution in Montería. In the period between September 2007 and February 2008 in the ICU of the hospital institution of Montería, we developed a descriptive, prospective study of cross sectional in the population of patients attending this service of the institution. To select patients for this investigation, three blood cultures were taken from each patient using the invasive techniques. Cultures were incubated in the BACTEC 9050 equipment; the samples were Gram stained and subcultures in solid mediums were made. Biochemistry tests for the identification were done to determine the aetiology and to confirm sepsis in the patient. The information was obtained from the clinic histories of the patients and from the results of the blood culture. Results were tabulated and analyzed with the excel program. One houndred twenty seven patient samples were taken. 28/127 (22,05%) were diagnosed with nosocomial sepsis; 17/28 (60,71%) acquired the infection in the ICU. The microorganisms most frequent were: *Staphylococcus aureus* (47,0%), *Acinetobacter spp*. (29,4%), *Staphylococcus epidermidis* and *Pseudomona spp.*, each one with (11,8%). The septic patients were submitted to invasive procedures as: bladder probe and mechanical ventilation in 100% of the cases, central catheter in 58,8% and parenteric nourishment in 88,2%.The high prevalence of Gram positive cocci can be related to post -surgical processes and direct infection of the patient by the personnel in charge of his care in the ICU. The multiple invasive methods were related to the appearance of sepsis, contributing together with the immunosupression in the deterioration of the clinic condition of these patients.

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"I TOLD YOU SO"- WORDS OF WISDOM FROM YOUR WIFE IN PROTECTION FROM DENGUE FEVER

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WHO facts: 50 million people infected with dengue each year with 500,000 hospitalizations and is the most prevalent mosquito borne viral disease. Patients may present with either minor flu-like illness (classic) or hemorrhagic shock (DHF) most are asymptomatic. There are 4 serotypes

and one does not offer cross protection to the others. There may be severe disease on a subsequent infection. Symptoms develop 4-7 days after the bite of an *Aedes egypti* mosquito. Second most common cause of fever in western travelers from developing countries. Presents with headache, eye pain and severe myalgias- breakbone fever. Exam nonspecific with occasional macular rash. Labs: leukopenia, thrombocytopenia and liver function abnormalities. Diagnosis by serology. There is no treatment. The best prevention is repellent and not bed netting this mosquito bites during the day. Aerial spraying is not effective the mosquito breeds indoors. An effective vaccine for all four serotypes is not available. We present a case of two married health care workers who returned from Trinidad each with a different outcome. The wife used repellant and her husband did not. Unfortunately, he suffered the consequences. The husband was a 60 year old man hospital worker with severe headache, generalized weakness and fever for 4 days. Fever persisted despite acetaminophen. Physical exam significant for temperature 38.5°C, few petechiae in the extremities. Admitting labs showed a platelet count of 33,000/cc^3. Platelets decreased to 9,000/cc^3 by day 6 and six units of platelet were given. Liver enzymes: ALT and AST increased to a peak of 448 mg/dl and 457mg/dl respectively on day 9 of symptoms. Dengue serology was positive: IgG 1.2 and IgM 7.6 (normal < 0.9 each). In conclusion, thrombocytopenia caused by: bone marrow suppression, platelet destruction, molecular mimicry between the dengue viral protein and coagulation factors like plasminogen. Risk factors for DHF:viral factors: dengue type 2 and "Asian" genotypes, and host factors such as prior infection, young age, well nourished and white. If severe thrombocytopenia, < 20,000/cmm platelet transfusions may be indicated; anti-D immune globulin still investigational. Newspaper reports over 1000 cases of DF in Trinidad and Tobago in the first 8 months of 2008 and 160 cases of DF in the first 15 days of September 2008 when our patient traveled. There is no adequate prophylaxis, repellant can be protective and our health care worker now heeds to his wife's advice.

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PHARMACOKINETIC PROFILES OF ARTESUNATE FOLLOWING MULTIPLE INTRAVENOUS DOSES OF TWO, FOUR AND EIGHT MG/KG IN HEALTHY VOLUNTEERS

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Safety, tolerability, and pharmacokinetics of artesunate (AS) were evaluated after ascending multiple doses at 2, 4, and 8 mg/kg with 2 minutes intravenous infusion in 24 healthy subjects daily for 3 days. Plasma samples for measurement of AS were analyzed by validated analytical procedures using a LC-MS/MS. Results showed that there was no dose-dependent increase in any adverse events. Drug concentration of AS on day 3 showed no significant difference to that on day 1, which is evidence of no accumulation and no decline of drug in the healthy volunteers. This suggests that the metabolic enzyme auto-induction shown *in vitro* may not be a characteristic of AS in healthy humans. After intravenous injection, AS rapidly declined and converted to dihydroartemisinin (DHA) with overall mean elimination half-lives ranging 0.15-0.23 hr for AS and 1.23-1.63 hr for DHA. It is known that AS is a prodrug of DHA, but the DHA/AS ratio was only 1.12-1.48 during the three days treatments. Concentrations vs. time data confirmed that pharmacokinetic model-dependent analysis is suitable to AS, while DHA is fitting both model-dependent and -independent methods. DHA concentration was slightly superior and also its half-life was much longer than AS, suggesting that AS-derived DHA plays an important role in the therapy of malaria. In addition, the AUC and C_{max} of AS and DHA were increased proportionally to the AS climbing multiple doses. The present data show that the administration of injectable AS at high dose of 8 mg/kg is safe and well tolerated. This increases the probability of success in treatment of severe malaria when dosing patients with large variability on pharmacokinetics and pharmacodynamics profiles.

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TRAVEL-RELATED DISSEMINATED *PENICILLIUM MARNEFFEI* INFECTION IN A RENAL TRANSPLANT PATIENT

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Penicillium marneffei is a dimorphic fungus that is endemic in South East Asia. A 67-year-old man with end stage renal failure secondary to vasculitis underwent renal transplantation in 2004. He was on chronic immunosuppressive therapy with tacrolimus, mycophenolate and prednisolone when he visited Vietnam in December 2008. Two weeks after return to Australia his serum creatinine increased from 124 to 190µmol/L and renal biopsy suggested recurrent vasculitis. Treatment was with 3 days of pulse IV methylprednisolone followed by an increased dose of mycophenolate and prednisolone. He subsequently presented to the outpatient clinic with 3 weeks of loose stool and 2 days of lower abdominal pain. Following admission he rapidly progressed to septic shock. Perforated sigmoid colon and intraperitoneal pus were found at laparotomy and Hartmann's procedure was performed. Histopathology revealed perforated colonic diverticulum and background CMV colitis. Plasma CMV viral load peaked at 2.32 x 10^4 copies/mL. *Penicillium marneffei* was grown in both blood culture and peritoneal fluid. He was treated with 2 weeks of IV liposomal amphotericin 3mg/kg/day, then changed to oral itraconazole 400mg daily, with close monitoring of serum itraconazole and tacrolimus concentrations. *P. marneffei* is an opportunistic pathogen common in HIV patients in endemic areas but rarely reported in solid organ transplant patients: all previously published case descriptions have been of patients living in endemic countries. Diarrhoea is a feature of disseminated penicilliosis in up to 30% of cases associated with HIV infection and intestinal penicilliosis is described. As with other invasive fungal infections in the immunosuppressed, it is possible that co-incident CMV reactivation and disease may have predisposed to both disease dissemination and colonic involvement. This case highlights the importance of careful pre and post-travel assessment and consideration of *P.marneffei* as a cause of opportunistic infection in solid organ transplant patients with relevant travel history.

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EMERGENCE OF ERYTHROMYCIN AND CLINDAMYCIN-RESISTANT *STREPTOCOCCUS PYOGENES* EMM 90 STRAINS IN HAWAII

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The epidemiology of group A streptococcal (GAS) infections are different in Hawaii than the continental U.S. with increased rates of impetigo, acute rheumatic fever and clusters of necrotizing fasciitis due to uncommon GAS emm types. Our previous studies showed a low prevalence of erythromycin resistance in Hawaii (~3.1%) and no clindamycin resistance among GAS isolates between years 2000 and 2005. During a retrospective review of GAS blood cultures collected between 2005 and 2007, a multi-drug resistant emm type (emm 90) was identified. Emm 90 commonly (12 of the 89 patients) appeared to be associated with severe disease such as sepsis, streptococcal toxic shock syndrome, necrotizing fasciitis and pneumonia. We compared the invasive and non-invasive emm 90 isolates and examined the drug-resistance mechanisms with clinical phenotypic tests. We emm typed, performed "E-test" (AB Biodisk, Piscataway, NJ), tested for drug-resistant genes (mef(A), erm(B), erm(A), and erm(TR)) and Prtf1 gene for invasiveness, then Pulse-Field Gel Electrophoresis (PFGE) for clonality with restriction enzyme smaI. We identified three subtypes: emm 90.1, 90.2 and 90.4b. Emm 90.4b was the only subtype that dominated and developed drug-resistance with MLSB phenotype. The drug-resistant isolates displayed only one pattern in the pulse-field gels. There is an association between erythromycin resistance and invasiveness in emm 90 strains identified since 2006 raises serious concern. The characterized drug-resistant genotype and phenotype are unusual to U.S. mainland and

Europe. Emm 90.4b is inherently persistant and adaptable because it is associated with uncomplicated pharyngitis to severe invasive GAS diseases and developed erythromycin and clindamycin resistance.

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COMPARATIVE FEATURES AND OUTCOMES OF *PLASMODIUM FALCIPARUM* MALARIA INFECTION IN GLUCOSE-6-PHOSPHATE DEHYDROGENASE-NORMAL AND DEFICIENT CHILDREN AT A TERTIARY CARE HOSPITAL IN IBADAN, NIGERIA

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Supporting data that possession of G6PD deficient gene (Gd-) protects against severe malaria are mainly from areas where malaria is less endemic and Gd- less common than Nigeria. We hypothesised that, being a haemolytic factor, G6PD deficiency makes severe malarial anaemia (SMA) commoner and more severe. In 930 children aged 0.5-12 years (458 boys), with microscopically-proven falciparum malaria, G6PD and haemoglobin were typed by the fluorescent spot test and electrophoresis, respectively. Molecular typing by PCR and restriction enzyme digestion was also carried out on 15% of randomly selected samples. All patients were treated according to WHO/Nigeria protocol. Severity of malarial was compared between G6PD-normal and deficient children. The prevalence of Gd- was 16.4% and 8.1% among boys and girls with malaria, respectively. Mean (SD) Haematocrits (PCV) of 22.8% (7.9) in Gd- was significantly higher than 21.0% (8.9) in Gd+ (p=0.041). In boys, 2.7% of Gd- had PCV ≤10% compared with 13.6% in Gd+ (OR=0.17, 95% CI: 0.04, 0.73, p = 0.005). Also, 21.3% of Gd- had PCV ≤15% compared with 39.4% in Gd+ (OR=0.42, 95% CI 0.23, 0.75, p = 0.003). However, no such contrast was found among girls. Overall, Hb AS was typed in 7.6% and was similar between Gd- (13.0%) and Gd+ (6.8%), p = 0.058. The geometric mean parasite counts (GMPC) was significantly lower in Gd- (15477.5/µl) than in Gd+ (19784.4/µl), p = 0.013, and it is independent of HbAS. In conclusion, Gd- male was unlikely to develop severe malarial anaemia, this prediction could not be made in female. Thus Gd- protects against emergency blood transfusion and its related risks in male.

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MALARIA MISDIAGNOSIS IN UGANDA - IMPLICATIONS FOR POLICY CHANGE

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In Uganda, like many other countries traditionally viewed as harboring very high malaria transmission, the recommendation is that febrile episodes are diagnosed as malaria. The policy implications of such recommendations are revisited in this study. A cross-sectional survey was undertaken at all health facilities in four Ugandan districts. Routine diagnostic and treatment practices were assessed and a research slide was obtained for later reading. Primary outcome measures were the accuracy of national recommendations and routine malaria diagnosis in comparison with the study definition of malaria (any parasitaemia on expert slide examination in patient with fever). Secondary outcome measures were the use, interpretation and accuracy of routine malaria microscopy. 1,763 consultations undertaken by 233 health workers at 188 facilities were evaluated. Malaria prevalence was 24.2% and ranged between 13.9% in patients ≥5 years in medium-to-high transmission areas to 50.5% for children <5 years in very high transmission areas. The sensitivity and negative predictive value of routine malaria diagnosis were high (89.7% and 91.6% respectively) while specificity and positive predictive value (PPV) were low (35.6% and 30.8% respectively). However, malaria was under-diagnosed in 39.9% of the children less than five years of age in the very high transmission area. The use of malaria slide examination was low (34.5%) without significant differences between age groups. Although 96.2% of patients with positive slide result were treated for malaria, also 47.6% with a negative result were treated. In conclusion, current recommendations and associated clinical practices result in massive malaria over-diagnosis, yet, under-diagnosis is also common in children <5 years. To address malaria misdiagnosis, a policy shift from presumptive to parasitological diagnosis encompassing introduction of malaria rapid diagnostic tests and strengthening of malaria microscopy.

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DETECTION OF $BLA_{CTX\text{-}M\text{-}15}$ EXTENDED-SPECTRUM B-LACTAMASE GENES IN *E. COLI* FROM HOSPITAL PATIENTS IN NIGERIA

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The aim of this study was to investigate the occurrence and relatedness of CTX-M type extended spectrum β-lactamases (ESBL) in *Escherichia coli* isolates from patients of a Nigerian Hospital. The study included 116 *E. coli* isolated from inpatients and outpatients from January 2006 to January 2007 at the Ladoke Akintola University Teaching Hospital in Osogbo, Nigeria. The phenotypical confirmation test revealed 12 ESBL positive isolates, which were investigated for the presence of $bla_{CTX\text{-}M}$ genes. Nine of these *E. coli* contained $bla_{CTX\text{-}M}$ group 1 genes and additionally harbored bla_{TEM} and bla_{OXA} group 1 genes. Pulsed field gel electrophoresis (PFGE) of these 9 strains revealed 6 clonal groups, as four of the isolates revealed identical PFGE patterns while the other five showed no relatedness. Sequencing of the $bla_{CTX\text{-}M}$ gene of one isolate from each clonal group always identified CTX-M 15. At present there are no published data about the genetic background of ESBL-producing *E. coli* in Nigeria. To our knowledge, this is the first report of *E. coli* carrying $bla_{CTX\text{-}M\text{-}15}$, bla_{TEM}, and bla_{OXA} genes in Nigeria.

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SHIGELLA SEROTYPES AND ANTIBIOTIC SENSITIVITY IN AN URBAN SLUM CLINIC IN NAIROBI, KENYA

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Shigellosis is endemic throughout the world. It causes an estimated 164.7million cases of which 163.2 million occur in developing countries. There is an increasing amount of resistance to various antibiotics currently in use for shigellosis. We characterized the burden, serotype distribution, and antibitiotic susceptibility in Kibera, the largest informal settlement within Nairobi. Stool samples were collected from consenting patients presenting with diarrhea and or dysentery in a field clinic established by KEMRI/CDC in Kibera, Nairobi, which is the referral site for participants of an active population-based surveillance system. The specimen were inoculated in cary-blair media, stored in a cool box and transported to KEMRI/CDC laboratories in Kisumu within 24 hours of collection where culturing, species identification and drug sensitivity studies were done. Between 1st October 2006 and 31st Jan 2009, 775 stool samples were cultured. Of these, 181 (23.4%) grew shigella species; 71 (39.2%) were from bloody diarrhea. Forty-two of 280 stools (15%) from children under

five years were positive for Shigella while 138 (29%) of 482 stools from persons older than 5 years were positive for Shigella. The species were identified as follows: 107 (51.1%)*Shigella flexneri*, 31(17.1%) *Shigella dysenteriae*, 18 (9.9%)*Shigella sonnei* and 4 (2.2%)*Shigella boydii 21 (11.6%) were* not differentiated. Susceptibility was ≥ 99% to ciprofloxacin, gentamycin, nalidixic acid, and ceftriaxone, 85% to kanamycin, 50% to chloramphenical, 45% to amoxiclavulinic acid, 31% to ampicillin, 17% to tetracycline, and 6% to trimethoprim- sulfamethoxazole, sulfisoxazole and streptomycin. In conclusion, *Shigella* is the most common cause of severe diarrhea and dysentery in adults and children in an urban slum area in Nairobi, where it caused almost one-quarter of all episodes overall. *Shigella flexneri* caused over half of infections, followed by *Shigella dysenteriae*. Antibiotic resistance to quinolones and ceftriaxone was low, but to sulfa-based antibiotics was high.

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PREVALENCE OF I CLASS INTEGRON AMONG *SHIGELLA FLEXNERI* AND *S. SONNEI* IN UZBEKISTAN

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Shigella infection is significant public health problem in developing countries. There are 140 millions cases of shigella infection registered annually with about 1 million fatal cases; about 60% of fatal cases occur in children under 5 years old. Recent years some shigella isolates obtain antibiotic resistance leading to reduction of therapy efficacy. One of the mechanism spreading and obtaining resistance is horizontal inheritance of genetic factors as integrons. This study was undertaken to determine the for presence of I and II class integrons in 52 strains of *Shigella flexneri* and *S. sonnei* isolates in Uzbekistan from dysentery patients and reveal of correlation with antibiotics resistance pattern. We have studied 52 strains of S. flexneri and *S. sonnei*. Antibiotic sensitivity test performed by dilution method following NCCLS (CLSI) recommendations. The integron presence studied by PCR. Strains used in the study were isolated in 1992-2007, including 22 (42.3%) strains isolated in 1992-1997 and 30 (57.7%) strains isolated in 2002-2007; 31 (59.65%) strains were *S. flexneri* and 21 (40.35%) strains were *S. sonnei*. 29 (55.8%) strains resistant to ampicillin also showed resistance to chloramphenicol. Among *S. sonnei* only one strain (4.8%) was resistant to ampicillin and chloramphenicol; the rest strains were sensitive to these antibiotics. 90.3% of *S. flexneri* were resistant to these antibiotics. All strains resistant to ampicillin and chloramphenicol were I class integron positive ($p<0.001$). In conclusion, for decades, ampicillin and chloramphenicol were first choice medication in Uzbekistan against shigella infection. Nowadays 90% of clinical isolates of *S. flexneri* that is most prevalent agent of bacterial dysentery obtained resistance to these medications. The correlation of simultaneous resistance to ampicillin and chloramphenicol and presence of I class integron confirm the role of this genetic structures in antibiotics resistance creation.

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ENTEROAGGREGATIVE *ESCHERICHIA COLI* (EAEC) IN CHILDREN FROM FORTALEZA, CEARA, BRAZIL: PREVALENCE, VIRULENCE FACTOR CODING GENES AND INFLAMMATION

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Enteroaggregative *E. coli* (EAEC) is an increasingly recognized cause of diarrhea worldwide. Despite of this, significant questions persist in the understanding of its epidemiology and pathogenesis. This work determines the prevalence of EAEC, its virulence-related genes and intestinal inflammation in children with (cases) and without (controls) diarrhea living in a shantytown in Brazil. A total of 252 children, 83 cases and 169 controls, aging 2-36 months, was analyzed in this study. DNA was extracted directly from stools by QIAamp DNA Stool Mini Kit (Qiagen, Valencia, CA). Diagnosis of EAEC was done by PCR for *aaiC* (chromosomal) and *aatA* (plasmidial) genes. Primers for virulence genes included *aggR* (transcriptional regulator), *aap* (dispersin), *pic*, *pet*, *EAST-1* and *Sat* (enterotoxins). *Aap*, *pic* and *EAST-1* genes were amplified by a multiplex PCR Kit (Qiagen). Aliquots of stool samples were evaluated for fecal lactoferrin (FLF) by ELISA (IBD Scan Kit, Techlab, Blacksburg, VA). EAEC (*aaiC*$^+$ and/or *aatA*$^+$) was found in 41.0% (34/83) of cases and 35.5% (60/169) of controls. Among the positive samples for EAEC, no difference was observed in the presence of the virulence genes *aggR* (47.1% of cases and 33.3% of controls), *aap* (52.9% of cases and 63.3% of controls), *pic* (97.1% of cases and 95.0% of controls), *pet* (41.2% of cases and 36.7% of controls), *EAST-1* (97.1% of cases and 86.7% of controls) and *Sat* (67.6% of cases and 61.7% of controls). Approximately 80.0% of the children presented elevated levels of FLF, regardless of the presence of illness and EAEC. All children with diarrhea associated with EAEC presented high concentrations of FLF. In conclusion, **t**he high percentage of EAEC in cases and controls suggests the participation of host variables in the course of the disease. These data show the heterogeneity of EAEC strains regarding to virulence genes. The high levels of FLF suggest that there are additional factors (microbial and/or host) which may trigger enteric inflammatory response.

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MICROBIOLOGICAL AND CLINICAL CHARACTERISTICS OF *SHIGELLA SP.* AS A CAUSE OF DIARRHEA AMONG CHILDREN IN INDONESIA

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Shigella has been an endemic enteropathogen and it is one of the important cause of diarrhea among children in Indonesia. The objective of this study was to estimate the prevalence of *Shigella* sp. among pediatric diarrhea patients in Indonesia. Passive surveillance of diarrhea among pediatric patients visiting hospitals and health centers in six cities of Indonesia from September 2005 through April 2008. Stool samples/ rectal swabs were transported into Cary-Blair and Buffered Glycerol Solution containers. A total of 12.670 patients were enrolled. Overall, 301(2.4%) of patients were positive for *Shigella* sp. The most common *Shigella* sp. isolated was *S. flexneri* in 156 samples (51.8%), followed by *S. sonnei* (46.8%), *S. dysenteriae* (0.7%), and non-typeable *Shigella* sp (0.7%), respectively. The age group with the highest degree of positive isolation was among 7 - 24 months in both *S. flexneri and S. sonnei*. Clinically, the diarrheal presentation was mostly non-specific diarrhea with only 15.3% having blood in their stool and only 19% also having fever. Antimicrobial susceptibility testing identified a high degree of antibiotic resistance toward first-line antibiotics (42%), with only a minimal amount of resistance toward third generation cephalosporins or fluoroquinolones (%). In conclusion, these data will provide important information to the Diarrheal Disease Control Program regarding clinical presentation and treatment of shigellosis in Indonesia.

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MULTI-RESISTANT *SALMONELLA* STRAINS ISOLATED FROM PATIENTS IN KUMASI, GHANA

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This study investigated the level of antibiotic resistance of *Salmonella* strains isolated from patients suspected of suffering from salmonellosis in Kumasi. The antibiotics tested included, the first-line antibiotics, chloramphenicol, ampicillin, tetracycline, and cotrimoxazole. Newer generation antibiotics including ciprofloxacin, norfloxacin, gentamicin, amikacin, cefuroxime, cefotaxime, ceftriaxone, and ceftazidime were also tested. A total of sixty (60) *Salmonella* strains were isolated from blood, urine and stool samples in Kumasi. It was found that 55 (91.7%) of the *Salmonella* strains were *S. typhi* and 5 (8.3%) were *S. typhimurium*. The study found that 85% of the strains (51 out of 60) were resistant to one or more of the antibiotics tested, with 58.3% (35 out of 60) showing multi-drug resistance (resistance to three or more antibiotics). The study confirms the existence of resistant/multi-resistant strains of *Salmonella* in Kumasi.

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CHANGING PATTERNS OF SHIGELLOSIS IN AN ANTIMICROBIAL RESISTANCE SURVEILLANCE PROGRAM IN PERÚ, 2005-2008

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Shigellosis is a major cause of diarrhea worldwide. *Shigella sonnei* is the most frequently isolated species, responsible for sporadic and epidemic enteritis in developed countries. *S. flexneri* is the most commonly isolated bacterial enteropathogen in most of Latin America, whereas *S. sonnei* predominates only in Chile. As part of an antimicrobial resistance surveillance program, we have noted a recent increase in the prevalence of *S. sonnei* isolates in Perú. We describe changes in the frequency of *S. sonnei* in relation to *S. flexneri* between 2005 and 2008 and the impact of this change on antimicrobial drug resistance in shigellosis. 8,507 stool specimens from patients with diarrhea were obtained from medical facilities in Perú from 2005-2008. 3,270 isolates were identified as *Shigella* by standard procedures. Antimicrobial susceptibility testing was performed by Kirby-Bauer disk diffusion methods per CLSI guidelines. Of 3,270 *Shigella* isolates, 2,206 were S. flexneri and 1,064 *S. sonnei*. The proportion of *S. sonnei* was stable at 26.1-30.3% from 2005-2007, before increasing to 46.7% of *Shigella* isolates in 2008. The proportion of *S. sonnei* isolates was highest in the summer months of November through February, exceeding S. flexneri isolates during November and December 2008. Shigella isolates in general and *S. sonnei* in particular were more common as a proportion of overall isolates from the contiguous districts of El Agustino, La Victoria, San Juan de Lurigancho and Lima Cercado, all in Lima. Rates of isolation were highest in children 1-3 years of age. No other differences by age or sex were noted. 79.4% of S. flexneri isolates were resistant to ampicillin, 80% to cotrimoxazole, 0.3% to ciprofloxacin, and 0.2% to azithromycin. *S. sonnei* was more resistant, with 95.6% resistant to ampicillin, 94.9% to cotrimoxazole, 0.4% to ciprofloxacin, and 4.3% to azithromcyin. In conclusion, *S. sonnei* appears to be increasing in prevalence in this passive surveillance network in Perú. The reason for this is unclear, although the increased antimicrobial resistance of this species suggests that selective drug pressure may be involved. Fluoroquinolones remain active in shigellosis, but resistance to other drug classes in *S. sonnei* may impact the management of pediatric patients. Continued surveillance of antimicrobial resistance in *Shigella* is warranted.

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TRENDS IN ANTIMICROBIAL RESISTANCE AMONG BACTERIAL ENTEROPATHOGENS IN A MILITARY POPULATION IN THE PERUVIAN AMAZON, 2003-2009

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Infectious diarrhea remains a major threat to the health of civilian and military populations worldwide. Antimicrobial drugs may reduce the morbidity and mortality associated with bacterial diarrhea, but the widespread use of these agents has lead to increasing resistance among once-susceptible pathogens. Prospective surveillance of diarrheal disease has been conducted since 2003 at the Vargas Guerra Army Base, a Peruvian Army facility in the Amazon basin with a high incidence of acute diarrhea. We present here the trends in antimicrobial resistance from selected pathogens isolated at this facility. We reviewed positive stool cultures for bacterial enteropathogens obtained in the Vargas Guerra project since 2003. Stool specimens were cultured on MacConkey, Campylobacter CVA, and Hektoen agar. Enterotoxigenic E. coli (ETEC) was detected by PCR. Identification was confirmed through routine biochemical and serologic procedures, with antimicrobial susceptibility testing performed by disk diffusion testing per CLSI guidelines. Among Shigella species in 2003-2004, resistance to ampicillin (AMP) was noted in 60/72 (83%), azithromycin (AZ) in 2/72 (3%), ciprofloxacin (CIP) in 1/72 (1%), ceftriaxone (CTX) in 0/72 (0%), and cotrimoxazole (CMZ) in 46/72 (64%). In 2007-2009, these rates declined to 26/71 (37%) for AMP, with no isolates resistant to AZ, CIP, or CTX found. Rates of resistance to CMZ increased to 54/71 (76%). Decreased resistance was noted in Campylobacter species over time. In 2003-2004, resistance to AMP was noted in 8/20 (40%), AZ in 1/20 (5%), CIP in 8/20 (40%), CTX in 2/20 (10%), and CMZ in 20/20 (100%). In 2007-2009, resistance to AMP was noted in 1/25 (4%), AZ in 0/25 (0%), CIP in 7/25 (28%), CTX in 1/25 (4%), and CMZ in 25/25 (100%). Among ETEC, resistance to AMP was noted in 24/52 (46%), AZ in 2/52 (8%), CIP in 6/52 (12%), CTX in 2/52 (4%), and CMZ in 14/52 (27%) from 2003-2004. By 2007-2009, resistance was noted to AMP in 13/32 (41%), AZ in 0/32 (0%), CIP in 0/32 (0%), and CMZ in 12/32 (38%). In conclusion, contrary to trends elsewhere, drug susceptibility in enteropathogens appears to be improving in this population in the Peruvian Amazon. This decrease may be the result of differential pressure from selected antibiotics, as noted in the increasing rate of cotrimoxazole resistance. Continued surveillance of drug resistance is warranted to optimize treatment of these common infections.

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ASSOCIATION OF SINGLE-NUCLEOTIDE POLYMORPHISMS (SNPS) IN THE *AGGR* GENE OF ENTEROAGGREGATIVE *ESCHERICHIA COLI* (EAEC) WITH ALTERATIONS IN THE CODIFIED AMINO ACIDS

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Enteroaggregative *E. coli* (EAEC) is the most recently recognized subgroup of *E. coli* to be associated with human diarrheal diseases. The *aggR* gene is a transcriptional regulator that controls the expression of chromosomal and plasmidial genes involved on the pathogenesis of EAEC infection.

The aim of this study was to investigate the relation of the occurrence of single-nucleotide polymorphisms (SNPs) in the *aggR* gene of EAEC with alterations in the resultant amino acids. Stool samples were taken from 7 children with diarrhea and 7 control children from a case-control study approved by a local IRB and conducted in Brazilian poor communities. Genotyping assays for *aggR* gene loci were performed using DNA extracted directly from stools (QIAamp DNA Stool Mini Kit, Qiagen, Valencia, CA). Samples were diagnosed for EAEC by PCR for the *aaiC* (chromosomal) and *aatA* (plasmidial) genes. Further PCR was performed to detect the presence of the *aggR* gene. Amplicons were purified by QIAquick Purification Kit (Qiagen) and analyzed by ABI Prism 3100 (Applied Biosystems, Foster, CA). From 798 nucleotides that constitute the *aggR* mRNA strand, 580 (72.7%) were sequenced. The analysis showed that 71.4% (5/7) of cases and 42.9% (3/7) of controls presented at least one SNP when compared to the sequence available in GenBank (National Center for Biotechnology Information - NCBI - National Institutes of Health, Bethesda, MD; accession number Z32523). These polymorphisms included homozygote and heterozygote genotypes. In total, 27 SNPs were observed, but only 6 of them yielded changes in the resultant amino acid and its biochemical characteristics. In addition, 16 SNPs did not cause changes in the resultant amino acids. Five of the 27 SNPs resulted in different amino acids but did not elicit a change in the biochemical features of the native amino acids. In conclusion, these results show the occurrence of SNPs in the *aggR* gene of EAEC. Further studies are necessary to determine the influence of these polymorphisms in the codified protein and, consequently, in the course of EAEC infection.

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PREVALENCE AND VIRULENCE GENES ASSOCIATED WITH *CAMPYLOBACTER* SP. INFECTION: A CASE-CONTROL STUDY ON CHILDREN FROM NORTHEASTERN BRAZIL

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Campylobacter jejuni and *C. coli* are important enteric pathogens resulting in diarrheal disease with significant morbidity worldwide. The main goal was to investigate the prevalence of *C. jejuni* and *C. coli* in children, aged 2-36 months, from urban Fortaleza, Ceara, Brazil, in an observational epidemiological case-control study. In addition, we evaluated the *Campylobacter* sp. infection and intestinal inflammation in these children. Children were enrolled after parental consent, according to the rules of the Federal University of Ceara's ethical committee. A total of 252 children, 83 diarrhea cases, and 169 controls without diarrhea, were analyzed in this study. Detection of *Campylobacter* sp. from frozen stool samples was performed by ELISA and PCR. We investigated the presence of *C. jejuni's* cytolethal distending toxin genes *cdtA*, *B* and *C* using a multiplex PCR. Intestinal inflammation was assessed by quantitative ELISA detection of fecal lactoferrin (FLF). *Campylobacter* sp. was detected in 23% (19/83) of cases and 26% (43/164) of controls using the ELISA *Prospect® Campylobacter*. *C. jejuni* (*hipO* gene) was found in 10% of cases (8/83) and 6% of controls (10/169). *C. coli* (*ask* gene) was detected in 6% of cases (5/83) and 1% of controls (1/169). The *cdtA*, *B* and *C* genes were detected in 72% (13/18) of samples *hipO* positive. There were no significant differences between cases and controls regarding to detection of *Campylobacter* sp. ($p>0.05$). Approximately 80% of these children presented high concentrations of FLF despite the detection of *Campylobacter* sp. or presence of diarrhea. In conclusion, the results suggest that ELISA is a potential test to use as a screening method to diagnosis *Campylobacter* spp., and specific PCR methods are necessary for diagnosis of *C. jejuni* and *C. coli*. There were high and equal prevalence of campylobacteriosis in both case and control groups, suggesting additional microbial and/or host factors associated with the disease process.

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APOLIPOPROTEIN E4 STATUS INFLUENCES GROWTH AND COGNITIVE RESPONSES TO MICRONUTRIENT SUPPLEMENTATION IN CHILDREN FROM NORTHEAST BRAZIL

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Apolipoprotein E4 (ApoE4) may obey antagonistic pleiotropy. If so, ApoE4 carriage would benefit early periods of life when the body is challenged by infection and nutritional decline, yet still be potentially detrimental later in life in the form of Alzheimer's disease. Two hundred thirteen Brazilian favela children with below median height-for-age z-scores (HAZ) were randomized to receive 200,000 IU of retinol (every 4 months), 40mg of zinc (twice weekly), neither or both for 1 year, with half of each group receiving 16g of glutamine for 10 days. HAZ, weight-for-age z-scores (WAZ), weight-for-height z-scores (WHZ) and lactulose:mannitol ratios (L/M) were assessed during the initial 4 months of supplementation for each child. An average of 4 years (range 1.4-6.6) later, the children had cognitive testing in non-verbal intelligence (TONI), coding tasks (WISC-III), verbal fluency (NEPSY), verbal learning (WRAML), and delayed WRAML. ApoE4 allele status was determined by PCR-RFLP analysis using the enzyme HhaI for 144 children (67.6%), yielding positive carriage in 37 children (25.7% of analyzed population) and an allele frequency of 13.9%. Among children receiving glutamine supplementation, ApoE4 (+) children showed significant positive Pearson correlations between the change in HAZ over 4 months and delayed WRAML ($r = 0.477$, $p = 0.029$), the change in WAZ over 4 months and TONI intelligent quotient scores ($r = 0.470$, $p = 0.032$), and the change in WHZ over 4 months and TONI intelligent quotient scores ($r = 0.502$, $p= 0.020$). ApoE4 (-) children, regardless of intervention, showed negative Pearson correlations for the change in L/M over 4 months with WRAML or TONI. During critical periods of childhood development, ApoE4 may work in concert with more gut-tropic nutrients, such as glutamine, to benefit immediate nutritional status that can translate into better long-term cognitive outcomes. Individuals without ApoE4, however, may require nutritional supplementation to improve intestinal barrier function that augments future cognitive development.

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ZINC SUPPLEMENTATION EXPOSES ASSOCIATIONS BETWEEN THE INTERLEUKIN 8 (-251 A/T) POLYMORPHISM AND MARKERS FOR HIGHER INTESTINAL INFLAMMATION IN CHILDREN FROM NORTHEAST BRAZIL

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Lactoferrin, well known for its anti-microbial effects in the primary and secondary immune responses, also modulates chemokine levels during the inflammatory process. Yet the direct relationship between lactoferrin and IL-8 levels remains unclear. We hypothesized that the -251 T to A polymorphism in the IL-8 promoter region causing greater IL-8 expression would consequently influence fecal lactoferrin (FLF) levels that would be an indication of intestinal inflammation and the body's attempt to subdue an IL-8 immune response. Two hundred thirteen Brazilian favela children with below median height-for-age z-scores were randomized to receive 200,000 IU of retinol (every 4 months), 40mg of zinc (twice weekly), neither or both for 1 year, with half of each group receiving 16g of glutamine for 10 days. IL-8 promoter region genotypes were

determined by PCR-RFLP analysis of 113 children (53.1%) yielding a mutant A allele frequency of 42.4%. While no significant associations were found between FLF levels and IL-8 promoter region genotypes at baseline, after 1 month of micronutrient supplementation, allele A homozygosity had significant associations with positive FLF titers as opposed to allele T positive individuals (*OR* = 8.0, *CI* = 2.3-27.8), AT heterozygotes (*OR* = 8.6, *CI* = 2.2-33.2), and T homozygotes (*OR* = 7.2, *OR* = 1.7-31.0). Closer analysis revealed that those children receiving zinc showed significant associations for allele A homozygosity with positive FLF titers as opposed to allele T positive individuals (*OR* = 36.0, *CI* = 3.4-384.0), AT heterozygotes (*OR* = 66.0, *CI* = 3.5-1255.0), and T homozygotes (*OR* = 26.0, *CI* = 2.2-304.9). The AA IL-8 promoter region genotype, and a resultant increase in intestinal IL-8 levels, may contribute to a nonspecific inflammatory state in the intestinal tract that is exposed by zinc supplementation.

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CRYPTOSPORIDIUM SPP. AND ENTEROAGGREGATIVE *ESCHERICHIA COLI* INFECTION AND LACTOFERRIN LEVELS IN CHILDHOOD DIARRHEA IN ACCRA, GHANA

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Enteric infections have been associated with elevated fecal lactoferrin levels, especially in malnourished children. Lactoferrin, a surrogate marker for neutrophils, is helpful in assessing intestinal inflammation. We studied *Cryptosporidium* spp. and Enteroaggregative *Escherichia coli* (EAEC) and quantified lactoferrin levels in these infections. Children with (n=172) and without (n=102) diarrhea consulting at a secondary hospital in Accra, Ghana between August 2007 and May 2008 were enrolled. Fecal DNAs were screened for *Cryptosporidium* and *Giardia* species and EAEC genes (aaP, aatA, aaiC and aggR). A stool specimen was considered positive for EAEC when at least three of the genes tested were detected. Lactoferrin was quantified by the IBD-SCAN. Cryptosporidium was detected in 14/172 (8.06%) of children with and 1/102 (0.98%) without diarrhea (p= 0.012). EAEC was detected in 85/172 (49.4%) of children with and 33/102 (32.4%) without diarrhea (p=0.004). *Giardia* was not detected in either study group. *Cryptosporidium* and EAEC co-infection was noted in 7/172 (4.1%) of those with diarrhea and none in the control group (p=0.039). Children ≤ 6 months with diarrhea have significantly higher fecal lactoferrin (n=24; 2576.9 ± 561.0 µg/ml) compared to children ≤6 months without diarrhea (n=29; 824.7 ± 303.0 µg/ml) (p=0.009). Children ≤ 6 months with either *Cryptosporidium* or EAEC have a 3.4-fold higher fecal lactoferrin than children ≤ 6 months without these pathogens (p=0.006). In conclusion, we found associations of *Cryptosporidium*, EAEC and fecal lactoferrin with diarrhea, although lactoferrin levels were not significantly higher with malnutrition. Further work is needed to clarify the role of enteric infection and inflammation in causing malnutrition.

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GEOGRAPHIC CHARACTERISTICS OF CHILDREN WITH MODERATE-TO-SEVERE DIARRHEA IN RURAL WESTERN KENYA, 2008

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Diarrhea is a leading cause of morbidity and mortality for children in the developing world, including sub-Saharan Africa. The Global Enteric Multicenter Study (GEMS) is a case-control study of children 0-59 months of age with acute moderate-to-severe diarrhea, attending one of 6 sentinel health centers within the GEMS Kenya site, an area of 500 km^2 area in rural western Kenya. We analyzed geographic characteristics of case and control children enrolled over a 1-year period at the GEMS Kenya site. A case was defined as 3 or more abnormally loose or watery stools accompanied by sunken eyes, loss of skin turgor, dysentery, hospitalization or intravenous rehydration. Age-matched controls without diarrhea in the previous 7 days were enrolled from the village closest to the case village. Stool samples from cases and controls were tested for a wide variety of enteric pathogens. From January 28, 2008 to January 25, 2009, 628 case-control pairs were enrolled; global positioning system (GPS) coordinates were available for the homes of 566 (90%). The median distance between the homes of cases and their matched controls was 0.9 km, with 16% living >2 km apart. The median distance to the nearest health facility participating in GEMS was similar (2.6 km) for cases and controls, with 19% living >4 km away. Among the 27 cases who died, the median distance to the nearest GEMS health center was 3.2 km, 2.6 km for their matched controls and 2.6 km for cases that survived (differences not statistically significant). Pathogens identified in stool specimens from cases included rotavirus (13%), *Cryptosporidium* (9%), enterotoxigenic *Escherichia coli* (15%), *Shigella flexneri*(5%), *Campylobacter* (5%), nontyphoidal *Salmonella* (5%) and enteropathogenic *E. coli* (11%). Cases by pathogen and who died were widely geographically distributed with the mean center of each group centrally located in the overall study area (within 4.5 km). In conclusion, cases of moderate-to-severe diarrhea occurred throughout the study area despite the presence of nearby health facilities.

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DENGUE VIRUS NONSTRUCTURAL PROTEINS INDUCE IL-8 TRANSCRIPTION: ROLE OF VIRAL PROTEINS IN DENGUE IMMUNOPATHOGENESIS

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Dengue virus (DENV) continues to spread worldwide and the incidence of dengue hemorrhagic fever (DHF) is on the rise. The immunopathology associated with DHF involves an over-production of chemokines and cytokines. DENV contains a plus-sense RNA genome encoding for three structural proteins (C, prM, and E) and seven nonstructural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5). Published data showed that NS5 induced transcription and protein secretion of interleukin-8 (IL-8), a DHF-associated chemokine, arguing that NS5 is a key viral protein involved in pathogenesis. We hypothesized that NS5 and possibly other NS proteins can induce transcription of IL-8 similar to that induced by DENV New Guinea C (NGC) strain. To test this hypothesis, we cloned the DENV NS genes of the NGC virus into the expression pcDNA3.1/V5-His TOPO® vector and either infected or transfected HEK293 cells with the NGC virus or the NS plasmids, respectively. Viable virus release was measured by plaque assay and viral copy number and IL-8 transcription

were measured using quantitative real-time PCR (qRT-PCR). Viral protein expression was measured using immunofluorescence staining and Western blot. Our data demonstrated a time-dependent increase (days 1-5) of IL-8 expression in DENV-infected HEK293 cells, correlating with viral plaque forming units and copy numbers. Two days after transfection, 30% of HEK293 cells expressing NS5 fusion protein showed a 2.6-fold induction of IL-8 transcription; whereas 22% of cells expressing NS4B fusion protein showed over 3.0-fold induction of IL-8 transcription. Moreover, 20% of cells expressing both NS4AB and NS2B(3)protease showed over 2.5-fold induction of IL-8. In conclusion, the induction of IL-8 might be due to the synergistic expression of DENV NS proteins and these NS proteins may play an important role during the immunopathology of DHF. Further studies are warranted to delineate the role of individual and various combinations of the NS proteins and their ability to induce DHF-associated chemokines and cytokines.

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AN ENTOMOLOGICAL STUDY ON RECEPTIVITY OF DENGUE VECTORS OF SELECTED PUBLIC HOSPITALS ADMITTING DENGUE PATIENTS IN METRO MANILA, PHILIPPINES

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Entomological investigations were carried out in 5 selected public hospitals admitting dengue patients in Metro Manila, Philippines. Results revealed the receptivity and presence of productive sites of *Aedes aegypti* mosquito,vector of Dengue / Dengue Hemorrhagic fever in these hospitals. The breeding sites of *Aedes aegypti* mosquito was mainly found in water plant vases, drum, basin, plastic cups, tin can, and empty paint cans. The water plant vase / bowl was the key container for *Aedes* breeding. The percentage positive rate of fresh water plant vase of the 5 public hospitals were RMC (40.69%), TMC (76.62%), SLH (80.60%), NCH (64.06%), and EAMC (40.40%). An analysis of data revealed that the Premise, Container, and Breteau indices varied from 0.0 to 4.0, 0.0 to 1.1 and 0.0 to 4.0 respectively, indicating thereby the low receptivity of the area to DF/ DHF transmission.. The egg density ranges from 0.0 to 48.5 showed the presence of *Aedes aegypti* vector in the 5 public hospitals. The presence of productive breeding sites indoor and outdoor in the five hospitals revealed that possible outbreak can occur in the future if no vector control plan is adapted and implemented. As a results of the study, vector prevention and control was submitted for implementation in these hospitals. Futures studies on mosquito virus detection using PCR and ELISA is recommended to confirm presence of dengue virus in these hospitals.

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NATURAL ATTENUATION IN A SOUTH PACIFIC OUTBREAK OF DENGUE TYPE-2

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Dengue is an arboviral disease that accounts for 50-100 million infections annually worldwide. The dengue virus, an 11 kb member of the Flaviviridae, has four serotypes (DENV-1 to -4) and is responsible for a spectrum of disease symptoms ranging from febrile illness to dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). Recent decades have seen an increase in dengue activity throughout the tropics, marked by more frequent and severe epidemics. While the causes of this reemergence are probably multifold, including both vector and virus expansions, the importance of virus strains with greater fitness, epidemic potential and possibly virulence, has been implicated and prompted us to investigate the role of virus molecular evolution in driving epidemics. Our study was a series of outbreaks of DENV-2 in the South Pacific beginning in 1971 in Tahiti and Fiji, which became increasingly severe in New Caledonia and Niue Island in 1972. In Tonga in 1974, however, it became dramatically attenuated, with near-silent transmission. To elucidate the relative contribution of viral genetic change in outbreak dynamics, we conducted whole-genome phylogenetic analysis of DENV-2 strains collected during the South Pacific sweep, taking advantage of the well-documented epidemiologic and clinical data; all islands were equally immunologically naive for dengue. Thus, this study offers an opportunity to isolate the effects of viral genetic variation from seropositivity rates on epidemic behavior. We studied 17 low-passage DENV-2 strains isolated during outbreaks on the islands of Fiji, Tahiti, New Caledonia, and Tonga. Each isolate was subjected to whole genome sequencing and imported into PAUP for phylogenetic analysis. We found variations in the coding portion of the dengue genome, particularly the premembrane gene (prM) and the nonstructural genes, NS2A and NS4A that correlate with the attenuation of the Tongan strains of virus. In conclusion, our analysis indicates a significant role for genotypic change in dengue epidemic severity.

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DENGUE IN DIFFERENT AGE GROUPS

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Dengue is the most common mosquito-borne virus causing disease in many countries worldwide. The diseases vary from asymptomatic infection, undifferentiated fever, dengue fever (DF), dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). The clinical manifestations and laboratory results may vary in different age groups. To study and understand the variety of these will help clinicians to achieve early diagnosis, proper management and reduce mortality. Clinical data in children with dengue infection, confirmed by serologically and/or virologically, admitted to King Chulalongkorn Memorial Hospital during 1987-2007 were analyzed. The patients were classified into 3 different age groups: 0-1 year, 2-12 years and 13-15 years. For all three age groups, fever, hepatomegaly and bleeding were the most common clinical manifestations of dengue infection. The respiratory tract and neurological symptoms were prominent in the 0-1 year age group, however, less common in older children. DHF was common in every age groups, but DSS was more common in 2-12 years age group. In conclusion, clinical manifestations and severity of dengue infection varied with age. Central nervous system manifestations were common in infants while DSS was less prominent. The study emphasizes that there is significant variance of clinical manifestations in different age groups, suggesting that proper treatment must take into account the different age-specific clinical manifestations.

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A POTENTIAL ROLE OF PLATELETS IN MATURATION OF DENGUE VIRUS

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Dengue virus infection is the most important mosquito borne human disease in terms of morbidity and mortality in urban tropical area. A wide spectrum of clinical manifestations in infected humans ranges from asymptomatic condition or mild dengue fever to severe and life-threatening dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). At present, *in vivo* underlying mechanisms of the early stage of dengue virus infection and development of DHF/DSS are not clearly understood. Thrombocytopenia (low platelet count) is one of the clinical hallmarks found in the infected patients and possibly caused by platelet dysfunction, increased destruction in association with immune complex-

mediated cell lysis, and/or decreased production from megakaryocytes. Detection of dengue viral antigens and viral RNA in the platelets has been reported previously; however, the association of platelets and dengue virus remains unclear. Our study aimed at investigating the role of platelets in the early event of dengue virus infection in both *in vitro* and *in vivo* models. Platelets from healthy volunteers were experimentally infected with dengue virus at an MOI of 0.03 and assessed for the presence of infectious virus. Real time RT-PCR showed that dengue viral RNA could be detected in both platelets and culture supernatants and the levels appeared to rise within 18 hr post infection and decline dramatically at the later time points in the period studied (48 hr). The decreased viral RNA copies were consistent with diminished levels of total RNA in the platelets and with reduced surface expression of platelet specific markers, CD41 and CD61, thereby suggesting a degradation of platelets in the cultures within a short period. The presence of isolated or a cluster of dengue viral-like particles was confirmed by electron microscopy (EM) in the *in vitro* infected platelets particularly within cellular vacuoles. Importantly, a sequential maturation of dengue viral-like particles was observed in the platelets from infected humans and non-human primates by EM. Finally, immuno-EM study revealed that the dengue viral-like particles were genuine dengue viral particles. Our findings therefore suggest that platelets may be a source of host cell providing the initial sites for replication and maturation of dengue virus during the early stage after virus inoculation.

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VALIDATION OF A MULTIPLEX MICROSPHERE-BASED IMMUNOASSAY FOR MEASUREMENT OF ANTI-DENGUE VIRUS IMMUNOGLOBULIN ANTIBODIES

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Dengue virus (DENV), a mosquito-borne flavivirus is a leading cause of hospitalization and death among children in south-east Asia. IgM antibody capture ELISA (MAC-ELISA) is primarily used as a test for DENV diagnosis, followed by a confirmatory plaque-reduction neutralization test (PRNT) to measure the level of DENV neutralizing antibodies. The objective of this study was to develop a rapid DENV IgG detection test using microsphere-bead based immunoassay (MBIA). 268 serum samples were obtained for diagnosis of DENV from patients with a wide range of age and geographic distribution within the Hawaiian Islands, and the Pacific Island Nations of Yap, Palau, French Polynesian and American Samoa. For determination of the negative cutoff value, serum specimens were obtained from 23 healthy individuals, and both suspected DENV- positive and -negative serum samples were confirmed using 90% PRNT on all 268 serum samples and DENV IgG indirect ELISA on 117 samples for the four DENV serotypes. A standard curve consisting of a known concentration of human IgG run with a serial dilution of Protein A bound to different bead sets was used to interpolate the concentration of IgG in serum samples. Of the 268 samples from patients and healthy controls, 168, were DENV MBIA and PRNT positive. Data obtained using MBIA, PRNT and IgG ELISA was 100% congruent. A validation study was coordinated between the Division of Vector-Borne infectious Diseases of the Centers for Disease Control and Prevention (DVBID) to determine (a) the reproducibility of the test between different laboratories, (b) the cross reactivity to St. Louis encephalitis (SLE), Chikungunya (CHIK), West Nile Virus (WNV), and Zika Virus (ZIKA), all of which share many clinical manifestations and are serologically similar when tested. The new MBIA assay was sent as a kit which contained everything necessary to complete the assay. Serum samples confirmed to be positive by PRNT and/or IgG ELISA for SLE, CHIK, WNV, ZIKA and DENV along with a negative panel were used by the CDC to run the MBIA assay. The kit was determined to be reproducible between two different laboratories. Some cross reaction was observed in SLE samples with minimum cross reactivity in CHIK, WNV and ZIKA samples. The newly developed DENV diagnostic assay will provide a rapid and sensitive alternative to ELISA for clinicians and public health workers, to prevent and control DENV infection and associated severe manifestations.

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ANALYSIS OF HUMORAL IMMUNOLOGIC RESPONSE IN MICE IMMUNIZED BY DNA VACCINE ENCODING PRM/E PROTEIN OF DENGUE VIRUS SEROTYPE 2 ADMINISTERED THROUGH GENE GUN AND INTRAMUSCULAR ROUTES

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Recent studies demonstrate that DNA vaccines containing the full-length prM and E (prM-E) genes of dengue-1 and dengue-2 viruses are immunogenic for mice, and that a dengue-1 prM-E DNA vaccine is also immunogenic and partially protective for rhesus and *Aotus* monkeys. In this study, we attempted to further evaluate how the immune response is influenced by different physical forms of DNA through different administration routes. Three groups of three 4-6 weeks BALB/c mice were immunized by DENV-2 prM-E DNA plasmids using intramuscular injection, gene gun-mediated delivery or both. Although the endpoint antibody titer is about the same among three groups, the mean anti-E antibody response at postimmunization days 36 and 51 in mice immunized by gene gun was higher than that in mice immunized by intramuscular inoculation of DNA or in mice immunized by both routes. Also, gene gun delivered plasmid immunization induced longer anti-E antibody responses for up to 119 days post-immunization than intramuscular delivery. Intramuscular DNA immunization induced T helper 1 (Th1) immune response, and the gene gun induced Th2 responses as demonstrated by IgG1/IgG2a ratio and the level of IL4 and IFN-γ. The functional immune response was evaluated by determining specific neutralizing antibodies and the results showed ninety percent neutralization titers ranging from 1:100 to >1:400 against the parental antigen, DENV-2 16681. We then tested whether the immune mouse serum also contain neutralizing capability against different DENV-2 circulating around the world. We used two local DENV-2 strain (KH1987 and PL046) representing genotype Asian II, strain 16681 representing genotype Asian I, and two local DENV-2 strain (20021222 and 8600759A) representing cosmopolitan genotype. Although the immune mouse serum can neutralize the Asian I and II strains very well, but it failed to neutralized the cosmopolitan strains. This is the first report of comparison of the immune response of different administration routes of delivering a DNA vaccine, which adds further support for the utility of dengue DNA vaccines and also strongly suggests using the contemporary virus for dengue vaccine design.

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STUDY OF THE REPLICATION OF DENGUE-2 VIRUS IN U937 CELLS UNDER THE ACTION OF CHLOROQUINE

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Dengue viruses represent the most important arboviral disease in humans. The only way to control dengue has been vector control since there is neither a vaccine that protects the individuals from this infection nor an antiviral for controlling virus replication. The objective of this study was to evaluate the action of the drug chloroquine under the dengue-2 virus replication. U937 cells were cultivated in a 24-well plate and the cellular suspension was infected with dengue-2 virus at a multiplicity of infection of 0.1. To analyse the inhibition of viral replication by this drug, suspension

of U937 cells were treated, concomitantly, 1 hour after viral infection, and at different intervals of the time (24-24; 12-12 hours) with chloroquine at concentration of 50 µg/mL. Supernatants of infected cells were collected at defined periods of 0, 24, 48, 72, 96, 120, 144 and 168 hours after viral infection, the total RNA was extracted from the supernatant, and viral replication was assessed by Real-time RT-PCR using primers specific to the 3' non-coding region of the dengue-2 virus genome and Sybr Green one-step RT-PCR master mix reagents Kits (Applied). When the number of viral RNA copies of DENV-2 was assayed on supernatants of U937-infected cells submitted to treatment with chloroquine at 12- and 24-hour intervals, a further and constant reduction in the viral yield was observed, when compared to untreated cells. The chloroquine induced an effective inhibition in DENV-2 virus replication when administered 24 hours after infection *in vitro*. In conclusion, our results suggest that the endosomal acidification is involved in the replicative cycle of DENV-2 in mammalians cells, and this observation could be used for considering chloroquine as an antiviral candidate to treat dengue virus infections.

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TRENDS IN PATTERNS OF DENGUE TRANSMISSION OVER FOUR YEARS OF A PEDIATRIC COHORT STUDY IN NICARAGUA

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Dengue is the most prevalent mosquito-borne viral disease in humans and a major urban public health problem worldwide. Dengue incidence and severity have increased in the Americas over the past three decades, yet few large-scale prospective studies have been carried out in the region. A community-based cohort study of ~3,800 children with an initial age range of 2-9 years was established in Managua, Nicaragua, in August 2004 to study the natural history of dengue transmission in an urban pediatric population. Healthy blood samples are collected each year prior to the dengue season, and capture of dengue cases occurs via enhanced passive surveillance at the study health center. The incidence of symptomatic dengue and the ratio of apparent to inapparent DENV infection varied substantially over the first four years of the study. Incidence of symptomatic infections was higher in Years 2 and 4 (1.8 and 1.7%) and lower in Years 1 and 3 (0.5 and 0.4%). A similar variation in the ratio of apparent to inapparent infections was observed, dropping from 1:3.4 and 1:2.2 in Years 2 and 4 to 1:9 and 1:11 in Years 1 and 3. These patterns match fluctuations in climate, as Years 1 and 3 received far less precipitation than Years 2 and 4. The dominant serotype switched from DENV-1 in Year 1 to DENV-2 in Years 2-4, accompanied by an increase in disease severity in Years 3 and 4 that is associated with a clade replacement event within DENV-2. Seroprevalence of anti-DENV antibodies increased across all age cohorts, but transmission slowed: 80% seroprevalence was observed in 6-year-old children in Year 1, compared to 47% seroprevalence among 6-year-olds in Year 4. This decrease in transmission parallels the increased dominance of DENV-2 and decrease in total infection incidence in Years 2 to 4, from 11.6 to 7.5%. The incidence of DENV infection in Year 3 was unexpectedly low (6.2%), which may be accounted for by the lowest precipitation in 50 years combined with a vigorous vector control campaign that year, resulting in the lowest indices of *Aedes aegypti* infestation in the four years of the study. Phylogeographic analysis of full-length DENV2 sequences revealed strong geographic clustering of dengue cases. This large-scale prospective cohort study of dengue in the Americas demonstrates year-to-year variation of dengue within a pediatric population, revealing expected patterns in transmission while highlighting the impact of interventions, climate, and viral evolution.

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NEUTRALIZING ANTIBODIES AGAINST DENGUE VIRUS TYPE-1 MAP TO DOMAIN III OF THE E PROTEIN AND PROTECT MICE FROM LETHAL CHALLENGE

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Dengue Fever is the most common arthropod-borne viral disease in humans and is caused by four distinct serotypes of dengue virus (DVEV). Infection by DENV causes a spectrum of clinical disease, ranging from an acute self-limiting febrile illness to dengue hemorrhagic fever/ dengue shock syndrome. Currently, there is no specific therapy or approved vaccine for human use. Previous studies with West Nile virus (WNV), a related flavivirus, demonstrated that monoclonal antibodies (MAbs) against the lateral ridge of domain III of the E protein strongly protect animals against infection. A humanized version of a DIII-specific neutralizing MAb against WNV is now entering human clinical trials. To develop analogous neutralizing MAbs against DENV, mice were infected with DENV serotype-1 (DENV-1) and subsequently boosted with recombinant domain III of DENV-1. 24 MAbs against DENV-1 were generated; 15 of these were domain III specific and 14 strongly neutralized DENV-1 by plaque reduction assay. Of these, 9 were type specific, 5 subcomplex-specific and 1 cross-reactive with all 4 serotypes of DENV. Although these neutralizing antibodies bound strongly to all 5 genotypes of DENV-1, some variation in neutralization of different genotypes was observed. To evaluate the protective capacity of these MAbs, a mouse infection model was developed with the different genotypes of DENV-1. AG129 (IFN- $\alpha\beta\gamma$R-/-) mice were highly susceptible to all DENV-1 genotypes with 100% mortality after intracranial route of infection. One strain (West Pac-74, genotype-4) caused lethal infection in AG129 mice by intraperitoneal route. To assess the *in vivo* efficacy of DENV-1 neutralizing MAbs, passive transfer experiments were performed. Among the 14 strongly neutralizing antibodies, 4 were completely protective whereas the majority of others showed partial protection. A detailed comparison of *in vivo* protection, neutralization potency in cell culture, and structural mapping is underway. These studies define the molecular profile of highly protective anti-DENV-1 antibodies, a first-step towards generating therapeutic antibodies or novel immunogens that elicit potently neutralizing antibodies against DENV in humans.

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INDEX CLUSTER STUDY OF DENGUE VIRUS INFECTION IN NICARAGUA

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Dengue, a mosquito-borne viral illness, is a major public health problem worldwide. Infection with one of the four serotypes of dengue virus (DENV) leads to subclinical infection, dengue fever, or the more severe,

life-threatening dengue hemorrhagic fever/dengue shock syndrome. Genetic, immunological, and virological indications of why some individuals remain asymptomatic while others progress to disease are unknown, largely because traditional study designs do not allow identification of acute asymptomatic cases. We conducted an index cluster study in Managua, Nicaragua, in which persons living within 50 m of an index case were evaluated for DENV infection. In the 2006 and 2007 dengue seasons, 18 dengue-positive and 4 dengue-negative index cases were identified through an ongoing pediatric cohort study. Paired day 1 and 14 samples were obtained from 442 contacts for serologic, virologic and molecular biological testing. The mean age of contacts was 30.3 (range 2-93). Post-enrollment (PE) and pre-enrollment DENV infections were confirmed in 12 (2.7%) and 19 (4.3%) contacts, respectively. Among PE infections, 10 (83%) were secondary and 7 (58%) were symptomatic. DENV-2 was identified by RT-PCR in 7 cases prior to symptom onset and in 2 of the 5 asymptomatic cases and was isolated from 6 contacts. Phylogenetic analysis was performed with full-length genomic sequence obtained from contacts, index cases, and dengue cases from the cohort study during the same time period. Incidence of PE infections was similar among contacts of negative (2.5%) and positive (2.8%) index cases, indicating a comparable rate of transmission in this urban setting regardless of proximity to a positive index case. The incidence of PE DENV infections in our study was similar to that reported in Indonesia but lower than that found in an index cluster study in Thailand. This is likely due to the age of the contacts (30 and 23 years old in Nicaragua and Indonesia, respectively, vs. 8 in Thailand); thus, many contacts in our study were likely DENV-immune. Case capture was limited by the exclusion of cohort participants (~30% of the 2-13 year-old population in the study area), among whom 9 symptomatic DENV infections were confirmed within 50 m of an index case during the cluster follow-up period. Nonetheless, this study demonstrates the feasibility of the study design for identification of acute asymptomatic and pre-symptomatic cases in an urban Latin American setting.

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EVALUATION OF AN IMPROVED ELISA FOR DETECTION OF DENGUE NS1

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Dengue is a flavivirus found largely in areas of the tropics and sub-tropics, with over half of the world's population living in regions at risk of dengue transmission. Traditional methods for diagnosis of dengue include detection of IgM and IgG antibodies to dengue. Detection of dengue Nonstructural Protein 1 (NS1) antigen by ELISA is a valuable procedure, as it allows detection of infection prior to seroconversion. Dengue NS1 can be detected in serum from day 1 after onset of fever and up to day 9. This compares to IgM antibodies that are not detectable until days 3-5. Earlier diagnosis of dengue allows earlier implementation of supportive therapy and monitoring. This reduces risk of complications such as dengue haemorrhagic fever (DHF) or dengue shock syndrome (DSS), especially in countries where dengue is endemic. The Panbio Dengue Early ELISA for the detection of dengue NS1 in sera was launched in 2006. Shortly after the release of the generation 1.0 product, development of a generation 2.0 product began, aimed at improving product performance. The redeveloped ELISA has been evaluated using characterised sera at three sites: in-house and at two reference laboratories in South-East Asia. The samples represent acute fever patients from days 1 to 7 post onset of illness, characterised virologically and serologically for dengue by a combination of PCR, viral culture, IgM and IgG ELISA. The positive samples are from patients with primary and secondary dengue resulting from infections by dengue virus serotypes 1, 2, 3 and 4. The samples were tested on the generation 1.0 and generation 2.0 Panbio Dengue Early ELISAs (E-DEN01P and E-DEN02P respectively) and another commercially available dengue NS1 ELISA. All sites reported significantly improved sensitivity (up to 15% increase) and similar specificity of the generation 2.0 ELISA and greater sensitivity than the other commercially available dengue NS1 ELISA. Our results show that the generation 2.0 Panbio Dengue Early ELISA is a useful tool in early diagnosis of dengue.

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THE MAKING OF A WORLD ATLAS OF INFECTIOUS DISEASES

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Many infectious diseases have a preference for certain world regions, depending on climate conditions, presence of vectors or specific reservoir species, local food habits, hygiene, antibiotic use, and other conditions. Currently there is no comprehensive illustrated overview of the geographic spread of infectious diseases and their underlying determinants. Such an overview of world infectious disease maps is crucial for teaching, research activities, and creating awareness. With experts from around the world we make and collect maps of over more than 100 infectious diseases and their preferred conditions. For this purpose we do extensive data searches to come to the best geospatial available data. Each map is accompanied by an explanatory text. All our maps are sent out for independent peer review to enable us to come to good quality maps. In the end the atlas will be published in book form by Wiley-Blackwell publishers and at a later stage, up-dated maps will be made available on-line under open-access agreement. These activities will evolve into a web-portal for global mapping activities, where all data are bundled together, visualized and made accessible to anyone interested and updated at regular intervals.

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ANEMIA OF INFLAMMATION IS RELATED TO COGNITIVE IMPAIRMENT AMONG CHILDREN IN LEYTE, THE PHILIPPINES

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Many studies have addressed the relationship between iron deficiency anemia (IDA) and cognitive impairment, but none have evaluated the role of non-iron deficiency anemia (NIDA). One of the main causes of NIDA in the developing world is anemia of inflammation, largely due to infectious diseases, whereby iron is shunted away from bio-available forms to storage forms, making it less accessible for use by host tissues. The objective of this study was to determine the effect of NIDA, due largely to anemia of inflammation in this context, on cognitive function after adjustment for potential confounders. Design: This cross-sectional study was conducted in Leyte, The Philippines among 322 children ages 7-18 years. Blood samples were collected and analyzed at the time of cognition testing. Three stool samples were collected and evaluated by the Kato Katz method for quantitative assessment for Schistosoma japonicum and geo-helminth infection. Socio-economic status (SES) was evaluated by survey. Linear regression models were used to quantify the adjusted relationship between performance in different cognitive domains and both IDA and NIDA. After adjusting for age, sex, SES and nutritional status, NIDA was associated with poor performance on the Philippines Non-Verbal Intelligence Test (PNIT) ($P = 0.04$) and the Wide Range Assessment of Memory and Learning (WRAML) verbal memory domain ($P < 0.05$). IDA was associated with poor performance on the PNIT after controlling for potential confounders ($P < 0.05$). In conclusion, NIDA, predominantly due to anemia of inflammation in this context, was related to lower performance on two tests of cognitive function. This is likely due to decreased delivery of iron to host tissues, including the CNS. This

work suggests that anemia of inflammation, caused by many infectious diseases in the developing world, may further limit children's ability to take advantage of limited educational opportunities, and can only be addressed by treatment of the underlying diseases.

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WORTH ITS WEIGHT IN GOLD- INTERVENOM- A GLOBALLY ACCESSIBLE INTERNET DATABASE TO DOCUMENT AND QUANTIFY THE GLOBAL BURDEN OF MORBIDITY AND MORTALITY FROM SNAKE ENVENOMATION AND TO IMPROVE MANAGEMENT AND OUTCOME

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Campaigning for snake envenomation reclassification as a neglected tropical disease is not without reason. Per annum worldwide research estimates up to 5.4 million snake bites, 2.5 million envenomings and 125,000 resulting deaths with high, undocumented morbidity rates among surviving victims. Research collection on snake bites remains inconsistent. With 169 countries being affected, 92 countries remain without any research data available due to geographical and resource challenges, hindering developments in the management of snake envenomation. Increased human-snake contact is causing a rising burden of disease and an increasing demand for antivenom. Spiralling costs alongside challenges in production, distribution and storage have caused the overall scarcity in supply of polyvalent antivenom, the gold standard in envenomation treatment and worth significantly more than its weight in gold. Intervenom, a globally accessible internet database, would allow data to be catalogued for individual envenomings including geographical information, species, photographs, anatomy affected, symptoms, results of investigations, traditional or alternative treatments tested and measurements in outcome including survival and disability scores. Furthermore data on the availability, type and quantity of antivenom could also be assessed. Simple online resources would aid in the identification of the correct species. Until now the potential role of telemedicine in snake envenomation has remained unexplored. An email and text based alert system could involve distant specialists to aid in acute management. Regular study and audits of the data collected would help to identify and optimise evidence based management methods. Reliable, uptodate antivenom shortage data would ensure a more appropriate distribution of antivenom and allocation of resources to areas in greatest need. New and better opportunities for international collaboration and networking would be created. The database would be trialled initially involving the principal international snake envenomation collaboration centres. Improvements could then be made before decentralisation phase providing universal access. Overall, Intervenom aims to collate evidence to support the reclassification of snake envenomation as a neglected tropical disease and to use the data collected to help identify new methods in tackling the burden caused by snake envenomation.

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GLOBAL HEALTH AS IT RELATES TO GASTROINTESTINAL ILLNESSES IN THE CARIBBEAN REGION

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According to a report done by the American Academy of Microbiology on resolving the global burden of Gastrointestinal Illnesses (GI), every single resident of any developed country is expected to become ill from an enteric infection at least once in the next 18 to 24 months, and yet gastrointestinal illness does not register heavily as a major public health problem. Infectious diarrhea is one of the leading causes of mortality and morbidity worldwide. Ironically it also persists as a leading cause of preventable death especially in children under the age of five in developing countries. Human caliciviruses (belonging to the family, Caliciviridae) have a worldwide distribution, with outbreaks and sporadic cases occurring in all age groups throughout the year. Two genera, Sapoviruses and Noroviruses infect humans and can be further divided into genogroups and genotypes. While Salmonella species is responsible for many food borne outbreaks, an etiologic agent for outbreaks is not usually identified. Recent studies suggest that Norovirus infections may account for a considerable proportion of these unidentified cases of acute gastroenteritis. This research aims at finding out if gastroenteritis is truly a public health problem and does it affect people and the environment. In 2007 Cricket World Cup (CWC) was held in nine Caribbean countries and represented an increased risk of illness and outbreaks as well as opened a potential platform for terrorist attacks. It raised the questions: - 'What were the chances of having a GI outbreak occur and if so could it be managed?' Fortunately no GI outbreaks occurred during this 47 day period but routine collection of samples thereafter showed otherwise. In fact both Norovirus genogroups I and II were identified through qualitative methods such as ELISAs and quantitative like real-time RT-PCR. Though it does not pose any immediate threat, it does pose a long term public health issue that may grow in magnitude if not monitored and addressed since the Caribbean acts as one of the major global tourist attractions.

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DETERMINANTS OF HEALTH SEEKING BEHAVIOR AMONG FAMILIES OF SICK YOUNG INFANTS IN A COMMUNITY SETTING IN KARACHI

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Young infants (infants in first 59 days of life) are at high risk of getting sick, and it can be life threatening, requiring tertiary care. Despite provision of transport and free care at a major pediatric hospital, 70-90% of the families in the slum areas of Karachi refuse care for their sick neonates. We conducted a longitudinal cohort study to determine the factors that influence the health seeking behavior of the population in these areas. The mothers/care-providers of 540 consecutive young infants, 0-59 days old, who were diagnosed with a serious illness at the above mentioned clinics between January 1, 2007 and December 31 2007, were interviewed at home, within one month of the onset of illness requiring referral. A pre-coded questionnaire was used to collect information on demographics, socioeconomic factors, and behavioral attitudes towards care-seeking for pregnant women and children. Multiple logistic regression analysis was used to predict the acceptance of referral according to behavioral, cultural, socioeconomic and clinical characteristics. Only 24.2% of the families accepted the referral to the major government-run pediatric hospital. The most influential factor affecting the family's was the child appearing very sick (OR = 17.3; 95% CI: 5.9, 52.2), followed by husband/father's concern for the health of his family (OR = 2.02; 95% CI: 0.90, 4.5), and the child being diagnosed with an illness other than sepsis and pneumonia (OR = 3.9, 95% CI: 1.6, 10.0). Gender of the child did not seem to affect a family's decision (OR = 1.00; 95% CI: 0.62, 1.6). In conclusion, the most influential factor influencing the referral decision seems to be clinical characteristics of the child, followed by behavioral factors, and socioeconomic characteristics of the family. Cultural factors seem to have the least affect on a family's decision to accept or refuse referral. These may provide possible avenues for interventions aimed at encouraging health-seeking behavior in a community similar to that of our study population.

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VALIDATION STUDY OF EDINBURGH POSTNATAL DEPRESSION SCALE IN MADAGASCAR

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Perinatal mood disorders are prevalent in 10-15% of females in the Western nations, and causes detrimental effects. Early screening can decrease the perinatal risks and adverse outcomes.The Edinburgh Postnatal Depression Scale (EPDS) is a sensitive screening instrument used to detect depression in postpartum women. It has been translated and validated in seventeen languages.The PHQ-9 derived from the Patient Health Questionnaire is based on DSM-IV items and used to screen for depression. There is no valid screening tool for detecting perinatal mood disorders. This study had two objectives: 1) to test the feasibility of using these scales translated into Malagasy on a sample of pregnant or postpartum women in Madagascar; and 2) to compare the scores of women on each of the questionnaires to determine how interchangeable they are. The questionnaires were translated into Malagasy and then back into English to verify the preservation of the meaning. The Malagasy versions of the EPDS and PHQ-9 were both administered to 25 subjects in two hospital settings in Madagascar.General demographics were obtained. The total scores of the questionnaires were compared by the Pearson correlation coefficient. A Kappa Statistic and a Test for Symmetry were used to test the degree to which the responses to seven pairs of similar questions from the surveys agreed. The Pearson correlation coefficient between the EPDS and PHQ-9 total scores was 0.448, explaining 20% of the variance between the scores. The Wilcoxon Signed Rank Test showed a significant difference in the responses between surveys. Five of the ten EPDS questions (#1,3,8,9,10) measure domains that are similar to those queried by questions #1,6,2,2, and 9 from the PHQ-9.PHQ question 9 and EPDS question 10 agreed with a Kappa statistic of 0.64. Despite the small sample size, the results showed a moderate positive correlation between the EPDS and PHQ-9.The differences represent the different domains measured. The PHQ-9 focuses on depression as defined by DSM-IV. In a non-industrialized international setting there were many barriers present. It was hard to conduct a study with little prior knowledge on customs, educational practices,and established medical practice. Communication barriers were present also.Blank items threatened scoring and data analysis. Time and recruiting difficulties made it hard to provide significant validation of EPDS and PHQ-9.

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INTEGRATING A CLINICAL TRIAL AND A PUBLIC HEALTH INITIATIVE: OPPORTUNITIES AND CHALLENGES

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As the numbers of clinical trials in developing countries increase, there are increased opportunities to merge public health studies with clinical trials. Such a hybrid model can save time, consolidate costs, and build relationships across the healthcare industry. For public health studies, working with a clinical trial can provide structure, visibility with doctors and government officials, and access to at-risk patient populations. For clinical research, the integration can bring credibility with partners and PDPs, insights for future program or product rollouts, and political capitol with stakeholders. Given that the objectives and approaches of public health studies and clinical trials differ, numerous challenges can emerge in the hybrid model. There were three difference in the study design related to: 1) flexibility (public health studies are adaptive, while clinical trials have a fixed protocol and the sponsor has no access to data during the trial); 2) focus (public health studies seek to develop broad programs while clinical trials focus on testing specific questions); and 3) sustainability (public health projects strive for long-term sustainability on supply chain and patient support structures while clinical researchers focus on proper trial conduct and scientific results). There are also two conflicting regulatory considerations: 1) Role of the sponsor (in public health studies, the sponsor has latitude to manage implementation and evaluation directly. In clinical trials, the role of the sponsor is strictly regulated); and 2) Patient interactions (public health studies rely on feedback from patients, *i.e.* exit interviews. In clinical trials, there are limitations on a sponsor's interactions with patients). And, finally, there are two Diverging goals for infrastructure and capacity-building: 1) training objectives. (In public health studies, training tends to be comprehensive, while clinical trial training focuses on the clinical protocol and GCP); and 2) site conditions (public health studies seek realistic site conditions. Clinical trial sites are infused with short-term staff and resources to meet strict GCP protocol standards). In conclusion, creative solutions, training innovations, and open dialogue are essential to overcoming these challenges.

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FACTORS INFLUENCING COMMUNITY DYNAMICS IN INTEGRATED NEGLECTED TROPICAL DISEASE INTERVENTIONS IN NIGERIA

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The Carter Center, with the Nigerian Ministry of Health, has integrated interventions for six neglected tropical diseases endemic to Nigeria. The primary mechanism for delivery integrated packages is the community health worker (CHW) who, as a result of integration, is distributing more drugs and products than before. CHWs and community leaders are key actors in the integration strategy yet little is known of their perceptions and acceptance of these new responsibilities. The objective of this study is to provide insight into the mechanisms associated with the implementation of integrated NTD interventions at the community level by analyzing how community cooperation and appreciation for integrated NTD interventions influence the CHWs' relationship with the community. The researchers developed qualitative in-depth interview (IDI) guides and interviewed 55 CHWs and 16 community leaders in 16 communities in 15 local government areas in Plateau and Nasarawa States, Nigeria. IDI data were analyzed using a grounded theory approach to understand the interactions of communities and CHWs and the integrated implementation package. Perceptions of drugs were highly influenced by outcomes associated with distribution. In areas were adverse reactions occurred (63% of communities surveyed), public opinion of the intervention was reported to have diminished which made decreased cooperation, while the remaining 37% of villages reported no adverse effects. Community cooperation with CHW drug distribution is also influenced by the level of perceived need for the drugs and by community sensitization towards the drugs. Distribution barriers include distribution occurring during farming season, shortages of drugs and materials, high CHW to population ratios and lack of incentives. Existing distribution barriers, including those listed above, are exacerbated by integration and unless these barriers are diminished the sustainability of integrated interventions at the community level is questionable. However, many CHWs give sacrificially to integrated program activities, recognizing that personal hardship is worth the health of the community; they will continue with CHW activities in the face of distribution barriers. The support of the community leader is of utmost importance, as he is able to sway his community towards accepting the drugs and providing incentives, which are necessary for the sustainable interventions.

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SPATIAL ANALYSIS AND DISEASE MAPPING: TOOLS FOR PLANNING AND EVALUATING INTEGRATED NEGLECTED TROPICAL DISEASE CONTROL IN SUB-SAHARAN AFRICA

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We aimed to determine spatial patterns of co-endemicity of the neglected tropical diseases (NTD) schistosomiasis and soil-transmitted helminths (STH) in large contiguous areas of East and West Africa where recent integrated NTD programmes have been initiated. In East Africa, parasitological surveys for schistosomiasis and STH were conducted in Uganda, Tanzania, Kenya and Burundi in 28,213 children in 404 schools, and in West Africa in Burkina Faso, Ghana, Mali and Niger in 32,280 children in 496 schools. Bayesian geostatistical models were used to interpolate prevalence of each infection across the study areas and interpolated prevalence maps were overlaid to determine co-endemicity. Additionally, *Schistosoma mansoni*-hookworm co-infection status in East Africa and *S. haematobium*-hookworm co-infection status in West Africa was established for each individual and prevalence of co-infection was interpolated. In East Africa, prevalence was 18.1% for *S. mansoni*, 50.0% for hookworm, 6.8% for *Ascaris lumbricoides* and 6.8% for *Trichuris trichiura*. *S. mansoni*-hookworm co-infections occurred in 10.1% of individuals. Hookworm was ubiquitous, whereas *S. mansoni*, *A. lumbricoides* and *T. trichiura* were highly focal. In West Africa, prevalence was 25.5% for *S. haematobium*, 3.1% for *S. mansoni*, 4.3% for hookworm, 0.6% for *A. lumbricoides* and 0.3% for *T. trichiura*. Prevalence of *S. haematobium*-hookworm co-infection was 0.9%. *S. haematobium* foci were distributed throughout the four countries but hookworm and *S. mansoni* were highly restricted to limited areas. Integration of schistosomiasis and STH control, and control of other NTDs, is only indicated in limited areas in East and West Africa. Due to the ubiquity of hookworm in East Africa, benzimidazole anthelminthics are required everywhere but praziquantel for schistosomiasis control is only required in foci adjacent large water bodies. In West Africa the situation is converse with praziquantel required in widely dispersed urinary schistosomiasis foci but benzimidazoles only required in limited hookworm-endemic areas.

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PARTNERSHIP WITH A LOCAL REFUGEE COMMUNITY TO DEVELOP STANDARDIZED CLINICAL SCENARIOS: AN EFFECTIVE TOOL FOR ASSESSING CULTURAL COMPETENCY IN THE HEALTH CARE SETTING?

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Cultural competency is recognized by the ACGME as a "core competency" for health care professionals. Standard criteria for objective assessment of cultural competency curricula have not been developed. To examine whether an Objective Standardized Clinical Examination (OSCE) could be useful in evaluating the ability of medical students to interact with patients from a different cultural background, we developed an OSCE case in partnership with a local refugee resettlement organization. The standardized patients in the encounter were members of the Sudanese community and the standardized clinical case was modeled after their experiences in the local health care system. The OSCE was included as part of the clinical skills examination (CSE) required of all fourth year medical students at SUNY Upstate Medical University. Additionally, the project aimed to quantify medical students' experiences in a global health setting by asking students to complete a validated survey. Students' performance on our OSCE was parametric, although skewed toward the lower end of the performance scale. There was a weak positive correlation ($r=.12$, $p<0.05$) between students' performance on the cultural competency encounter and the CSE. 97% of students completed the post-encounter survey. Overall, 36% (55/151) of students reported participating in a global health experience either before or during medical school. 42% report an interest in having "global health" be a significant portion of their career. The scores of students on the cultural competency case were higher for the students with global health experience, trending towards significance. In conclusion, these data demonstrates that an OSCE provides new information regarding a student's ability to interact with patients from another culture. Furthermore, the ability to interact with patients from different background is important, as a plurality of fourth year students plan to incorporate "global" health into their careers.

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THE PREVALENCE AND IMPACT OF THE CO-MORBIDITY OF SCABIES AND OTHER NEGLECTED TROPICAL DISEASES ON A COHORT OF CHILDREN IN THE SOUTH PACIFIC

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Many people living in tropical settings in the developing world are burdened with neglected infectious diseases which remain unaddressed by the health sector. This study was the first comprehensive survey to investigate the prevalence and impact of common health issues such as skin infections and parasitic diseases among children in the Pacific Island nation of Tuvalu. Baseline and follow-up surveys using consecutive convenience sampling were conducted on a cohort of 900 children of Tuvalu to determine the prevalence and scope of skin infections and intestinal parasites. Selected health indicators such as height, weight and peripheral blood haemoglobin were also used to assess the overall health of the children. Results indicated a high prevalence of infectious disease. Intestinal parasites were present in 67% of participants, over 60% had at least one skin infection with pyoderma and scabies (40%) being the most common presentations, with a high proportion having co-infection. Clinically, one third of all participants were anaemic and one third had proteinuria or heamturia. In conlusion, the high proportion of multiple infectious diseases found in the children, along with the clinical evidence suggests both acute and possible long-term negative health implications for this community. Clearly, this and similar communities could benefit from addressing these existing conditions to reduce their prevalence and impact on the population. The current epidemiological pattern of co-infection with multiple pathogens will require a strategic intervention which addresses the complexity of co-morbidity in an already resource- constrained setting. Therefore, the opportunity exists to develop an integrated community-based disease control programme employing multiple interventions in order to effectively and efficiently reduce the burden of disease in this population; rather than the standard single-disease, single-programme model currently in use in most of the developing world.

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JOSEPH JAMES KINYOUN: FOUNDING FATHER OF AMERICAN MICROBIOLOGY

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Little remembered today, in the late 19th century Joseph James Kinyoun exerted a lasting impact on American microbiology, public health, and tropical medicine. Upon establishment of the germ theory in the 1870s, Kinyoun, the son of the Assistant Surgeon General of the Confederate Army, sought training under both Koch and Pasteur, joined the United States Marine Hospital Service (now the United States Public Health Service), and personally drafted federal legislation to establish, in 1887, the United States Hygienic Laboratory, the forerunner of the National Institutes of Health. At age 28 Kinyoun became the laboratory's founding director and within a year made the first isolation of the cholera vibrio in

the United States. In addition to producing smallpox and rabies vaccines, and diphtheria antitoxin, Kinyoun was apparently the first scientist anywhere to treat smallpox with immune serum. By the 1890s, Kinyoun was a minor national celebrity, but a swift downfall ensued in 1900 when plague struck San Francisco. Kinyoun was sent by Surgeon General Walter Wyman to run the outbreak investigation and control efforts, which were resisted by California's governor in a long-standing States Rights dispute. In the midst of lawsuits and high-level politics, Kinyoun was made the scapegoat. Although a federal commission headed by Simon Flexner corroborated Kinyoun's diagnoses of plague deaths, the California governor first denied the existence of plague, and then accused Kinyoun of importing it in a bioterrorist attack upon the State. In a tragic compromise, the State of California and President McKinley agreed to have Kinyoun sacrificed. He was demoted in shame and left federal service thereafter. When World War I began Kinyoun, 58 years old and dying of lymphosarcoma, enlisted in the US Army as an epidemiologist. He served on the cantonments in North and South Carolina, was transferred to the Army Medical Museum in Washington, D.C., and died unknown on Valentine's Day, 1919.

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THE EPIDEMIC SITUATION OF SEVERAL MAIN INFECTIOUS DISEASES AND THEIR THREATS TO GLOBAL PUBLIC HEALTH SECURITY

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We generally covered the epidemic situation in the 21st century of several main infectious diseases,including AIDS, SARS, avian influenza, multi-drug resistant tuberculosis, extensively drug-resistant tuberculosis and polio, and their threats to global public health security in the background of globalization. And we summarized the progress made in detection, monitoring, prevention and treatment, as well as the difficulties being confronted, stressing out the importance of both international cooperation and governmental actions on infectious disease prevention and treatment in the future.

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END-TO-END DISEASE SURVEILLANCE IN DEVELOPING NATIONS

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Developing nations are limited in their ability to closely monitor their populations for outbreaks, due to resource constraints and underdeveloped public health and communications infrastructures. We sought to address this challenge by building a suite of open-source disease surveillance tools, with support from the US Department of Defense Global Emerging Infections Surveillance and Response System, and in partnership with the Naval Medical Research Center Detachment in Lima, Peru. These tools may be used by themselves or in concert. When used in concert, they provide health authorities with an end-to-end disease surveillance capability that automates the collection of disease reports from remote locations, visualizes collected data, and analyzes data for unusual disease activity. Since the costs to field and maintain a disease surveillance capability have traditionally been prohibitively expensive, these tools have been developed with the intent to minimize deployment costs, and impose no recurring software licensing costs. We decided to exploit the worldwide proliferation of cellular phones as a means to instantaneously collect health data from rural areas that lack most other forms of technology. Rural health workers enter data over the phone with an automated system, which is based upon interactive voice response (IVR) technology. Health authorities can then visualize, analyze, and query against the collected data using a secure open-source Web application called "OpenESSENCE". OpenESSENCE is written from the ground-up to support user interfaces in more than one language. To build OpenESSENCE, we tapped years of experience with the ESSENCE disease surveillance system, which is deployed in jurisdictions across the United States. In closing, we will discuss the successes, lessons learned, and future directions from the initial deployment of this suite of disease surveillance tools in Peru.

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A SUSTAINABLE NATIONWIDE LYMPHOEDEMA PROJECT IN TOGO

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Lymphatic filariasis (LF) elimination is based on two major strategies: the interruption of transmission and morbidity control. In 2000, the Ministry of Health (MoH) in Togo began LF elimination activities through mass drugs administration and training of surgeons for a hydrocele program in 2005. In 2007, a national lymphoedema (LD) management program was launched with technical support from CDC and funding from USAID/IMA. The goal of the project is to make treatment available to everyone with LD. Patients are advised to come to the dispensary to receive training on self-management. During their first visit, a kit with soap and towels and an information booklet, in addition to a practical training, is provided. The project employs a sustainable, decentralized approach so that the project can scale-up rapidly and can continue after external funding runs out. A training-of-trainers approach was adapted. Implementation in the districts is regularly monitored by a central coordinating unit through regular supervision visits. The project was piloted in LF-endemic districts and scaled-up rapidly nationally after one year, resulting in the first nationwide LD project in Africa. At least one health worker in each dispensary in Togo is trained to teach Community Health Workers (CHW) and those affected with LD on self-management. Ninety district management staff, trained in five workshops, instructed 500 dispensary nurses, who trained more than 1,600 CHW. The project also focused on educating the community about the existence and benefits of the proposed treatment. The project disseminates messages about LF and opportunities for the management of LD through television, posters, local radio stations and newspapers. Traditional healers are also informed. To date, providers are following more than 800 cases of LD, 24% living in areas considered as currently non-endemic for LF. Evaluation by CDC seemed to indicate that coverage is adequate, but that LD prevalence is lower than previously thought. Additional evaluation is ongoing to verify this. Most of the patients reported to be very satisfied with this approach and believe that their daily lives have improved significantly since the program began. A 5-year cohort study to evaluate the impact of the program is ongoing. The cost of implementation of this approach is around $0.30 per inhabitant. This program shows that it is feasible for a MoH to implement a nationwide morbidity management program.

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PERSISTENCE OF *WUCHERERIA BANCROFTI* IN THE INSECT VECTOR DESPITE APPARENT ELIMINATION OF INFECTION IN THE HUMAN POPULATION

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A pilot study of community-based mass drug administration (MDA) was instituted in six *Wuchereria bancrofti* (Wb) endemic villages of the southern part of Mali to provide baseline data and guidance prior to the initiation of the National Lymphatic Filariasis Elimination Program. Six annual MDA with albendazole and ivermectin have been completed to date. Transmission was assessed by dissection of mosquitoes collected by man landing catch from July to December at baseline (in 2001) and each year after the MDA. The prevalence of microfilaremia was determined at baseline and yearly thereafter by calibrated thick smear (60 microliters) of night blood obtained from a finger prick at baseline. Finally, the prevalence of infection was determined using ICT cards. We have previously presented the data for the first 3 rounds of therapy, which demonstrated a significant decrease in prevalence of microfilaremia from 24% (234/1141 subjects tested) in 2002 to 6% in 2005, but persistent low levels of transmission with an entomological infection rate (EIR) of 0.14% in 2005. We now report the results following the 6th round of MDA (overall coverage rate 70%). Microfilaremia was not detected in any of the 686 subjects tested 12 months after the 6th round of MDA. More importantly, circulating antigen was not detected in any of the 121 children under the age of 5 years tested in 2008, as compared to 53% (103/194) prior to the insitution of MDA in 2002. Lymphatic filariasis was transmitted primarily by *Anopheles gambiae* sensu lato (~90% of vector population) and *Anopheles funestus* (<10%). The number of infective bites per man per year decreased considerably from 4.8 in 2002 to 0.04 in 2007. Thus, despite the absence of detectable infection or evidence of transmission in the human population, analysis of the vector population suggested that transmission was still occuring albeit at a very low level. Whether this low level is suffcient to cause recrudescence of the infection when MDA is stopped remains to be seen.

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THE NATIONAL PROGRAM FOR THE ELIMINATION OF LYMPHATIC FILARIASIS IN TOGO: A SUCCESS STORY

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In an effort to determine lymphatic filariasis (LF) prevalence in West Africa, a rapid mapping survey was used to identify the endemic districts. This survey indicated that seven out of 35 districts (1.1 million persons) in Togo were LF endemic. In 2000, the MoH in Togo began a national LF elimination program addressing all the activities of the WHO recommended strategies: interruption of transmission by annual mass drug administration (MDA), and morbidity alleviation by a national lymphoedema (LD) and hydrocele program. This was accompanied by a stringent monitoring and evaluation plan. MDA with donated ivermectin and albendazole started in Binah district in 2000; by 2003, all 7 districts were covered. The total number of treatment cycles received in 2008 varied by district from 6 to 8. The annual reported treatment coverage ranged from 85% to 87%. In 2005, the program began to address the hydrocele problem by training 15 surgeons as part of the West African Hydrocele program. This was followed by a number of surgical campaigns in the north of the country. In 2008, eight additional surgeons were trained, and surgeries are now routinely conducted. Since 2005, more than 410 cases have been treated surgically. Since 2007, Togo has had a national LD program. More than 400 health workers and over 1000 community health agents in all districts, endemic and non-endemic, have been trained in LD management. To date, providers are following more than 800 cases. The program used different performance indicators to monitor performance. Reported coverage was confirmed by surveys. Sentinel sites selected before the first round of treatment were used to monitor nocturnal microfilaremia. Additional spot check sites were also added. Antigen surveys among children indicated a very low filarial antigenemia by ELISA. An ongoing cohort survey is being conducted to evaluate the impact of the lymphoedema program.

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IS LYMPHATIC FILARIASIS TRANSMISSION INTERRUPTED IN TOGO?

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Lymphatic filariasis (LF) in one of the few diseases targeted by WHO for elimination by 2020. In 2000, the Ministry of Health (MoH) in Togo started a national elimination program after initial mapping showed that seven districts were endemic. The MoH, with support from drug donations, Health and Development International and Centers for Disease Control and Prevention (CDC), organized up to eight rounds of mass drug administration (MDA) with Ivermectin and Albendazole. Based on the findings described here, the MoH thinks that LF transmission has been interrupted and MDA can be stopped. In 2005, WHO updated its guidelines about monitoring the impact of MDA on transmission and steps to be taken before stopping MDA. Currently, the guidelines are under further revision. For the duration of the program, Togo followed, as resources allowed, the WHO guidelines to measure MDA coverage and its impact. Since 2003, all endemic areas have been targeted by MDA. The reported coverage each year was >80% (81-87%) and this was confirmed by surveys in 2003 (82%) and 2006 (92%). Before the first round of MDA, nocturnal microfilaremia (mf) varied between 0.6 to 22.6%. After eight years, a drastic reduction in mf was observed, ranging between 0 and 0.6% at the 22 evaluations conducted in 11 sentinel sites and 10 spot checks. An innovative passive monitoring strategy, based on review of nocturnal malaria slides selected in a network of laboratories covering the whole country, was implemented in 2006 and 2007. After reading 8364 slides, only two positives were discovered. Further mf investigation in the village of origin of one case proved that it was an isolated case; the other case, a nomad, was lost to follow-up. Seven 30-cluster surveys were carried out in 2008: 4230 children between the ages of 2-6 years were tested. The LF antigen prevalence by ICT card test (Binax) was 2.1%. Confirmatory testing by ELISA (Ag Og4C3) at CDC showed a circulating antigen prevalence of 0.02%. Following the recommendation of the WHO expert committee, the MoH in Togo decided to stop MDA in 5 of the 7 districts. In addition, the LF program assured financial and technical support to carry out additional research, including the expansion of passive monitoring by adding testing in dispensaries to increase geographic coverage, developing an alternative surveillance system, and enhanced testing of previously endemic and non-endemic areas and cross border transmission.

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EVIDENCE FOR STOPPING MASS DRUG ADMINISTRATION FOR LYMPHATIC FILARIASIS (LF) IN SOME, BUT NOT ALL LOCAL GOVERNMENT AREAS OF PLATEAU AND NASARAWA STATES, NIGERIA

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Since 2000, ivermectin and albendazole have been distributed annually to eliminate LF from Plateau and Nasarawa States, Nigeria. All thirty local government areas (LGAs) within the states received at least six rounds of mass drug administration (MDA). Monitoring in sentinel sites indicated reduction in antigenemia and microfilaremia. From 2007-2008 population-based surveys were implemented in all LGAs to determine the prevalence of *Wuchereria bancrofti* antigen. Additionally, non-sentinel, baseline mapping sites were re-evaluated in LGAs where antigen prevalence from population-based surveys suggested that MDA might be suspended. In total, 36 215 persons were examined. Among 7 295 children 6-10 years of age, antigen prevalence was 1.35% (exact upper 95% CI = 1.62%) with a range by LGA of 0-7.57%. Overall antigen prevalence among all persons over 2 years of age was 3.17% (exact upper 95% CI = 3.49%) with a range by LGA of 0.20-14.9%. The exact upper 95% confidence limit of antigen prevalence among persons over 2 years of age was less than 2.0% in ten LGAs. No children less than 11 years of age were antigen positive in an additional LGA where only the point estimate of antigenemia was less than 2.0%. Antigen prevalence in baseline mapping sites of these eleven LGAs was 2.64% (range 0-9.0%) with no microfilaria positive persons identified. Data from these impact assessments suggest interruption of transmission in eleven LGAs. However, due to the co-endemicity of onchocerciasis in five of the eleven LGAs, we recommend continuing MDA in these five. MDA will also continue in the other 19 LGAs where antigen prevalence remains high. In six LGAs however, MDA will cease, but activities to monitor LF prevalence will be implemented to detect and respond to any recrudescence of infection.

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MICROARRAY-BASED ANALYSIS OF THE EFFECT OF DOXYCYCLINE ON *BRUGIA MALAYI* MICROFILARIAE

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Lymphatic filariasis is caused by the mosquito-borne, lymphatic-dwelling nematode parasites disease, mainly caused by *Wuchereria bancrofti* and *Brugia malayi.* The blood-borne microfilarial-stage is associated with complex disease pathology and host immunomodulation. Antimicrobial compounds, such as doxycycline, have been successfully used to sterilize and/or kill adult worms, thereby reducing microfilaria density, preventing disease transmission and improving disease morbidity. Moreover, doxycycline also has the effect on the obligate intracellular gram-negative bacteria, *Wolbachia*, a mutualistic endosymbiont that appears to exert influence on filarial nematode embryonic and larval development, adult female fertility, and filarial survival. However, little is known about the molecular effect(s) of doxycycline on the blood-dwelling microfilaria. We used microarray analysis to investigate temporal gene expression changes in *B. malayi* microfilariae exposed *in vitro* to 20 µg/ml doxycycline as compared to untreated control. By 61 hours post-exposure, doxycycline-treated microfilariae exhibited a significantly altered gene expression signature. We observed up-regulation of genes involved in protein folding and transcription/translation such as, small heat shock protein, heat shock protein 90, and 28S ribosomal RNA. In contrast, genes encoding for enzymes involved in the parasite mitochondrial electron transport chain, such as subunits of NADH dehydrogenase and cytochrome oxidase, were down-regulated. Our data suggests that doxycycline alters larval homeostasis either through a direct effect on the worm or through an indirect effect on the parasite's endosymbiont.

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COMPARATIVE ANALYSIS OF GENE EXPRESSION PROFILES IN INFECTIVE LARVA OF *BRUGIA MALAYI* AND *B. PAHANGI*

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Brugia malayi (Bm) and *B. pahangi* (Bp) are closely related filarial species that can be maintained in jirds and *Aedes aegypti*. Previous reports have identified developmental and biochemical differences between vector-derived infective larvae (vL3) from the two species. The purpose of this study was to compare gene expression profiles present in Bm vL3 and Bp vL3 for 15, 412 *Brugia* elements in the v2 filarial microarray. Eight hybridizations were performed for each 65-mer element on the array. Expression signals were detected for 4,788 elements with one or more L3 types. 330 elements up-regulated in Bm vL3 relative to Bp vL3 included genes that encode enzymes for energy metabolism, various proteases (cathepsins) and protease inhibitors (chymotrypsin/elastase), antioxidants (GST, oxidoreductase), signaling molecules, and immunomodulators (TGF-b, cystatins, ES-62 homolog, endochitinase, larval allergen) and many novel proteins. Bm vL3 had dramatically higher (> 100 fold) expression of genes encoding NADH dehydrogenase and cytochrome subunits. 321 elements up-regulated in Bp vL3 relative to Bm vL3 included genes related to energy metabolism, antioxidant-GPX, and immunomodulators (serpin, CPI-1). KEGG analysis showed that L3 from both species had strong expression of genes related to alanine and aspartate metabolism and to regulation of actin cytoskeleton. Mitochondrial energy metabolism and carbohydrate metabolism were enriched in Bm vL3. In contrast, nitrogen metabolism, metabolism of co-factors, and biosynthesis of secondary metabolites were enriched in Bp vL3. These differences suggest that the two species may have different metabolic adaptations and approaches to parasitism. Gene ontology analysis also suggested biological functions related to energy use were enriched in Bm vL3 while cellular physiological processes (protein polymerization, amino acid and nucleotide biosynthesis) were enriched in Bp vL3. Homologues of 18% of Bm vL3 and 21% of Bp vL3 up-regulated genes have severe RNAi phenotypes in *C. elegans.* vL3 gene expression is likely to be important for establishment of infection and immune evasion. Differences in Bm and Bp vL3 gene expression may be related to differences in host preference, tissue tropism, and pathogenicity observed between these species.

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ANALYSIS OF *WOLBACHIA* GENE EXPRESSION IN FILARIAL PARASITES BY *IN SITU* HYBRIDIZATION

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Wolbachia are symbiotic α-proteobacteria present in many medically important filarial nematodes. *Wolbachia* are necessary for parasite development, reproduction, and (in some species) viability. The molecular basis for the dependence of filarial worms on *Wolbachia* is unknown. Animal studies and clinical trials have shown that anti-*Wolbachia* treatments such as doxycycline first sterilize and then kill adult filarial worms. Effects of antibiotic treatment on *Wolbachia* can be assessed by microscopy (decreased bacterial numbers) and by quantitative PCR. However, filarial *Wolbachia* cannot be maintained in culture, and improved methods are needed for assessing the viability and metabolic activity

of these bacteria. We have developed in situ hybridization assays to study *Wolbachia* gene expression in filarial worms. Digoxygenin-labeled RNA probes of 300-500 bp length were used to detect ribosomal and specific messenger RNAs of *Wolbachia* in frozen sections of adult *Brugia malayi*. A probe for 16s rRNA produced intense signals corresponding to Wolbachia in parts of the lateral chord of male worms and in the hypodermis, the lateral chords and in developing embryos in females. This probe is a sensitive marker for *Wolbachia* that demonstrates the abundant but uneven distribution of the bacteria in filarial worms. A probe for a *Wolbachia* surface protein (wsp1) produced weaker signals in the same areas as the 16s probe. This localization pattern was also seen when wsp-1 protein was stained with a monoclonal antibody against wsp1. We conclude that *Wolbachia* endobacteria and their gene products can be sensitively detected by in situ hybridization. Expression signals vary by gene, developmental stage, and tissue type. Messenger RNA is likely to be more labile than DNA. Quantitative gene expression assays should be more useful than DNA assays for studying changes in metabolic activity of *Wolbachia* in different developmental stages and for assessing changes in bacterial viability after chemotherapy.

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COULD SNPS IN *MDR-1* GENE CONTRIBUTE TO OCCASIONAL SEVERE ADVERSE EFFECTS, FOLLOWING IVERMECTIN TREATMENT IN ONCHOCERCIASIS PATIENTS, FROM CAMEROON, THAT WERE CO-INFECTED WITH *LOA LOA*?

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Mass distribution of ivermectin (IVM) to human populations infected with *Onchocerca volvulus* is the mainstay of programs for onchocerciasis control. In Africa, lymphatic filariasis control is conducted using IVM and albendazole combination. IVM is exceptionally safe in humans. However, cases of encephalopathy, which can be fatal, have been reported in a small number of individuals (mostly in Cameroon and Democratic Republic of Congo) who harbored large numbers of *Loa loa* microfilariae (mf) in the blood. The pathophysiological and/or pharmacological basis for these rare serious adverse events (SAEs) is not fully understood. It is critical to clarify the mechanisms associated with the SAEs, in order to identify, if possible, those individuals who are at risk for SAEs, and to identify the most appropriate treatment to manage them. The SAEs could be the result of the effect of IVM on the *Loa* microfilariae, that would lead, in case of high microfilaremias, to embolisms of the mfs in the brain micro-circulation. One possible alternative explanation could involve the pharmacology of IVM. The drug is safe in humans because it is excluded from the CNS by the drug-transporting P-glycoprotein (PgP) of the blood-brain barrier. An absence of a functional PgP can lead to the penetration of the drug into the brain, and cause coma and sometimes death. This is the case in some dog breeds and in mice harboring a loss-of-function mutation in the PgP gene, *mdr-1*. We have investigated the possibility that a similar alteration in the *mdr-1* gene exists in humans who experience a post-IVM SAE. Blood samples from 4 patients who recovered from a SAE and from 8 individuals that never experienced a SAE following IVM (matched on sex, age and village of residence), were collected in Cameroon. RNA from leukocytes was extracted. The full length of *mdr-1* cDNA for each patient was amplified. SNPs were identified. While some of the SNPs led to amino acid changes other were silent. These SNPs are compared to known SNPs in *mdr-1*.

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IDENTIFICATION OF PROTEINS BINDING TO THE ESSENTIAL PROMOTER DOMAIN OF *BRUGIA MALAYI* 12 KDA SMALL SUBUNIT RIBOSOMAL PROTEIN (BMRPS12) GENE USING PHAGE-DISPLAY

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There is little information available about gene regulation in the human filarial parasite *Brugia malayi*. Only two *B. malayi* promoters have been mapped in detail so far, BmHSP70 and BmRPS12. The BmRPS12 promoter contains a 44- nucleotide tandem repeat sequence, the deletion of which results in 80% loss in promoter activity in the transient tranfection assays. This essential promoter domain lacks the binding sites for most general transcription factors present in other eukaryotic promoters but contains several GATA transcription factor binding sites encoded within this repeat. In the present study, we employed the T7 phage display technique to identify putative transcription factors that interact with this repeat domain. Using a *B. malayi* adult female T7 phage cDNA library, we have identified 5 different candidate proteins that were represented more than or equal to 5 times out of total 100 clones sequenced after final round of biopanning. Two of these proteins contain the RNA recognition motif (RRM) and constituted the most abundant group when equal number of phages displaying all five proteins was subjected to selection using stringent conditions. RRM-domain containing proteins have been shown previously to bind to single stranded as well as double stranded DNA, and have been found in several transcription factors. The RRM containing proteins that we have identified could thus constitute a new class of transcription factors in *B. malayi*.

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THE *BRUGIA MALAYI* ANKYRIN DOMAIN CONTAINING *WOLBACHIA* PROTEINS AS POTENTIAL MEDIATORS OF ENDOSYMBIOSIS

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Most human filarial parasites harbor an endosymbiotic bacterium of the genus *Wolbachia*, which appear to be essential for their survival as elimination of *Wolbachia* results in irreversible sterilization of the adult female worm. *Wolbachia* provides therefore an attractive new chemotherapeutic target for the treatment of human filarial infections by exploiting the vulnerability of the filarial parasites to the elimination of the *Wolbachia* endosymbiont. Our aim is to identify and characterize *Brugia malayi* (Bm) and *Wolbachia* (wBm) proteins that are essential for the endosymbiotic relationship. Bioinformatic analysis of the *Wolbachia* genome has identified a number of putatively secreted proteins, some of which contain ankyrin domains. The ankyrin domain is known to mediate protein-protein interactions and has been implicated in host-pathogen interactions in other bacteria. To study the potential role of wBm ankyrins in host-bacterium interaction and to identify their binding partners in Bm, we expressed and purified three wBm recombinant proteins corresponding to the Wolbachia ankyrin domain containing proteins Wbm0287, Wbm0394 and Wbm0447.We used the recombinant ankyrin domain of Wbm0394 with varying concentrations of Bm crude extracts in a modified ELISA assay to first verify whether it binds specifically to Bm extracts in comparison to extracts from *A. viteae* adult worms, which lack the *Wolbachia* endosymbiont. Our preliminary data indicate that the recombinant ankyrin domain of Wbm0394 binds to the Bm extract in a concentration-dependent manner. Moreover, the binding of the recombinant ankyrin protein levels off at higher concentrations suggesting a possible saturation, a characteristic of specific interaction. We are in the process of identifying its specific interacting partner(s) in the filarial host by using panning of a Bm cDNA phage display library and proteomic

approaches. The putative binding abilities of the other two wBm ankyrin domain proteins to Bm will also be presented.

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INFECTIVITY AND GENETIC ANALYSIS OF HYBRID *BRUGIA* LARVAE

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The differential infectivity of *Brugia malayi* and *B. pahangi* for *Armigeres subalbatus* has generated many questions about the basis of mosquito immunity against filarial nematodes. We have employed a genetic approach to identify filarial nematode determinants that coincide with infectivity for *Ar. subalbatus*. Here we report the successful generation of F1 progeny from reciprocal crosses of *B. pahangi* x *B. malayi*, and their phenotypes in *Ar. subalbatus* (melanize *B. malayi*, susceptible to *B. pahangi*), and in the black-eyed, Liverpool *Aedes aegypti* control (LVP; susceptible to both parental species). We examined hybrid larvae for melanotic encapsulation on day 5 post-infection, and quantified L3 development on day 11. Of the Bm male x Bp female hybrid larvae that penetrated the *Armigeres* midgut, 26-49% were fully melanized; and in the reciprocal cross, 14% were melanized. This suggests that the infectivity trait in *Brugia* may be under control of multiple genes. Efforts are ongoing to identify genetic loci shared between the *B. pahangi* parental strain and *Armigeres*-derived L3 hybrids by random amplification of polymorphic DNA, and sequencing of shared amplicons.

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DEVELOPMENTALLY REGULATED GENE EXPRESSION IN *BRUGIA MALAYI*

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Filarial parasites undergo major developmental changes as they move between insect and mammalian hosts and as they mature from microfilariae to sexually mature adult parasites (L5). We used microarray analysis to study changes in gene expression across the *Brugia malayi* life cycle. mRNA were prepared from worms at different life cycle stages (microfilariae-L1, infective larvae-L3, 2 week-L4, 6 week, 12 week-L5); male and female 6 and 12 week parasites were studied separately. Equal amounts of cDNA from each stage were pooled to produce a common reference sample. Microarray analysis was performed with the BmV2 array with 15,405 65-mer *Brugia malayi* cDNA elements. 12,054 elements were scored as "present" (2 fold over background in at least 3 of 4 hybridizations) in at least one stage, and 4,033 elements were "present" in all stages. 12 wk female worms had the highest number of elements present (10,962) and L4 had the lowest number (5,453). L3 and microfilaria are considered as developmentally arrested stages in the life cycle, surprisingly, they have expressed fairly amount of transcriptoms (6,765 and 7,680, respectively). More than two thousand elements were stage-specific ("present" in only one parasite type by microarray), and a majority of these (73%) were in 12 wk females. Advanced analyses including ANOVA, principal component analysis, and hierarchal clustering provided a global view of gene expression relationships among the different developmental stages of the parasite. KEGG and GO analyses are in progress to identify pathways and functions associated with different parasite stage. This study has provided the first broad view of developmentally regulated gene expression in a nematode parasite. Additional studies will be needed to determine how these changes in gene expression relate to important functions such as growth, molting, reproduction, and adaptation to different host environments.

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NOVEL LIPID BIOSYNTHETIC PATHWAYS IN LEISHMANIA CHEMOTHERAPEUTICS

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Leishmania infections represent a diverse clinical spectrum of disease and currently lack adequate therapy. Prior studies with *L. major* (Zhang *et al*, in preparation) have demonstrated strong synergistic interactions between inhibitors of sphingolipid synthesis (Myriocin, MYR) and some inhibitors of ergosterol synthesis (Itraconazole, ITZ), with fractional inhibitory concentration indices of less than < 0.1. While promising, ITZ has variable efficacy against *Leishmania* species and MYR is immunosuppressive and/or toxic. Furthermore, *L. major* salvage sphingolipids from host cells, bypassing *de novo* synthetic inhibitors. Thus to circumvent these issues and develop new compounds as both investigational and lead compounds, we developed a high throughput synergy screen to identify new synergistic partner compounds. Our screen utilizes *L. major* promastigotes stably expressing high levels of luciferase (LUC) following integration into the rRNA locus. Promastigoes were assayed under conditions where LUC expression is proportional to cell growth. The use of the reporter, LUC-*L. major*, was validated with control inhibitors and showed good quality for screening (Z'factor >0.65). LUC-*L. major* promastigotes were screened against compounds in basal media or in combination with sub-toxic concentrations of MYR or ITZ, and scored for LUC expression after 48 hours of growth. To identify novel synergistic partners, parasites showing inhibition in the presence of the MYR or ITZ but not in their absence are considered hits. Thus far we have screened more than 1,400 compounds at 10 μM. In primary screening, roughly 11% were toxic under all conditions and about 2% were hits. Secondary tests are underway; several selected compounds have been validated and show comparably strong synergy to that seen with MYR and ITZ. We are currently completing the secondary screening and initiating studies pursuing the mechanism by which synergy is attained.

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DIAGNOSIS OF VISCERAL LEISHMANIASIS BY POLYMERASE CHAIN REACTION OF DNA EXTRACTED FROM GIEMSA STAINED SLIDES COLLECTED FROM NEPAL

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Visceral leishmaniasis (VL) is a parasitic disease caused by *Leishmania donovani* and transmitted by sand flies. VL is responsible for about 59,000 deaths per year and 2.4 million disability-adjusted life years lost in worldwide. India, Nepal and Bangladesh account for 300,000 cases annually and thus shoulder 60% of the global burden of the disease. Nepal is an endemic area for VL which causes major public health problems in the Terai (lowlands) in southeast regions. The aim of this study was to evaluate the sensitivity of polymerase chain reaction (PCR) in the detection of *Leishmania* DNA from Giemsa-stained bone marrow slides. We also compare PCR with direct microscopy and parasite culture. 96 patients with suspected VL as determined by clinical diagnosis between 2003-2006 were enrolled in this study. A few drops of bone-marrow aspirate were obtained from iliec-crest punctured. Bone-marrow aspirates were used to prepare smears on glass slides and to make cultures. DNA was extracted from bone-marrow smears using a cell and tissue extraction kit. Kinetoplast minicircle DNA was used as the target gene for PCR analysis. Results: PCR analysis shows that all the positive samples were of *L. donovani* species. PCR showed the highest sensitivity 76/96 (79%) than microscopic. Microscopic examination detected 57/96 (59%) and

culture 20/96 (21%) of the samples. In addition, PCR was able to detect VL of 19 patients (19.8%) which were negative by microscopy. PCR of DNA extracted from Giemsa-stained bone-marrow slides is a suitable tool for confirming diagnosis in patients with VL and may be useful in the diagnosis of difficult cases. Bone-marrow smears were easily stored, and can be easily sent to research centers where PCR is available. This makes PCR is good option for diagnosis in the field.

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ISOLATED AND PURIFIED NOVEL ANTILEISHMANIAL DRUG CANDIDATE FROM *HIMATANTHUS SUCUUBA*

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Cutaneous Leishmaniasis is a major parasitic disease worldwide health problem. Although a number of antileishmanial drugs are available, painful and lengthy treatments with standard drugs such as Antimonials and Amphotericin B remain being used even though it is well known that they have toxic side effects and growing chemotherapy failure, reasons why new drugs are actually needed. In previous studies, we have isolated, purified and bioassayed a spirolactone iridoid compound, Plumericin, from the Amazonian plant *Himatanthus suucuba*. Results, using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) micromethod, showed that Plumericin has a potent antileishmanial activity. Thus, *in vitro* axenically cultured amastigotes disclosed an IC_{50} value of 0.21 µM versus 0.52 µM of Amphotericin B. In addition, Plumericin also demonstrated lower citotoxicity when challenging healthy peritoneal mice macrophages *in vitro* than with Amphotericin B (IC50 of 1.86 µM versus >10 µM, respectively). Those potential positive features prompted us to identify its biochemical target in the amastigote form. Plumericin effects on *Leishmania amazonensis* DNA and RNA nucleic acids metabolism were studied by comparing the incorporation of labeled analogues [^{3}H] thymidine and [^{3}H] uridine, respectively, after treating axenic amastigotes for 30 minutes with Plumericin. Interestingly, Plumericin was also active on inhibiting cellular DNA synthesis with a subsequent recovery, but especially on the inhibition of cellular RNA synthesis which after 120 minutes of evaluation, it maintained a progressive decrease of RNA precursor molecules incorporation. Therefore, Plumericin would severely interfere in nucleic acids metabolism of the parasite. If so, we propose this compound as an interesting anti-leishmanial drug candidate for future studies.

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ORGAN SPECIFIC ACCUMULATION AND DISTRIBUTION OF STRUCTURALLY RELATED ANTI-TRYPANOSOMAL COMPOUNDS: A POSSIBLE ROLE IN RENAL TOXICITY

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Human African trypanosomiasis (HAT), also called African sleeping sickness, is a neglected tropical parasitic disease occurring in sub-Saharan Africa. HAT progresses through a relatively asymptomatic, hemolymphatic first stage into a fatal central nervous system (CNS) infection in the second stage. Current treatments for second stage HAT are limited by severe toxicity and lack of efficacy. Diamidine compounds such as pentamidine and furamidine are potent anti-trypanosomal molecules. Clinical trials of pafuramidine, a prodrug of furamidine for potential use in first stage HAT, were recently terminated due to unexpected delayed nephrotoxicity. CPD0801 is a related diamidine compound in development by the UNC based Consortium for Parasitic Drug Development. CPD0801 is curative in the second stage of a HAT murine model, and is currently under development as a treatment for second stage human disease. Rats were administered furamidine or CPD0801 (10 µmol/kg; single bolus dose) through a femoral vein cannula. Kidneys and livers were harvested for fluorescence microscopy and HPLC-MS/MS quantification. Livers contained similar quantities of both compounds while exhibiting slightly different distribution patterns. In contrast, CPD0801 accumulated in kidney at concentrations approximately 10 times less than furamidine when measured 48 hours after a single dose. Mechanistically-relevant, differential renal distribution patterns were also observed. Dissimilarities in kidney accumulation and distribution over time should reveal potential mechanistic differences in uptake and/or excretion of the compounds. Additional distribution/localization studies using the prodrug forms of CPD0801 and furamidine (pafuramidine) are in progress. The low accumulation and distribution of CPD0801 compared to furamidine may indicate a lesser risk of delayed nephrotoxicity in humans.

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ANTIBODY DROP IN NEWBORNS CONGENITALLY INFECTED BY *TRYPANOSOMA CRUZI* TREATED WITH BENZNIDAZOLE

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Vector control leads to drastic drop of the prevalence of Chagas disease in Latin America, giving importance to congenital Chagas disease. Diagnosis and treatment of infected newborns becomes a priority. Recovery is confirmed by the disappearance of *T. cruzi* antibodies several months after the treatment. During a trial aiming at comparing two treatment modes of congenital Chagas disease, we compared the decrease of antibody titers in congenitally infected newborns after treatment and evaluate the time to recovery. The decrease of *T. cruzi* antibody titers measured by ELISA tests was followed during the first year of life in congenitally infected newborns treated with different doses of benznidazole and compared to *T. cruzi* antibody titers in non infected newborns. Confirmation of recovery was given by two negative serological tests: Chagas Stat-Pak® (immunochromatography) and Chagatest® v3.0 (ELISA). In non infected infants from infected mothers, antibodies from maternal origin disappeared in less than 8 months while in infected infants *T. cruzi* antibodies decreased more slowly and disappeared in 9 to 16 months allowing to confirm the recovery. All Chagas Stat-Pak® tests were negative before the 9th month while about 10% of ELISA tests remained positive at the 12th month. Recovery may be confirmed in most cases at 10 months. The Chagas Stat-Pak® test appeared to give a reliable response earlier than the Chagatest® v3.0 ELISA. The decrease rate of antibodies does not depend on treatment modes.

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ACUTE INFECTION WITH *TRYPANOSOMA CRUZI* IN WISTAR RATS INDUCES GROWTH RETARDATION AND DEVELOPMENT OF MORPHOLOGICAL ANOMALIES IN THEIR FETUSES

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The present study shows the development of the fetuses in two groups of Wistar rats. Rats (R) were injected intraperitonealy with $2x10^5$ of bloodstream trypomastigotes I/PAS/VE/00/PLANALTO and ASM *Trypanosoma cruzi* strains. To obtain pregnancies, rats were maintained in estrus the menstrual cycles and them mated with males at days 12 after infection, noninfected pregnancy rats were used as control. The results showed high levels of parasitemia in the rats between days 12 to 34 of infection with 0, 6, 12 and 20 days of gestation. Some of the rats were sacrificed and the number and aspects of fetuses extracted was revised. Between 1 and 8 fetuses were collected from 4 infected rats with PLANALTO *T. cruzi* strain; R1 showed 4 fetuses in right side and 4 immobile fetuses in left side of uterus (intrauterine growth retardation was seen in these fetuses), R2 showed fetal resorptions and placenta remains in right side and 1 fetus in left side of uterus, R3 showed 3 fetus in right side and 2 fetus dead in left side of uterus together to inflammated and necrosed placenta, and R4 showed 1 fetus in right side and 3 fetus in

left side of uterus. Structural muscular-skeletal anomalies were seen in some the fetuses collected from 4 infected rats with ASM parasite; these rats developed between 6 and 8 fetuses; R5 showed 3 fetuses in right side and 5 fetuses in left side of uterus, R6 showed 2 fetuses in right side and 4 fetuses in left side of uterus, in R7 protuberances were found on the head and body dorsal side of 3 fetuses in right side of uterus, other fetus developed his anterior right footpad with big size, the left side of uterus presented one fetus with a protuberance on his body dorsal side and 3 fetuses of normal aspect; R8 showed 2 fetuses in right side and 5 fetuses in left side of uterus of normal aspect. Morphometric analysis revealed differences (P<0,01) between the fetuses size of infected rats and healthy pregnancy rats. In conclusion, the morphological anomalies seen in the fetuses such as the development of protuberances on head and the dorsal body side in 4 (57,11) fetuses of same rat, as well as fetal growth retardation, death and resorptions were probably due to the highest parasitemia by *T. cruzi* in the rats during 20 days of gestation.

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IN VITRO PHARMACODYNAMICS AND MECHANISM OF ACTION STUDIES OF OXABOROLE 6-CARBOXAMIDES: A NEW CLASS OF COMPOUNDS FOR THE TREATMENT OF AFRICAN TRYPANOSOMIASIS

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Human African Trypanosomiasis (HAT) is a fatal disease caused by *Trypanosoma brucei spp*. There is a need for new treatment for HAT because current treatments are costly, difficult to administer and frequently toxic. We have identified several oxaborole 6-carboxamides that demonstrate potent activity against *T. brucei brucei in vitro* and exhibit efficacy against both the acute and chronic CNS stages of HAT in mouse models. Exposure of *T. b. brucei* to oxaboroles leads to significant changes in shape, reduction in cellular size and detached flagella at the time of death. *In vitro* studies performed to characterize the relationship between killing of *T. brucei* and oxaborole exposure, demonstrate an early (6-9 hrs) onset of trypanocidal activity as shown by the inability of the parasites to generate ATP. Parasite commitment to death *in vitro* occurs with similar kinetics even when compound is washed out 2-3 hours following exposure. For mechanism of action studies, fluorescently tagged oxaborole analogues have been synthesized and incubated with *T. brucei* parasites to identify sub-cellular localization. In addition, representative compounds have been immobilized on agarose matrices for use in affinity capture of parasite target proteins which will be identified by mass spectrometry and data base searches. Collectively these studies will provide a better understanding of how oxaboroles exert their trypanocidal effects and enable us to develop valuable PK/PD models to ensure appropriate drug delivery for treatment of HAT.

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TOWARDS RATIONAL DRUG DESIGN IN LEISHMANIASIS: PTERIDINE REDUCTASE 1 AS A TARGET FOR ANTIFOLATE CHEMOTHERAPY

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Leishmania are protozoan parasites of the order Kinetoplastida that cause a variety of diseases in humans, from dermal lesions to visceral infections. Since the discovery of the first drug for leishmaniasis treatment (i.e. pentavalent antimonials), until the current days, the search for cost effective compounds with antileishmanial activity, without toxic effects and able to overcome the emergence of drug resistant strains still remains as the current goal. The rational development of medicines is a reality that offers new perspectives for discovering new drugs and /or improving those that already exist. Consequently, we report the identification of a new antifolate lead molecule (a dihydropyridine analogue) using pteridine reductase 1 enzyme in the *Leishmania* parasite as the therapeutic target for which there is no equivalent in the human host. The amastigote stage in leishmaniasis is of prime importance in perpetuating the disease. This lead molecule inhibited the proliferation of amastigotes in infected macrophages both *in vitro* and in experimental animal model with no host cytotoxicity. In addition, this drug maybe orally used. We were also able to produce highly selective inhibition of the recombinant enzyme. Structure activity relationship based on homology model drawn on our recombinant enzyme additionally established the high affinity of this ligand to the enzyme active site. It was seen that the leishmaniacidal effect of this lead molecule is triggered by programmed cell death. To extend our target- and drug-discovery aspect further, we carried out expression profiling to allow identification of genes within this targeted pathway which may be therapeutically exploited and/or lead to further hypotheses as the basis for follow-up experiments.

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SYBR GREEN-BASED REAL-TIME PCR DIFFERENTIATION OF *LEISHMANIA SPP.* IN CLINICAL SPECIMENS

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More than 1.5 million people are affected by leishmaniasis each year in tropical, subtropical and Mediterranean regions of the world. More than 20 species cause human disease, including the *Leishmania. donovani, L. mexicana,* and *L. tropica* complexes and the subgenus *Leishmania Viannia*. Microscopic examination can detect *Leishmania* amastigotes, but it lacks sensitivity and does not discriminate *Leishmania* at the species or complex level, which is important for clinical management and treatment. Cellulose Acetate Electrophoresis (CAE) is the gold standard for species identification. However, CAE needs significant parasite mass obtained through *in vitro* culture, making it time-consuming and labor-intensive. Molecular tests offer an alternative for specific detection of *Leishmania* spp. in clinical samples. In this study, we developed a SYBR green real-time PCR assay, using primers spanning part of the ITS1, followed by a melting curve analysis to discriminate *Leishmania* spp. within different groups. DNA was extracted from 54 clinical samples containing organisms of *L. donovani* complex (n=17), *L. mexicana* complex (n=9), *L. major* (n=6), *L. tropica* (n=5), *L. aethiopica* (n=1) and subgenus *Viannia* (n=16) identified at species/complex level by CAE analysis. Using this method, we were able to discriminate these *Leishmania* positive samples within 3 groups: G1 represented by the subgenus *Viannia*; G2 which included *L. donovani* complex and *L. tropica;* and G3 which included *L. mexicana* complex and *L. major*. Only two samples identified *L. braziliensis* (subgenus *Viannia*) and *L. major* by CAE were negative by this SYBR green assay. These primers did not amplify DNA extracted from different trypanosomatids (*Trypanosoma cruzi* [n=5], *T. caninum* [n=1], *T. rangeli* [n=1], *T. rhodesiense* [n=1]) or from human blood and tissue negative for *Leishmania* sp. (n=15). The use of this method results in preliminary identification of *Leishmania* spp. and decreases the time to appropriate clinical interventions.

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MALARIAL RETINOPATHY AND MICROCIRCULATION IN ADULTS WITH CEREBRAL MALARIA

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A specific retinopathy has been described in African children with cerebral malaria, but in adults this has not been extensively studied. Since the structure and function of the retinal vasculature greatly resembles the cerebral vasculature, study of retinal changes can reveal insights into the pathophysiology of cerebral malaria. Obstruction of microcirculatory blood flow is thought to be important in causing both malarial retinopathy and cerebral malaria. A detailed observational study of malarial retinopathy in Bangladeshi adults with cerebral and non-cerebral severe falciparum malaria was performed using high-definition portable retinal photography. Control groups were patients with uncomplicated malaria or sepsis and healthy volunteers. Markers of systemic impaired microcirculatory blood flow, including serum lactate, rectal mucosal capillary flow, red cell deformability and HRP2-derived parasite biomass were correlated with the severity of different features of malarial retinopathy to investigate the role of microcirculatory dysfunction in its pathogenesis.

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PLACENTAL MALARIA IN AREAS OF DIFFERENT ENDEMICITY: A MODIFIED PATHOLOGICAL GRADING SCHEME

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During placental malaria (PM), infected erythrocytes sequester in the placenta and cause an inflammatory response that is harmful to the fetus and the mother. Histologic parameters can serve as a surrogate endpoint for interventional trials. Placental malaria episodes have been histologically classified as acute or chronic, however chronic placental malaria is a broad category that encompasses several distinct features of infection: namely placental inflammation and malarial pigment deposition. Using frozen section histology in Tanzania, we describe a pathologic grading and staging scheme to evaluate placental malaria at delivery. For comparison, samples from Thailand were selected from the small fraction of women with *Plasmodium falciparum* at least two weeks prior to delivery. In the Tanzanian cohort, the inflammation grade was associated with levels of inflammatory markers at delivery, and the pigment deposition stage was independently associated with birth weight. The samples from Thailand had similar inflammation but decreased pigment deposition when compared to samples from Tanzanian first time mothers, and the inflammation grade was associated with decreased birth weight. The proposed pathological grading system is simple yet captures increased complexity of PM episodes, suggesting that combining these two measures can improve immunocorrelation for clinical trials across areas of differing endemicity.

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ANGIOPOIETIN EXPRESSION IN POSTMORTEM HUMAN BRAIN IN CEREBRAL MALARIA

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The precise pathophysiological mechanisms underlying cerebral malaria (CM) remain unclear. It is not known how parasitized red blood cells (PRBC), remaining within the vascular space, influence parenchymal function to induce coma and death. Recent studies have implicated the dysregulation of angiopoietins (Ang) in the pathogenesis of severe malaria. The circulating levels of Ang-1, Ang-2 and the Ang-2/1 ratio have shown to be promising biomarkers for CM and predictors of severity and death. The Ang-1-Tie 2 ligand-receptor system plays a key regulatory role in the maintenance of functional integrity of the Blood Brain Barrier. Normally Ang-1 mediated Tie-2 signalling maintains endothelium in a quiescent state, but Ang-2 can destabilise this and enhance endothelial response to exogenous stimuli including inflammatory and angiogenic cytokines such as TNF and VEGF. We have investigated protein expression of the Angiopoetins Ang-1 and Ang-2, and their ligand Tie-2 receptor, using quantitative and semi-quantitative immunohistochemical techniques, in post-mortem human brain samples from 23 cases of Vietnamese adults dying of severe malaria and 19 non-malaria control cases. The total number of intraparenchymal vessels in each case were quantified and compared with the numbers of vessels expressing these angiogenic proteins. Cerebral malaria cases show variable upregulation of Ang-2 expression in blood vessels. Different patterns of Ang-2 expression were observed in neurons and glial cells. Ang-2 immunostaining was also observed in PRBC. A full clinicopathological correlation will be presented.

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EFFECT OF CHLOROQUINE, METHYLENE BLUE AND ARTEMETHER ON HEPATIC OXIDATIVE STRESS AND ANTIOXIDANT ENZYMES IN *PLASMODIUM YOELII*-INFECTED MICE

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The liver is responsible for multiple functions including the detoxification of compounds. Reactive oxygen species are important mediators of tissue injury during malaria infection. Most antimalarials are thought to be pro-oxidative in action, thus affecting the antioxidant defense system of both host and parasite. However, little is known of the effect of these drugs on the cellular antioxidant defense system and extent of lipid peroxidation in the hepatic tissues of the host during malaria chemotherapy. This study therefore aims at evaluating the antimalarial efficacy of chloroquine (CQ), methylene blue (MB) and artemether (ART) plus their effect on the malondialdehyde (MDA) level, catalase and superoxide dismutase activities in hepatic tissues of the host during *Plasmodium yoelii* infection. One hundred and twenty mice were grouped into six treatment groups and CQ (10mg/kg), MB (10mg/kg) or ART (4mg/kg) was administered to both the infected and uninfected mice for three consecutive days after established *P. yoelii* infection. Two groups of animals were used as positive (with malaria) and negative (without malaria) controls respectively. Lipid peroxidation and antioxidant enzymes were determined in liver samples using standard procedures. CQ, MB and ART caused significant increase (MB $\rightarrow$CQ$\rightarrow$ART) in MDA level in both infected and uninfected mice during therapy. Similarly, catalase and superoxide dismutase activities were

significantly increased during administration of the three drugs in both *P. yoelii*-infected and uninfected mice. In conclusion, malaria infection as well as CQ, MB and ART induce oxidative stress and disrupt the antioxidant enzyme activities of the host.

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DYSREGULATION OF ANGIOPOIETINS IN LOW BIRTH WEIGHT OUTCOMES OF PLACENTAL MALARIA

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Placental malaria doubles the risk of low birth weight, which is caused predominantly by intrauterine growth restriction. In turn, low birth weight increases the infant mortality rate. Despite a clear epidemiological association of low birth weight with both placental malaria and higher mortality rates, the physiological mechanisms of intrauterine growth restriction induced by malaria infection of the placenta remain undefined. Angiopoietin (Ang)-1 and -2 are important in the formation of placental vascular system, and their dysregulation has been associated with cerebral malarial. In an experimental mouse model of placental malaria, we show that *Plasmodium berghei* ANKA infection of pregnant mice leads to decreased Ang-1 and increased Ang-2 levels in serum and placenta concurrent with fetal growth restriction. These results were extended to and validated in malaria-exposed pregnant women in Cameroon. In a prospective study over the course of pregnancy, maternal plasma Ang-1 levels were decreased, and the Ang-2:Ang-1 ratio increased, in the presence of peripheral *P. falciparum* parasitemia in pregnant primigravid women. In a cross-sectional study of primigravid women at delivery, women with placental malaria had increased Ang-2 levels and Ang-2:Ang-1 ratios associated with delivery of low birth weight infants. Thus, Ang-1 and Ang-2 levels may be clinically informative biomarkers to identify *P. falciparum*-infected mothers at risk of low birth weight deliveries. These data support the hypothesis that angiopoietins contribute to intrauterine growth restriction in the context of placental malaria.

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HAPLOTYPES AND DIPLOTYPES OF INTERLEUKIN-18 PROMOTER POLYMORPHISMS ARE ASSOCIATED WITH SUSCEPTIBILITY TO SEVERE PEDIATRIC MALARIAL ANEMIA

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Interleukin-18 (IL-18), a pro-inflammatory cytokine secreted primarily by activated monocytes/ macrophages, is important in regulating innate and acquired immunity and plays a key role in autoimmune, inflammatory and infectious diseases. Previous studies demonstrated associations between IL-18 production and clinical outcomes in children with *Plasmodium falciparum* malaria. Since the role polymorphic variability in the IL-18 promoter in conditioning severe malarial anemia (SMA) has not been reported, the effect of two functional IL-18 promoter variants (-137G>C and -607C>A) were investigated. Children (3-36 mos.) with acute malaria (n=523) were recruited at Siaya District Hospital, western Kenya, a holoendemic *P. falciparum* transmission area. Complete hematological and parasitological profiles were measured, and hemoglobin (Hb) levels used to stratify children into SMA (Hb<5g/dL; n=123) and non-SMA (Hb>5g/dL, n=400). IL-18 promoter genotypes were determined by TaqMan 5′ allelic discrimination assay and PCR-RLFP. Genotype, haplotype and diplotype frequency distributions were determined. Multivariate logistic regression analyses, controlling for the appropriate confounders (age, gender, sickle-cell and HIV-1 status, and presence of bacteremia) demonstrated that the -137G/ -607C (GC) haplotype was associated with a two-fold higher risk of developing SMA [OR=2.07 (95%CI=1.05-4.08), p=0.036]. In contrast, homozygous polymorphic individuals at the -607 A allele (relative to the CC genotype) and those with the -137GG/ -607AA (GA/GA) diplotype were significantly protected against SMA [OR=0.44 (95%CI=0.21-0.90), p=0.027 and OR=0.34 (95%CI=0.12-0.99), p=0.048, respectively]. Taken together, these findings demonstrate that variation at -137 and -607 in the IL-18 promoter are associated with susceptibility to SMA in children living under intense *P. falciparum* malaria transmission.

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ASSOCIATION BETWEEN POLYMORPHIC VARIATION IN THE IL12B 3′ UTR AND PROTECTION AGAINST *PLASMODIUM FALCIPARUM*-INDUCED SEVERE MALARIAL ANEMIA IN KENYAN CHILDREN

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Plasmodium falciparum malaria remains a leading cause of morbidity and mortality among African children. Low levels of circulating interleukin (IL)-12 are associated with severe malarial anemia (SMA). However, the functional role of genetic variation within IL-12 genes in determining disease outcomes in children naturally exposed to *P. falciparum* infection remains unexplored. To determine the impact of genetic variation in IL12B in conditioning malaria disease outcomes, the association between variants at the IL12B 3′UTR and SMA (Hb<6g/dL) was investigated in children <3 years of age in western Kenya. Parasitemic children (n=544) were enrolled at Siaya District Hospital. Complete hematological measures were obtained with a Beckman Coulter Counter®, while Giemsa-stained slides were used to determine parasitemia. Children were divided into two groups based on Hb status; non-SMA (Hb≥6.0 g/dL) and SMA (Hb<6.0 g/dL). DNA was extracted from blood spotted on filter paper using the Chelex method. IL12B 3′UTR genotyping was carried out using Taqman 5′ allelic discrimination Assay-By-Design. Genotypic distributions were: 39.3% AA; 41.7% AC and 18.9% CC, with A and C allele frequencies of p=0.59 and q=0.41. Multivariate logistic regression analysis, controlling for potential confounders, demonstrated that relative to homozygous T, the GG genotype was associated with a 39% reduced risk of developing SMA (OR; 0.61, 95% CI, 0.37-0.99; P=0.047). These results demonstrate that variation in IL12B 3′UTR is associated with protection against SMA in young children resident in this *P. falciparum* holoendemic transmission area.

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LACK OF ASSOCIATION BETWEEN VARIATION AT TNF-A-1031(T/C) AND SEVERE MALARIA ANEMIA

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TNF-α is a multifunctional pro-inflammatory cytokine that regulates a wide spectrum of biological processes. Overproduction of TNF-α has been associated with several pathologies, including autoimmune diseases and severe malaria. However, the association between TNF-α -1031T/C and malaria disease outcomes have not been reported. In this study, the

relationship between TNF-α -1031T/C variants and severe malarial anemia (SMA; Hb<6.0g/dL) was investigated in children from a *Plasmodium falciparum* holoendemic transmission area of western Kenya. In this study, children (n=503), matched by age and gender, were enrolled at Siaya District Hospital in western Kenya. Complete hematological counts were obtained with a Beckman Coulter Counter, and Giemsa-stained slides were used to determine parasite densities. TNF-α -1031T/C genotypes were determined using a gene-specific polymerase chain reaction assay, followed by allele-specific restriction enzyme digestion using BbsI. Prevalence of TT and TC genotypes was: 90.1% and 9.9%%, respectively, with allele frequencies of T=0.90 and C=0.10, respectively. In a binary logistic regression model controlling for age, gender, bacteremia, HIV-1 and sickle cell status, polymorphic variability at TNF-α -1031T/C was not associated with severe malarial anemia [SMA, Hb<6.0g/dL; OR: 1.50, 95%CI 0.80-2.81, p=0.209]. Additional analysis based on WHO definition of SMA (Hb<5.0g/dL, with any parasite density) did not show any association with SMA (OR: 0.98, 95%CI 0.49-1.98, p=0.963). These results indicate that polymorphic variability at -1031 in the TNF-α promoter does not appear to be associated with malarial anemia severity in children from this holoendemic region of western Kenya.

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MEASUREMENT OF *PLASMODIUM FALCIPARUM* SEQUESTRATION IN HUMAN TISSUE

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The devastating nature of falciparum malaria is attributed to the ability of *Plasmodium falciparum* to cause infected erythrocytes to adhere to vessel walls. Despite the clinical importance of the sequestered parasite population, there is a lack of available tools to i) quantify parasite load in human biopsied or post-mortem tissues, and ii) distinguish circulating from sequestering parasite populations in these tissues. Our aim has therefore been to evaluate and develop methods for the measurement of these two important parameters. We have conducted this work using diverse tissue types derived from patients of an ongoing autopsy study on fatal pediatric malaria in Blantyre, Malawi. Initial quantification involved the current standard for making these assessments: manual counts of parasites per high power field on H&E stained tissue sections. The limitations of this method include the difficulty in accurately identifying and counting parasites and the inability of obtaining a representative picture of a large and heterogenous tissue. We therefore performed immunohistochemistical staining (IHC) for the detection of Plasmodium lactate dehydrogenase (pLDH), as a more specific method of identifying parasites in the tissue sections. In parallel, we performed ELISA assays for the quantification of pLDH in a tissue homogenate, allowing us to assess parasitemia is a larger section of tissue. In addition, we established a quantitative Real Time PCR (qPCR) assay for the assessment of parasitemia based on parasite genome copies in the tissue homogenate. Importantly, we have also used qPCR to examine the distribution of circulating vs. sequestered parasites through a measurement of stage-specific parasite transcripts. Altogether, we have combined protein and nucleic acid based assays to measure parasitemia and distinguish between circulating and sequestering parasite populations in the human body.

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IDENTIFYING THE COMPONENTS OF INTRAERYTHROCYTIC *PLASMODIUM FALCIPARUM* THAT INTERACT WITH GROWTH-PROMOTING LIPIDS

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Malaria remains a devastating disease, particularly in the tropics. New chemotherapeutic approaches are needed from a better understanding of parasite biology and interaction with the host. Crucial and novel targets for malaria chemotherapy can be found from factors that induce growth of *Plasmodium* spp. and parasite factors interacting with growth-promoting agents. We recently reported on a chemically defined medium formulated with recombinant protein and structurally defined lipids for erythrocytic growth of *P. falciparum*. To determine the chemical actions that underlie growth promotion in the parasite, we identified parasite factors that interacted with the growth promoting lipids detected at the molecular level with a fluorescent analogue of the lipids combined with the LC MS/MS technique. 13 parasite components were confirmed to interact with the agent, including a predominately merozoite surface protein (MSP)-1.

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ANEMIA DURING PREGNANCY AND LOW BIRTH WEIGHT IN AN ENDEMIC MALARIA AREA IN BENIN

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Anemia and malaria are highly prevalent in pregnant women in Benin. We studied the relationship between anemia in pregnant women treated for malaria and low birth weight (LBW) in Benin. A retrospective cohort study was conducted based on data from a randomized controlled trial in a semi-rural area in Benin from July 2005 to April 2008 on intermittent preventive treatment of malaria during pregnancy showing equivalence between sulfadoxine-pyrimethamine and mefloquine. Among the 1601 pregnant women recruited in the trial between 16 and 28 weeks of gestation, 1440 observations have been analyzed including HIV non-infected women. The hemoglobin concentration (Hb) was assessed at least once during pregnancy (at enrolment during the second trimester, at least one month later and/or at delivery) and infant's weight collected at birth. Anemia was defined as severe (Hb < 80 g/l), moderate (Hb ≥ 80 and < 100 g/l), mild (Hb ≥ 100 and < 110 g/l) and no anemia (Hb ≥120 g/l). Gestational age was assessed by the Ballard score in 80% of birth and by the best measure available for others. The proportions of women with severe, moderate, mild and no anemia were 4.1%, 28.8%, 31.2% and 35.9% during the second trimester, 4.1%, 30.2%, 29.8 and 36.0% during the third trimester, and 2.5%, 15.7%, 21.5 and 60.4% at delivery, respectively. The prevalence of LBW was 9.1%. Compared with women without anemia during the third trimester, women with severe anemia during the third trimester were at higher risk of LBW after adjustment for malaria, gravidity, BMI at inclusion, infant sex, maternal age, maternal hypertension, intervention group and number of antenatal care visits (OR=3.4; 95%CI [1.4-8.1]). A dose-response relationship was found between Hb in four categories and LBW (p-trend=0.05; p-trend=0.02 in primigravidae). In conclusion, in the context of malaria prophylaxis and iron/folic acid supplementation during pregnancy, the prevalence of anemia was very high in pregnant women and its severity during the third trimester of pregnancy was associated with a higher risk of LBW.

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PHASE DEPENDENT SUB-CELLULAR FREE CALCIUM CONCENTRATION WITHIN *PLASMODIUM FALCIPARUM* INFECTED ERYTHROCYTES

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Calcium is a ubiquitous messenger in eukaryotic cell signaling. During the malaria parasite's intraerythrocytic cycle, free Ca^{2+} likely mediates various events including invasion, maturation, and gametocytogenesis, and must therefore be tightly regulated. Several groups have developed methods to quantify free Ca^{2+} within iRBCs, however, agreement between reported results is poor. We report new methods to characterize free Ca^{2+} within the parasite cytoplasm and the digestive vacuole (DV) for highly synchronized iRBC culture. iRBCs loaded with Fura-2 AM are used for quantifying cytoplasmic free Ca^{2+}, and dextran-conjugated Fura-2 is pre loaded into RBC for localization to the DV. We have studied the photochemical properties of the incorporated probes for both HB3 (chloroquine [CQ] sensitive) and Dd2 (CQ resistant) strains under perfusion with physiological perfusate. After calibration, growth phase dependent changes in Ca^{2+} for cytoplasm and DV were computed. We also measured the effects of bolus CQ treatments on free Ca^{2+} concentration in these compartments, at different growth phases.

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PLASMODIUM FALCIPARUM CULTIVATION IN A SODIUM-FREE MEDIUM

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The plasmodial surface anion channel (PSAC) is a broad permeability ion channel on human erythrocytes infected with *Plasmodium falciparum*. This channel increases the infected cell's permeability to organic and inorganic solutes, including Na^+. This permeability leads to a gradual increase in erythrocyte cytosolic Na^+ concentration, creating a gradient across the parasite plasma membrane that may be used to import other solutes. To test this hypothesis, we designed and evaluated Na^+-free media for *in vitro* growth of parasites. A novel medium containing sucrose as the primary osmotically active impermeant solute supported parasite survival and expansion at rates similar to those in standard RPMI 1640-based formulations. We did not detect a lag in parasite growth upon transfer to this medium, excluding a need for adaptation. Growth required supplementation with small amounts of human serum, which was the sole source of Na^+ in this medium. Because this medium's measured Na^+ concentration (~ 6 mM) is lower than that estimated for uninfected erythrocyte cytosol, net Na^+ efflux (not uptake) occurs in this medium over the course of the intracellular parasite cycle. These findings argue against physiologically significant Na^+-coupled solute transport across the parasite plasma membrane and exclude a role for Na^+ uptake in erythrocyte rupture at the end of the intracellular cycle. Increased host cell permeability appears to function primarily in parasite nutrient acquisition; Na^+ uptake is an unimportant byproduct.

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THE USE OF ARTEMISININ-BASED COMBINATION THERAPIES (ACTS) IN PUBLIC SECONDARY HEALTH FACILITIES IN LAGOS, NIGERIA - AN INTERVENTION STUDY

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The objective of this study was to assess the impact of an educational intervention program on the prescription pattern of Artemisinin Combination Therapies (ACTs) in public secondary health facilities in Lagos State, Nigeria. Five out of the ten General Hospitals that were studied initially, were selected for the post intervention study. A total of 1070 retrospective prescriptions of out-patients from March, 2008 were systematically sampled and assessed and the result compared with those of the baseline study. Intervention consisted of a national antimalarial policy sensitization programme for all stakeholders. The percentage of prescriptions containing ACTs had increased from 5.9% to 91.4% The prescription of artemisinin derivatives as monotherapy had reduced from 18.2% to 4.57% The prescription of chloroquine had decreased significantly from an average of 48.8% to 0.68% ($p \leq 0.05$). The percentage of prescriptions containing correct antimalarial doses had increased significantly from to 87.9%. to 96.9%. Prescribers were more favourably disposed to the ACTs policy change, which tallied with prescribing pattern. Those who attended ACTs training seminar were more likely to prescribe correct doses than those who did not. A sustained educational intervention programme coupled with "Eko free" antimalaria programme have impacted positively in terms of rational use of ACTs and the overall adherence to the national antimalarial treatment policy.

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INTERMITTENT PREVENTIVE TREATMENT USING ARTEMISININ-BASED COMBINATION THERAPY REDUCES MALARIA MORBIDITY AMONG SCHOOL-AGED CHILDREN IN MALI

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Among school-aged children, malaria causes symptomatic illness and anemia, resulting in absenteeism and impaired cognitive development. Intermittent preventive treatment (IPT) is effective for children under five years, but few studies have included school-aged children. We conducted an open randomized controlled trial of seasonal IPT among students from 6 to 13 years of age in Kollé, Mali. The study began in September 2007 and completed follow-up in May 2008. Students were randomized to one of three study arms: Sulfadoxine-pyrimethamine plus artesunate (SP/AS), amodiaquine plus artesunate (AQ/AS), or vitamin C. All students received two full treatment doses, given two months apart during the season of high transmission from September to December. Groups were compared with respect to incidence of febrile malaria, asymptomatic parasitemia, and blood hemoglobin concentration. 296 students were randomized, and retention in the study was 99.3%. Febrile malaria incidence in the SP/AS and AQ/AS arms was reduced by 66.6% and 46.5%, respectively, versus vitamin C ($p<0.001$). There were fewer acute clinic visits for any cause among the children receiving SP/AS or AQ/AS ($p=0.024$). The prevalence of asymptomatic parasitemia was >5-fold higher in the vitamin C arm than either SP/AS or AQ/AS at each post-treatment evaluation ($p<0.001$). At the end of the transmission period, children treated with IPT had lower rates of anemia (SP/AS 17.7%, AQ/AS 16.0%, vitamin C 29.6%; $p=0.039$). In conclusion, IPT reduced the rates of febrile malaria, all-cause acute clinic visits, asymptomatic parasitemia, and anemia among school-aged children.

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CURCUMIN ENHANCES NON-OPSONIC PHAGOCYTOSIS OF *PLASMODIUM FALCIPARUM* BY UP-REGULATING CD36 SURFACE EXPRESSION IN MONOCYTES/MACROPHAGES

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The class B scavenger receptor CD36 on monocytes/macrophages plays an important role in malaria innate immunity through opsonin-independant phagocytosis of *Plasmodium falciparum* parasitized erythrocytes (PE). Up-regulation of CD36 expression by peroxisome proliferator activated receptor γ-retinoic-X-receptor (PPAR-γ-RXR) agonists has been shown to enhance phagocytosis of PE and inhibit proinflammatory cytokine production. Curcumin, a natural product from *Curcuma longa* has demonstrated antimalarial activity *in vitro*, with an IC_{50} of 5~9μM against drug sensitive and resistant *P. falciparum*, arresting the intraerythrocytic development at the trophozoite stage. In this study, we report for the first time that curcumin increases CD36 expression in human monocytes at the protein and mRNA level and enhances the non-opsonic phagocytosis of *Plasmodium falciparum* parasitized erythrocytes. Curcumin-induced CD36 expression takes place following the production of reactive oxygen intermediates (ROI) and can be inhibited by the antioxidant N-acetylcysteine but not totally abrogated by the PPARγ antagonist, GW9662 indicating that CD36 expression on monocytes following curcumin exposure, is partially dependent on the activation of this nuclear transcription factor. The role of nuclear related {erythroid-derived 2} factor 2 (Nrf2), a redox-sensitive nuclear transcription factor, was investigated and shown to be an alternative PPARγ-independent pathway for CD36 induction by curcumin. Our study supports the view that harnessing innate immunity without harming the host can be useful in the management of microbial infection. This "host targeted approach" represents a novel strategy to complement the direct anti-parasitic effect of compounds with antimalarial activity and as such could be a valuable tool in limiting the emergence of drug-resistant parasites.

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MALARIA-INFECTED MICE LIVE UNTIL AT LEAST DAY 30 AFTER A NEW MONOMERIC TRIOXANE COMBINED WITH MEFLOQUINE ARE ADMINISTERED TOGETHER IN A SINGLE LOW ORAL DOSE

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In only 5 simple steps and 48% overall yield from natural trioxane artemisinin, the thermally stable fluoroanilide analog 4b was synthesized. A single oral dose of only 6 mg/kg of monomeric trioxane 4b combined with 20 mg/kg of mefloquine hydrochloride caused five out of five mice infected with the malaria parasite *Plasmodium berghei* to live until at least day 30 post infection. Of the surviving mice, four were cured (parasite-free) and one mouse had 4% blood parasitemia. A single oral dose of 13 mg/kg of trioxane fluoroanilide 4b plus 13 mg/kg of mefloquine hydrochloride also prolonged survival of all of the mice until day 30, with four mice cured and one mouse having 2% blood parasitemia. The four cured mice were reinfected and experienced an extended survival time of approximately four days longer than the infected control group.

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SEX-RELATED DIFFERENCES IN THE STEADY-STATE PHARMACOKINETICS OF PRIMAQUINE IN HEALTHY SUBJECTS

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Primaquine is the only drug available for either radical cure or post-exposure prophylaxis of *Plasmodium vivax* malaria. Limited data has indicated that sex-related differences may exist in the pharmacokinetics of primaquine at steady-state. The aim of this study was to determine whether gender affects the steady-state pharmacokinetics of primaquine in healthy subjects. Seventeen male and 17 female healthy Vietnamese subjects were administered 30 mg (base) of primaquine daily for 14 days. Plasma primaquine and its major metabolite, carboxyprimaquine concentrations were measured by HPLC at the end of the 14 day regimen. Females had significantly higher median values of C_{max} (212 vs. 122 ng/mL, $P < 0.001$), AUC_{0-24} (1,909 vs. 917 ng·h/mL, $P < 0.001$), but smaller Vss/F (3.42 vs. 4.59 L/kg, $P = 0.03$) and slower CLss/F (0.31 vs. 0.55 L/h/kg, $P < 0.001$) of primaquine compared with males. Similar to primaquine, females had significantly higher median steady-state values of C_{max} (2,409 vs. 1,957 ng/mL, $P = 0.01$) and AUC_{0-24} (47,085 vs. 36,511 ng·h/mL, $P = 0.005$) of carboxyprimaquine compared with males. Although the average weight of the females was 10 kg lighter (50.4 vs. 60.3 kg) than the males, the sex-related differences in the disposition of primaquine cannot be explained solely by weight differences. The primaquine pharmacokinetic data suggest that females have increased exposure to primaquine, which may put them at increased risk for toxicity when administered the same maintenance dosage as males.

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EFFECT OF EXOGENOUS ANGIOPOIETIN DURING EXPERIMENTAL CEREBRAL MALARIA

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Cerebral malaria is a neurovascular pathology that still carries an unacceptable fatality rate despite the use of artemisinin based combination therapies. Adjuvant treatment is expected to be new opportunities for survival increase during the acute phase of the disease. Most of previous adjuvant trials in humans focused on the inflammatory response to the infection. Targeting the vascular component of the disease could be a new strategy. Promising actors involved in the control of vascular homeostasis are the angiopoietin - Tie system. It exerts important maintenance functions of the quiescent vasculature and is involved in vascular development and remodelling to control vessel assembly, maturation and differentiation. The angiopoietin-Tie system is involved in septic and ischemic processes that could lead to clinical benefit during cerebral malaria: angiopoietin 1 reduces the mortality induced by endotoxin during lung edema; angiopoietin 2 plays a critical role in blood flow recovery after femoral artery occlusion. More interesting, is the recent demonstration of these angiogenic factors to be associated with outcome of severe *falciparum* malaria, as reported previously. In order to decipher the complex mechanisms involved in neurovascular interactions during severe malaria, we used exogenous drugs to modulate the angiopoietin system in infected mice. Mice were treated with recombinant angiopoietin 1 at the onset of the symptoms for 3 days. Clinical stages, parasitemia and weights were collected during the follow-up. Kaplan Meier survival analyses were performed to analyse effects of the treatment on survival compared to control mice. Angiopoietin balance modulation is a vascular alternative for adjuvant therapy that is needed to be extensively explored.

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EMERGENCE, SEX RATIOS OF FIRST-APPEARING AND CLEARANCE OF *PLASMODIUM FALCIPARUM* GAMETOCYTES IN CHILDREN TREATED WITH MEFLOQUINE OR ARTESUNATE-MEFLOQUINE

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The gametocyte sex ratio of *Plasmodium falciparum* may play a role in transmission but little is known of effects of chemotherapy on sex ratios of newly- emerging (first-appearing) gametocytes. We evaluated the emergence, sex ratios of first appearing gametocytes, the temporal changes in sex ratios in cohorts of first appearing gametocytes, and clearance of gametocytes following treatment with mefloquine or artesunate-mefloquine in 350 children with acute falciparun malaria. Ninety one and ninety six percent of all gametocytes appeared by day 7 and day 14, respectively following treatment. The overall risk of gametocytaemia was 31%. The risk of gametocytaemia was significantly higher in mefloquine than in artesunate-mefloquine treated children if no gametocytaemia was present a day after treatment began (25.3% versus 12.8%, P = 0.01). Gametocyte clearance was significantly faster with artesunate-mefloquine (1.8 ± 0.22 [sem] *v* 5.6 ± 0.95 d; P = 0.001). Pre-treatment sex ratios, the sex ratios of emerging gametocytes, and temporal changes in the sex ratios of cohorts of emerging gametocytes were similar and were male-biased in both treatment groups. Artesunate may significantly modify the emergence and clearance of gametocytes but may not significantly modify the sex ratios of emerging gametocytes when combined with mefloquine in children from this endemic area.

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CLINICAL STUDIES ON PRIMAQUINE-INDUCED HEMOLYSIS IN G6PD-DEFICIENT PATIENTS

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8-aminoquinolines (8AQs) are the only effective class of drugs against *Plasmodium vivax* (Pv) hypnozoites and mature stage 5 *P. falciparum* (Pf) gametocytes. Primaquine (PQ) is the only approved and commercially available 8AQ drug and may play an important role in malaria control and elimination. Unfortunately PQ is known to cause hemolytic anemia (HA) in individuals with glucose-6-phosphate dehydrogenase (G6PD) deficiency (G6PDd) thus limiting its role in settings in which screening for G6PDd cannot be reliably or logistically performed. Because concern over HA prevents the routine use of PQ, G6PDd data were identified and reviewed.We conducted a systematic review of the published and unpublished literature of G6PDd subjects receiving PQ in clinical studies to identify the risk of serious adverse events in G6PDd persons. We also performed a separate search to define all identifiable deaths from PQ. Papers in languages other than English were also accessed if an English translation was available. Data were entered and analyzed in Excel (2007) and SPSS for Windows, version 16. Between 1948 and January 2009, a total of 84 clinical studies (157 clinical series) on PQ in humans (total patients N = 68,521) were identified. Of these, 37% (31/84) included G6PDd patients (N = 534). Among them, 303 (57%) were reported to develop hemolysis. The reduction in hematocrit ranged from 4.1% - 23%. Twelve patients in 5 studies were reported had clinically significant HA resulting in discontinuation of PQ. Seven cases from 3 studies required blood transfusion. Five cases from 4 studies developed acute renal failure, 3 of whom needed dialysis. In the overall literature, only four deaths attributable to PQ were identified in one publication. All four were children in Sri Lanka given the adult equivalent dose of 15-30 mg PQ (two G6PDd, two not able to be tested). In conclusion, this extensive evidence-based review will contribute to the risk-benefit evaluation of PQ and the need for G6PDd screening when the use of PQ is planned in different epidemiologic settings.

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INCREASING ACCESS TO ARTEMESIN-BASED COMBINATION THERAPY (ACT) IN POST-CONFLICT ENVIRONMENTS: THE EXPERIENCE OF SOUTHERN SUDAN

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Over 21 years of conflict collapsed what little infrastructure and health system development existed in Southern Sudan. The signing of the Comprehensive Peace Agreement in January 2005 led to greater stability, but medical needs remain critical. The complexity of this situation is compounded by an influx of internally displaced people and refugees, extremely low population density, and difficult access, especially during the wet season. Additionally, an absence of logistics data, inadequate monitoring and evaluation, and a weak drug procurement and supply management systems have resulted in country-wide shortages of antimalarials. Malaria is the leading cause of morbidity and mortality in Sudan, accounting for 20-40% of outpatient clinic visits and approximately 30% of hospital admissions. ACTs were adopted as policy in 2004. The President's Malaria Initiative supported this policy through the provision of 1.5 million ACT treatments and technical assistance to facilitate their clearance, receipt, storage, and distribution. Faced with existing impediments, the Ministry of Health of the Government of South Sudan (GOSS) partnered with the UN to deliver ACT shipments by air to seven states and used humanitarian NGOs for onward distribution to service delivery points. The GOSS is committed to developing a logistics system that includes rational procurement planning and procedures based on annual product quantifications, regular supply plans, and a harmonized LMIS at all levels. By understanding the complexity of Southern Sudan and its successful ACT implementation efforts, policy makers and program managers can learn how to expand access to ACTs in other post-conflict environments.

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EFFECT OF MICROWAVE FREQUENCY ON THE *IN VITRO* GROWTH OF *PLASMODIUM FALCIPARUM*

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All malaria parasites digest hemoglobin. Because the heme moiety of hemoglobin is toxic due to the presence of iron as Fe^{2+}, the parasite processes it into hemozoin pigment, a more inert form which contains iron as Fe^{3+}. Malaria parasites can effectively process and store most of the intracellular iron initially found in the red cell. We reasoned that this property of malaria parasites may make them sensitive to microwave frequency for two reasons: 1) powder metals easily absorb microwave radiation and heat up preferentially over other constituents of blood and 2) the Fe^{3+} is more susceptible to magnetic field than the Fe^{2+}. Therefore, we hypothesized that microwave radiation can be used to target the iron in the food vacuole of malaria parasites leading to thermal ablation. To test our hypothesis we exposed *Plasmodium falciparum* ring and trophozoite stages in culture to microwave single (magnetic) and combined (electric and magnetic) fields of 2.45 GHz frequency. Both rings and pigment-containing trophozoite stages appeared to be sensitive to short exposure of combined electric and magnetic fields in a dose-dependent manner with no apparent damage to the red cells. We will present parasite survival data as well as ultrastructural data of the effect of

microwaves on the parasite. These preliminary experiments suggest that exposure to low frequency microwave is worth evaluating as a possible treatment modality for malaria.

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PHARMACODYNAMICS OF HYDROXAMATE-BASED HDAC INHIBITORS IN *PLASMODIUM FALCIPARUM*

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Histone deacetylase (HDAC) inhibitors are receiving increasing attention as potential new antimalarial drug candidates. A possible advantage of this class of compounds in the context of targeting drug resistant parasites is that they may affect multiple parasite pathways via global changes in gene transcription or inhibit deacetylation of non-histone proteins. Hydroxamic acid-based compounds, which are also being developed for the treatment of other diseases including cancer, represent one of the most promising classes of antimalarial HDAC inhibitors studied to date. A better understanding of the pharmacodynamics of these compounds will help drive drug discovery efforts in this area. We have previously shown that a range of hydroxamic acid-based HDAC inhibitors display potent (low nM) and selective (up to ~600 fold) antimalarial activities *in vitro* against *Plasmodium falciparum*, and one of these compounds has shown promising *in vivo* efficacy in a mouse model of malaria. Here we have carried out *in vitro* pharmacodynamic studies on these compounds and show that they inhibit histone deacetylase activity in *P. falciparum* nuclear extracts (IC_{50} <100 nM), that they preferentially target trophozoite-stage over ring stage parasites, and that that they can act within four to six hours. Significant ultrastructural changes are apparent in trophozoites treated with these compounds, including alterations in nuclear integrity. Genome wide microarray analyses show that up to ~20% of genes in trophozoites are transcriptionally altered following exposure to hydroxamate-based HDAC inhibitors. Affected genes include homologues of eukaryotic genes that are recognized molecular markers of HDAC inhibition. Taken together these data extend our understanding of how hydroxamate-based HDAC inhibitors act in malaria parasites and this may contribute to further development of this class of compounds for use against malaria and to the identification of new molecular markers for rational drug discovery approaches.

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TARGETING PFHSP90 IN *PLASMODIUM FALCIPARUM* MALARIA: A STRATEGY TO REVERSE RESISTANCE

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Hsp90 is a critical chaperone in the trafficking of a vast array of client proteins in eukaryotic cells. Our strategy aims to identify inhibitors of Hsp90 in *Plasmodium falciparum* (PfHsp90) using a protein-based high throughput screen (HTS) strategy. Hits will be confirmed using both cell-based and animal model studies where hits are combined with known antimalarial in order to determine whether synergy exists. In parallel, human Hsp90 inhibitors currently in clinical trials will be assessed for PfHsp90- and antimalarial activity and optimized for more specific anti-malarial activity. This study had two goals: (i) to design a high-throughput PfHsp90 protein-based *in vitro* chemical library screen; and (ii) to screen human Hsp90 inhibitors currently in phase II clinical trials for antimalarial activity. Three methods were utilized: (1) The ATP-binding domain of PfHsp90 was expressed using prokaryotic system. The protein was purified using DE52 anion-exchange and Ni-NTA chromatography; (2) An *in vitro* ATP-binding inhibition assay using bis-ANS (a fluorescent ATP analogue) was developed; and (3) A flow cytometry-based SYBR green assay was adapted for the *P. falciparum* cell-based screen. The ATP binding domain of PfHsp90 was successfully cloned, expressed and purified. The bis-ANS ATP-binding screen was adapted to a robotic facility capable of screening 1000 compounds daily. In the initial screen of a natural compound library, compounds such as quinacrine and Gentian violet were identified as potent Hsp90 ATP-binding domain inhibitors. The initial results of the PfHsp90 protein screen and antimalarial sassay for the natural compound and small drug-like compound libraries (65,000 compounds total) will be presented. In parallel, PU H71, a phase II anticancer agent that targets human Hsp90 demonstrated potent ATP-binding inhibition of PfHsp90 and significant antimalarial activity *in vitro* (IC50 of 11.6nM). Efforts are underway to synthesize a more specific antimalarial inhibitor using the PU H71 scaffold with PfHsp90 modeling and crystal structure as guide. In summary, our two-pronged HTS and synthetic chemical strategy have identified potent and novel antimalarial hits that target PfHsp90.

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EVALUATION OF A FACS-BASED METHOD USING AUTOFLUORESCENCE AND DNA STAIN YOYO-1 FOR PARASITE QUANTIFICATION IN PERIPHERAL BLOOD FROM CHILDREN IN RURAL MOZAMBIQUE

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An increasing volume of clinical trials and epidemiological studies in malaria calls for new and better tools for detection and quantification of *Plasmodium falciparum* infection. A flow cytometry-based method of quantifying parasitemia has been developed in the mouse models of malaria. This method (FACS FL2/YOYO-1) exploits the autofluorescence patterns of fixed blood stained with YOYO-1, a DNA-specific dye, to identify *Plasmodium*-infected cellular events. This study aimed to assess the FACS FL2/YOYO-1 method in human samples acquired in a malaria-endemic setting and assess its utility in epidemiological studies of malaria. Small volumes of blood were collected from 100 children admitted to the inpatient department of a health center in rural Mozambique. Blood was analyzed for hematology and parasite density by microscopy. 2 µL of blood was fixed in glutaraldehyde, permeabilized, treatmented with RNase, and stained with YOYO-1. Stained samples were acquired on a FACSCalibur. A unique stage-specific population pattern of infected erythrocytes was identified by adjusting the compensation of FL1 into FL2 (FL2 -%FL1). Regions were placed around the infected erythrocyte and leukocyte populations to quantify parasitemia and parasite density. Assessment of parasite density by the FACS FL2/YOYO-1 method correlated with microscopic assessment of parasite density and increased limits of quantification and detection. The FACS FL2/YOYO-1 method may be a valuable tool for malaria epidemiological studies and clinical trials due to its high range of quantification and detection. Additionally, the high throughput nature of the method makes it practical for large-scale studies.

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SIMILAR EMBRYONIC TOXICITY AND COMPARABLE PHARMACOKINETICS OF ARTESUNATE FOLLOWING MULTIPLE INTRAVENOUS AND INTRAMUSCULAR DOSES IN PREGNANT RATS

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Artesunate (AS) has been used as treatment for malaria in Asia and Africa for decides and has been generally recognized as being rapid, effective and safe. However, concerns about its safety in pregnancy have been raised following detection of embryotoxicity in animal species exposed. In the present study, mature SD female rats were mated with males and selected by sperms in vaginal smears, and dosed AS (GMP product) intravenously and intramuscularly. Pregnant animals were injected at 0

(vehicle control), 0.50, 0.55, 0.60, 0.65, 0.75, 1.0, 1.25, and 1.5 mg/kg/d for 13 days from gestation day (GD) 6 to 18. Dams were euthanized on GD20 and fetuses were examined. After i.v. and i.m. dose range studies, the moderate-effective toxic dose was subsequently tested in the first (GD6-10), second (GD11-15) and third (GD16-20) periods of pregnancy. A bioavailability study was also conducted to evaluate pharmacokinetics of AS following i.v., i.m. and subcutaneous administrations in normal rats. No significant adverse effects were found in dams. All or several of fetuses were resorbed by placentas in all treated pregnant uteri except in control group, and the survived rate of fetuses is dose-dependent. The maximum dose that did not show fetuses resorbed was 0.4 and 0.5 mg/kg, and the dose to resorb a half of fetuses was 0.60 and 0.61 mg/kg following i.v. and i.m. injections, respectively. The highest toxic effect or the most sensitive period for injectable AS (i.v. and i.m.) was confirmed between GD11 and GD15. When calculated with total concentrations of AS and DHA, the bioavailabilities of 82.5 and 97.8% after subcutaneous and intramuscular, respectively, administrations were fulfilled FDA Guidance in a bioequivalence with intravenous treatment. Injectable AS applied during pregnancy exhibited a clear embryotoxicity in rats at relatively low doses. The similar embryotoxicity finding after i.v. and i.m. AS seems related to their comparable pharmacokinetic profiles.

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IDENTIFICATION AND VALIDATION OF THE *PLASMODIUM* CELL-CYCLE IN BLOOD STAGE BY FLOW CYTOMETRY IN COMPARISON TO OPTICAL MICROSCOPY

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The asexual blood stages of the *Plasmodium berghei* (PB) life cycle are attractive targets for transmission blocking drugs. Improved understanding of PB blood-stage growth and development would provide new opportunities to evaluate antimalarial drugs. The aims of the present study were to obtain full evaluation and validation of the PB model with life cycle by flow cytometry (FCM) technique, and compared with light and fluorescent microscopes. Blood stage samples from C57BL/6 mice infected with PB sporozoites were singly stained with YOYO-1 DNA dye and measured by FCM. Slides were made from same samples, and stained with diluted Giemsa's and YOYO-1 fluorescent stains, respectively. Correlated three results were evaluated by Beckman FC500 flow cytometer, light and fluorescent microcopies. The results for the stage determination by microscopy are shown the mean percentage of ring and early trophozoites was 47.55%, and followed by 28.55% of later trophozoites including 2-3 multiple infections, 16.69% of immature schizonts including 3-5 multiple infections, and 7.22% of mature schizonts during the 48 hours life-time. Flow cytometry demonstrated a clear separation between various stages. When compared to light microscopes, a strong correlation (r^2 = 0.9253-0.9739) between the two methods was observed in determining ring, trophozoites and schizonts phases, suggesting that flow cytometry technique can replace microscopy for stage monitoring of PB parasites in mice. Studies demonstrated that the flow cytometer, being superior in terms of speed, lower sampling error, accurate and reliable identification of rodent erythrocyte parasites in life stages based on the principles of FCM, makes an ideal alternative to the microscope. Flow cytometry offers several advantages over conventional methods including microscopy. Due to these advantages, the life stage development model for *P. berghei* in mice blood has been launched by flow cytometry, which could widely use for drug and vaccine research in malaria therapy and prophylaxis.

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IMPROVING RELATIVE BIOAVAILABILITY OF ORAL WR299666 BY REDUCING PARTICLE SIZE USING HOMOGENIZER AND ULTRA-SONICATOR

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Particle size is an important determinant of gastrointestinal absorption in human and animal species by oral administration. Although particle size reduction increases the bioavailability were reported, the affect of a reduction in particle size on the bioavailability of WR299666, a new antimalarial compound, is unclear. Three oral formulations of WR299666 were made by glass drive, high-speed homogenizer and ultra-sonicator, and their particle sizes were measured by a LA-930 laser particle size analyzer. The two new formulations at 50 mg/kg were administrated intragastrically to mice, and blood samples were collected for LC-MS/MS analysis. The results indicated that the particle size was shown significant difference in the formulations made by different techniques. Glass grinder method manually decreased the particle size of W299666 with minimal change in particle diameter of 42.22 µm. Sonicator method evenly distributed pulses of sound to break apart particle diameter with a mean of 1.5 µm. Particle size was made at 5000 RPM with homogenizer and resulted in into average of 1.44 µm. If set a bioavailability as 100% for small particle size (1.50 µm) formulation in mice, the relative bioavailability of WR299666 with big particle size (42.22 µm) was shown only 55.79%. The initial data were demonstrated by the conclusive evidence that the particle size of drug in oral suspension formulation is technique dependent. Particle size can be reduced by using glass grinder, high speed homogenizer and ultra-sonicator. However, high speed homogenization is not suitable for the drug of WR299666 because the speeding generated bubbles that interfered with homogenizer knife. Decreased homogenizer speed can eliminate issues from air bubbles. Therefore, homogenizer speed must be taken into account to reduce air bubbles and formulation complication. In the present report, sonication was found to be the more effective technique in particle size reduction. Similar to other findings, the reduced particle size of WR299666 can significantly increase its oral bioavailability in rodent animals.

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METABOLIC STABILITIES AND METABOLITE PROFILES OF ARTESUNIC ACID (AS) AND ARTELINIC ACID (AL) IN CRYOPRESERVED HUMAN HEPATOCYTES

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Human hepatocytes are the *in vitro* system of choice to study and predict drug properties in man. The present study was to clarify their metabolic profiles by evaluating the power of the metabolic profiles of artesunate (AS) and artelinic acid (AL), determined in human hepatocytes. A standard and validated culture technique with human cryopreserved hepatocytes is established and the radiolabeled drug was dissolved in the mixture of the BD medium and then incubated at 37°C with shaking. After incubation, suspension samples were taken at 0.1, 5, 10, 20, 40 min; then 1, 2, 4, 6 and 24 hr. These suspension samples, after spiked with 1:9 ethyl acetate/n-butyl chloride, were dried as free fraction. The remaining aliquot suspensions were hydrolyzed by glucuronidase/arylsulfatase as conjugation fraction. All fraction samples were analyzed by HPLC with gradient elution and radioflow detection. Artesunic acid displayed unstable properties such as a short clearance half-life of 0.82 hours, a total of 5.60% left as unchanged drug, and a rapid clearance at 13.79 ml/hr. There was a high conversion rate (17.11% of total drug) of AS to dihydroartemisinin (DHA), a compound with same antimalarial efficiency of AS. However, AL holds most of its parent compound after metabolism, retaining 89.55% of unchanged AL, and a low clearance rate (0.35 ml/hr) was also found. The slower metabolizing of the compounds in AL yield a great increase in the half life (33.74 hours), increasing higher stability. Also, DHA converted

from AL parent drug was limited (0.53%). Regarding metabolites, artesunic acid was discovered to have 12 major metabolites with each of them composing over 3% of the total drug. Among the metabolites found, two long lasting metabolites in AS were found with 17.4 and 23.63 hours of the half-life. Meanwhile, only 9 metabolites were detected in AL and no single metabolite was over 3% of the total drug. Rapid elimination and high rate of conversion of AS to DHA showed a powerful antimalarial potency and excellent safety in malaria treatment, due to short exposure time. Although AL have not been tested in humans yet, the metabolic profile of AL can predicate that AL would be less efficient and have higher toxicity because of the limited conversion of AL to DHA and long drug exposure time.

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TRIANGULAR TEST DESIGN TO EVALUATE TINIDAZOLE IN THE PREVENTION OF *PLASMODIUM VIVAX* RELAPSE

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There are very few drugs that prevent the relapse of *Plasmodium vivax* malaria in man. Tinidazole is a 5-nitroimidazole approved in the United States for other indications. It has shown cure in 1 of 6 Rhesus monkeys studied with an apparent mild delay to relapse in the other 5 of 6 in a relapsing *P. cynomolgi* model and one study has claimed activity against *P. vivax* in man. We report on a phase II, randomized, open-label study conducted along the Thai-Myanmar border designed to evaluate the ability of tinidazole to prevent relapse of *P. vivax.* We use a modified triangular test sequential analysis which allows repeated evaluation during the course of enrollment while maintaining a specified power and type 1 error and minimizing recruitment of subjects. Enrollment was to be halted when a pre-specified success/failure ratio was surpassed. The study was designed to have a 5% type 1 error and 90% power to show whether tinidazole would produce a relapse rate of less than 20% or greater than 45% through day 63 of weekly follow-up after initiation of treatment and initial parasite clearance with 3 days of a oral weight based dosing of chloroquine and 5 days of 2 grams/day of tinidazole. Results: All subjects cleared their parasitemia by day 3. Six of the first seven subjects treated with tinidazole relapsed prior to day 63 (average day 48.3 (range 42-56)). This exceeded the upper boundary of the triangular test and enrollment to receive tinidazole was halted. A concurrent cohort definitively treated with primaquine and chloroquine showed no episodes of reinfection (n=5). Conclusions: Tinidazole is ineffective in preventing relapse of *P. vivax* at the dose used. The Rhesus relapsing model appeared to correctly predict outcome in humans. Use of the modified triangular test allowed minimal enrollment and limited unnecessary exposure to the study drug and reduced costs. This adds weight to the ethical and economic advantages of this study design to evaluate similarly situated drugs.

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A NOVEL SERIES OF *PLASMODIUM FALCIPARUM* DIHYDROOROTATE DEHYDROGENASE INHIBITORS WITH *IN VIVO* ANTIMALARIAL ACTIVITY

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Dihydroorotate dehydrogenase (DHODH), an enzyme of the *de novo* pyrimidine biosynthetic pathway, catalyzes the formation of dihydroorotate through a coupled redox reaction utilizing a mitochondrial respiratory chain ubiquinone. *Plasmodium falciparum* is unable to salvage pyrimidines and must rely on *de novo* biosynthesis for survival. The anti-malarial agent atovaquone indirectly inhibits DHODH activity by disrupting the electron transport chain. A chemical library of approximately 217,000 compounds was screened for inhibitors of *P. falciparum* DHODH (*Pf*DHODH) activity, and a potent compound was identified from which analog synthesis is currently ongoing. A series of 5-benzimidazolyl-N-alkylthiophene-2-carboxamides has demonstrated double-digit nanomolar *in vitro* potency against the enzymes from *P. falciparum, P. vivax,* and *P. berghei*, with corresponding efficacy against the *P. falciparum* 3D7 and Dd2 parasites. The mechanism of action of this series was confirmed using a transgenic *P. falciparum* strain expressing a divergent homolog of DHODH, this parasite strain being resistant to all compounds tested to date. Several of the most potent compounds demonstrated good tolerability and oral exposure in the mouse, and oral b.i.d. dosing of Genz-666658 at 200 mg/kg/day in the 4-day murine *P. berghei* model resulted in a highly significant 50% reduction in parasitemia ($p<0.001$). Further analog synthesis is proceeding with the aim of increasing potency while improving physical properties and metabolic stability. Scale-up of relevant compounds for further *in vivo* tolerability and efficacy studies is continuing and an iterative lead optimization process is underway.

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THE DEVELOPMENT, OPTIMIZATION AND VALIDATION OF A HIGH THROUGHPUT SCREENING ASSAY FOR ANTI-MALARIA DRUGS DISCOVERY

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The emergence and spread of *Plasmodium falciparum* parasite resistant to many antimalarials, particularly chloroquine (CQ) and pyrimethamine (PY)-sulfadoxine (SD), is largely responsible for the recent global resurgence of malaria, which severely hampers our capacity to roll back malaria. As the last line of defense against drug resistant malaria, the development of resistance to artemisinin and its derivatives would be a disaster for global malaria control efforts. Therefore, development of novel antimalarial therapies is an urgent issue. Here, we present the development, optimization and validation of a novel High-Throughput Screening (HTS) assay for antimalarial drug discovery and development. Using a recently developed transgenic *P. falciparum* strain expressing firefly luciferase reporter gene, this assay was first adapted in 96-well plate, and then miniaturized in 384-well plates. Assay conditions, including the parasite numbers, parasitemia, hematocrit, DMSO concentrations, were optimized using artimisinin as the positive drug control. Assay robustness, including Z'-value, coefficient variation (CV) and signal-to-background ratio (S/B ratio), were also determined. A 3,000 small compound library, with known antimalarial drugs as positive controls, was used to validate the assay. Our results showed an average Z'-value as up high to 0.8, CV of 5% or lower,

and S/B ratio of 20. This result demonstrated that this assay is robust, and can be used for future HTS against large compound library. Our long term goal is to develop novel antimalarial drugs to counter the wide spread burden of malaria.

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3D-QSAR MODELS TO PREDICT *IN VITRO* ANTIMALARIAL EFFICACY AND METHEMOGLOBIN TOXICITY OF 8-AMINOQUINOLINE ANALOGS AND DERIVATIVES

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8-Aminoquinolines (8-AQ) such as primaquine, tafenoquine etc are important antimalarials because of their activity against the exoerythrocytic forms of *Plasmodium* species. Therefore, they are also an excellent choice for causal prophylaxis against malaria. However, their use has been severely hampered because of reported hemolytic toxicity, especially in the glucose-6-phophate dehydrogenase (G6PD) deficient individuals. Thus, the objective of this study was to develop approaches for design of novel 8-aminoquinolines with reduced hemolytic toxicity and improved antimalarial efficacy, resulting in higher therapeutic indices. Towards this end, we computed 3D-Quantitative Structure Toxicity Relationship (3D-QSTR) models for an *in vitro* marker (methemoglobin) of hemolytic toxicity and 3D-Quantitative Structure Activity Relationship (3D-QSAR) models for *in vitro* efficacy against erythrocytic form of *Plasmodium falciparum*. The data set of 37 8-AQs analogs for *in vitro* methemoglobin generation in normal human blood in presence and absence of mouse liver microsomes was used for generation of QSTR models. The chemical functions of 30 8-AQ analogs (the training set) were analyzed through toxicophore and physicochemical properties based 3D-QSTR models for the prediction of hemolytic toxicity. The 3D-QSTR models were generated with correlation coefficients ranging from 0.97 to 0.99 and the predictive r^2 ranging from 0.7 to 0.9. The models were validated with test-data set of 7 analogs. The data set of 68 8-AQs on *in vitro* antimalarial efficacy against blood stage *P. falciparum* was obtained from WRAIR database. The chemical functions based pharmacophore and physicochemical properties based 3D-QSAR models were generated with training data set of 37 analogs. Remaining 31 analogs were used as test-set to validate the models. The 3D-QSAR models were obtained with correlation coefficients ranging from 0.8 to 0.9 and the predictive r^2 ranging from 0.5 to 0.8. The QSTR and QSAR models may be further validated and employed for design of better therapeutic index analogs.

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A HIGH-THROUGHPUT *IN VITRO* SCREEN TO IDENTIFY INHIBITORS OF THE *PLASMODIUM FALCIPARUM* HEAT SHOCK PROTEIN 90 BINDING ACTIVITY

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Heat shock proteins (HSPs) are a class of evolutionary highly conserved molecular chaperones that facilitate normal protein folding and that serve as a buffer for proteins, which have become unstable due to environmental stress and to prevent cellular toxicity caused by misfolded and aggregated proteins. One of the best-studied members of the HSP family is HSP90. A number of independent studies have demonstrated that HSP90 is essential in organisms ranging from yeast to mammals and that inhibition of HSP90 activity by small molecules is lethal. HSP90 proteins have been shown to be highly expressed in *P. falciparum* and the sensitivity of *P. falciparum* to known HSP90 inhibitors such as Geldanamycin (GA) suggested that inhibition of pfHSP90 activity may present an opportunity for a targeted anti-malarial drug development program. This was further encouraged by our identification of small molecule modulators showing differential inhibition of human HSP90 over pfHSP90, despite the high sequence homology between the host and parasite enzymes. In an effort to identify novel compounds with selectivity for pfHSP90 we developed and optimized an *in vitro* fluorescence polarization assay, which was used to screen >200,000 compounds. As secondary screens, we have developed a novel chemiluminescence based ELISA to exclude false positives caused by fluorescent compounds. More than 700 compounds were discovered to achieve > 40% displacement of the GA/pfHSP90 complex at 10 uM and subsequent hit confirmation resulted in the selection 196 compounds for specificity testing in an hsHSP90 counter screen. Selected compounds were tested in a parasite growth assay to confirm *in vitro* activity.

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LINKING ANTI-*PLASMODIUM* COMPOUNDS TO THEIR PROTEIN TARGETS VIA HIGH-THROUGHPUT ENZYME ACTIVITY ASSAYS

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Target-based approaches to drug discovery research are often limited by the inability of target inhibitors to reach their targets *in vivo*, while phenotype-based (cell-based) approaches are hampered by an ignorance of cell-active compounds' mechanisms of action. To expedite development of new antimalarial drugs, we have taken a hybrid approach consisting of identification of anti-*Plasmodium* compounds followed by identification of some of these compounds' possible targets. A screen of a library of ~650,000 small molecules revealed about 5,500 compounds that inhibit the growth of *P. falciparum* cultures with EC50 values below 1.25 µM (see ASTMH abstract "Generation and characterization of a 5K antimalarial compound collection" by K. Kuhen et al.). These 5,500 cell-active compounds were then tested in high-throughput assays against enzymes believed to have excellent potential as drug targets. These enzymes included several involved in nucleotide metabolism (e.g., adenylosuccinate synthetase, dUTPase, guanylate kinase, OMP decarboxylase) as well as components of other key metabolic pathways (e.g., choline kinase, glutamate dehydrogenase, S-adenosylhomocysteine hydrolase). IC50 values of preliminary hits against enzymes were then checked with repurchased compounds. In cases where a compound's IC50 value against a particular enzyme is much lower than its EC_{50} value against *P. falciparum* cultures, the compound may be hypothesized to kill the parasite via its inhibition of the enzyme. Our results suggest that high-throughput enzyme inhibition assays with cell-active compounds can be useful in identifying possible targets of these compounds, provided that the number of compounds and enzymes tested is sufficiently large. Our results also underscore the importance of follow-up studies (e.g., increases in EC50 value following overexpression of the putative target) to confirm tentative compound-target associations.

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DETECTION OF *IN VIVO* CHLOROQUINE RESISTANCE IN *PLASMODIUM FALCIPARUM* IN CHENNAI, INDIA

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Malaria has persisted in Chennai city in spite of various control measures undertaken over the years. *In vivo* studies on chloroquine resistance undertaken revealed therapeutic failure (36%), out of the 50 study subjects completed and followed up. Reappearance of the parasites was observed between Day 14 and Day 28 indicating Late Treatment Failure (LTF). However, all of them responded well to Sulfadoxine-Pyrimethamine (S-P) combination. The results of *msp*[1], *msp2* and *glurp* genotyping of paired samples of *Plasmodium falciparum*, collected on day 0 and the day of recrudescence from 16 of the apparent treatment failures, indicated that 15 (93.7%) of the 16 were probably true treatment failures. Development of high level of chloroquine resistance in *P. falciparum* in Chennai may be due to the intense drug pressure along with perennial transmission by the urban malaria vector, *Anopheles stephensi*. The present study warrants a change in the drug regimen for effective control of malaria to prevent drug resistant foci to other areas.

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IN VITRO MALIAN *PLASMODIUM FALCIPARUM* SUCEPTIBILTY TO ARTEMISININ AND PREVALENCE OF PFATP6 S769N MUTATION

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Artemisinin-based combination therapies are now first line malaria therapies in most malarious areas. Recent studies in French Gyanna showed that mutation was associated with raised IC50s of artemether. We investigated the *in vitro* susceptibility of Malian *P. falciparum* isolates to artemisinin and measured the prevalence of *PfATP6* S769N. Fresh *P. falciparum* positive blood samples were collected in Bougoula-Hameau, Mali. In vitro 3H-hypoxanthine based method was used to assess IC50s for artemisinin. A nested PCR followed with restriction digestion was developed to analyze the mutation of *PfATP6* S769N. Forty five (45) *P. falciparum* field isolates were collected in 2004-2005 from Bougoula-hameau. Two (4.9%) cases of PfATP6 769N were detected in 41 samples successfully analyzed. Mean IC50 for artemisinin was 1.9 nM. In conclusion, we show the presence of *PfATP6* 769N mutation in Mali. All tested isolates were fully susceptible *in vitro* to artemisinin.

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A MOUSE-SCREENING MODEL FOR RESISTANT *PLASMODIUM FALCIPARUM* DHFR-TS INHIBITORS

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Pyrimethamine, an antifolate inhibitor of dihydrofolate reductase (DHFR), was the mainstay of anti-malarial chemotherapy for many years. Although Pyrimethamine use is discontinued owing to the spread of resistance-causing mutations, new antifolates to be used in combination with other compounds has renewed interest in the development of better antifolate inhibitors. In the absence of a robust *Plasmodium falciparum in vivo* drug screening model, we have developed an *in vivo P. berghei* surrogate model. The *P. berghei* dihydrofolate reductase-thymidylate synthase gene was replaced by its *P. falciparum* homologue. Independent parasite lines were generated expressing the *P. falciparum* double, triple and quadruple-mutant Pyrimethamine-resistant DHFR variants. The ability to test inhibitors against a *P. falciparum* enzyme in this rodent system will greatly facilitate rapid compound evaluation for drug development. Here, we report *in vivo* data on the drug-sensitivity profiles of these parasite lines against a variety of standard antimalarials, such as Sulfadoxine-pyrimethamine, Chloroquine, Amodiaquine and Artesunate. This model will allow testing the efficacy of new therapies against resistant forms of dhfr-ts of *P. falciparum*. We are using this model to test for new dhfr inhibitors from our library of synthesized compounds.

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ARTEMISININ-BASED COMBINATION THERAPY (ACT) DRUG RESISTANCE TRENDS IN *PLASMODIUM FALCIPARUM* ISOLATES IN SOUTHEAST ASIA

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Plasmodium falciparum, one of the parasites that cause clinical malaria, is a continuous public health concern, especially in Asia and Africa. Unfortunately, the parasite has developed resistance to many drugs created to treat and prevent the disease. Artemisinin and its derivatives are the new gold standard for treatment of malaria, yet treatment failures in clinical studies are starting to be reported. Clearly, artemisinin resistance needs to be characterized and dealt with accordingly. In support of the Gates Foundation Artemisinin Consortium, we conducted a blinded study to elucidate the phenotypic response of artemisinin derivatives of parasites derived from patient blood samples from Cambodia and Thailand. Blood samples containing *Plasmodium falciparum* were cultured and then assayed using SYBR green as an indicator to obtain drug IC_{50}s. The data suggested that many isolates are not demonstrating resistance to artemisinin. However, a select few are showing some resistant characteristics in the form of elevated IC 50s, especially to some of the drugs already identified in previous studies as drugs having resistant characteristics. Compared to studies conducted within the past ten years, no significant changes in parasite susceptibility to the artemisinin drugs have been observed. Additional analysis of clinical outcomes, therapeutic drug levels, and molecular markers needs to be completed before it can be assumed that artemisinin resistance has emerged.

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VARIATIONS IN PFUBP-1, ATPASE-6 AND PFMDR-1 GENES ENCODING DRUG RESISTANCE FOLLOWING ARTEMETHER-LUMEFANTRINE TREATMENT IN SUDANESE PATIENTS

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Development of drug resistance to antimalarial drugs has posed a great challenge in the control of malaria in endemic areas. Artemisinin based combination therapy (ACT) has recently been deployed in many malaria endemic areas with the rationale for combination therapy being to delay development of resistance. Artemether-lumefantrine (AL) is one of the few co-formulated ACTs available in tropical Africa. However, treatment failure following administration of this drug has been reported in both *in vitro* and in the field. Studies on the genetic determinants of *Plasmodium falciparum* resistance to AL have identified a selection of pfmdr-1 alleles in the failure cases. An earlier study reported association with the pfcrt gene. Reports of association with pfmdr-1 amplification have also been made.In vitro work has demonstrated a role for the atpase-6 gene SNPs in increaed IC50 to artemisinins. However, recent field reports failed to show