1

GLYCOSYLATION OF THE DENGUE 2 VIRUS E PROTEIN AT N67 IS CRITICAL FOR VIRUS GROWTH *IN VITRO* BUT NOT FOR GROWTH IN INTRATHORACICALLY-INOCULATED *AEDES AEGYPTI* MOSQUITOES

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To determine the importance of N-linked glycosylation of the dengue 2 virus (DEN2V) envelope (E) protein for viral growth, mutations in the infectious clone of strain 16681 (30P-A) were made at one or both of the N-linked glycosylation motifs: E1(N67Q), E2(N153Q), E1/2 (N67Q and N153Q). We found that while the E2 mutant replicated in mammalian and C6/36 mosquito cell culture, the E1 and E1/2 viruses were unable to grow in mammalian cells, and were only able to be minimally passaged in C6/36 cells before a compensatory mutation, K64N, occurred restoring the glycosylation motif in this area. In fact, when daily virus samples from infected C6/36 cells were sequenced, the compensatory mutation was present at day four post-infection (p.i.). This was the same day that virus titers began to increase to detectable levels as measured in a $TCID_{50}$ assay in C6/36 cells. In contrast, all E glycosylation mutants replicated similarly to 30P-A virus in intrathoracically-inoculated mosquitoes, and no compensatory mutation at amino acid 64 was detected at day 14 p.i. These results show that while N-linked glycosylation of the E protein is not necessary for DEN2V replication in mosquitoes, N-linked glycosylation at amino acid N67 (or nearby N64) is critical for survival of the virus in either mammalian or insect cell culture.

2

HIGHER FREQUENCY OF DENGUE VIRAL RNA DETECTED IN PLATELETS THAN IN PLASMA IN THE LATE STAGE OF DENGUE VIRUS INFECTION

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This study investigated the presence of positive and negative stranded dengue viral RNA in specimens collected from late stage (four to eight days after the onset of fever) dengue patients. Platelets were freshly isolated from EDTA-treated whole blood by using OptiPrep density gradient centrifugation. The purity of the isolated platelets was confirmed with immunofluorescent staining and flow cytometric analysis for a platelet-specific marker, CD41. Dengue viral RNA was isolated from platelets and plasma and subjected to RT-PCR. The detection of positive and negative stranded dengue viral RNA was performed using specific primers for dengue viral E and the 3' untranslated region, respectively. The products were verified with conventional agarose gel electrophoresis and nucleotide sequencing. All specimens were confirmed to be dengue infections by serological assays. A surprising high percentage (81%, 13/16) of specimens containing positive strands of dengue viral RNA was observed in platelets collected at late stage of infection. Only 31% (5/16) of plasma samples had positive strands of RNA during the same period (Fisher exact test, P=0.01). In addition, we were able to detect negative strands of dengue viral RNA in three specimens from isolated platelets, suggesting possible ongoing viral replication. The amplified viral RNAs were confirmed by nucleotide sequencing. Within a very limited number of patients and samples we demonstrate for the first time that anuclear platelets are potentially a late target of dengue virus infection. This finding may partially explain the drop in platelet count in dengue virus infections at the onset of defervescence.

3

PRIMARY HUMAN SPLENIC MACROPHAGES ARE THE PRINCIPAL TARGET CELLS FOR DENGUE VIRUS INFECTION EX VIVO

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Understanding the pathogenesis of dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) requires the precise identification of dengue virus (DV) permissive target cells. In a previous study using un-fractionated human peripheral blood mononuclear cells, we found that monocytes, but not B or T cells, were the principal DV-permissive cells in the absence of pooled dengue-immune human sera (PHS), and major mediators of antibody-dependent enhancement in the presence of PHS. To further identify DV-permissive target cells in other tissues and organs, we isolated human splenic mononuclear cells (MNC), inoculated them with DV2 (strain 16681), in the presence or absence of PHS, and assessed their infection either directly using flow cytometry and RT-PCR assays; or indirectly by plaque assay. We found that, in the absence of PHS, a small proportion of primary macrophages among the MNC appeared to be DV-permissive in comparison to staining controls by the flow cytometric assay (0.77±1.00% vs. 0.18±0.12%; p=0.07, n=10); and that viral RNA in these cells was detectable by the RT-PCR assay. Additionally, supernatant from the infected-MNC contained infectious virions that were readily detectable by plaque assay even when the percentage of infected cells was small. Moreover, the magnitude of macrophage infection was significantly enhanced in the presence of highly diluted PHS (5.41±3.53% vs. 0.77±1.00%; p=0.001, n=10). In contrast, primary T and B cells were not infected either in the presence or absence of PHS (peak values of 0.10±0.18% and 0.15±0.14% for T and B cells, respectively). These results provide evidence, for the first time, that human primary splenic macrophages, rather than B or T cells, are the only DV-permissive cells; and that they may be uniquely important in the initial steps of immune enhancement that lead to DHF/DSS in some DV-infected individuals.

4

WHOLE BLOOD TRANSCRIPTIONAL PROFILES ASSOCIATION WITH DENGUE SHOCK SYNDROME

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The pathogenesis of severe dengue remains poorly understood. Here we describe the ex vivo gene expression profile of whole blood from 18 Vietnamese children with acute dengue virus infections. SAM (Significance Analysis of Microarrays) was used to identify gene expression patterns associated with mild dengue or dengue shock syndrome (DSS). In children with acute DSS, there was marked attenuation of a range of immune response genes, particularly those associated with cytokine and chemokine pathways, apoptosis and Type I interferon-induced responses. This phenotype was independent of length of illness or haematological parameters. We used quantitative PCR to validate a subset of 187 genes in a second sample set of 34 children with dengue infections, including 20 with DSS. Many, but not all genes identified in the microarray were validated. These data suggests that immune responses, and in particular Type I interferon mediated processes, are attenuated during acute DSS, or that the kinetic of the response is different in acute DSS cases. The significance of these observations for our understanding of disease pathogenesis will be discussed.

5

DIFFERENTIAL AND TEMPORAL MODULATION OF ENDOTHELIAL BARRIER FUNCTION BY HEMORRHAGIC FEVER VIRUSES

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Hemorrhagic fever viruses produce a syndrome in humans characterized by endothelial dysfunction. The exact mechanisms underlying vascular leakage in viral hemorrhagic fevers are unknown. We used a transwell assay to study the effect of two hemorrhagic fever viruses, Sin Nombre (SNV) and Dengue viruses (DV), on macromolecule permeability across endothelial cell monolayers. Virus infection decreased TNF-α and IFN-γ mediated hyperpermeability across endothelial cell monolayers 2-3 days after infection. This effect was mediated by live virus-induced Type I interferon. However, at later time points (seven days after infection), virus infection increased TNF-α mediated hyperpermeability across endothelial cell monolayers. This differential effect on barrier function involved complex virus effects on endothelial cytoskeletal components. Our research provides new insights into mechanisms of vascular leakage caused by infection with hemorrhagic fever viruses.

(ACMCIP Abstract)

6

DEVELOPING A MOUSE MODEL OF DENGUE IMMUNOPATHOGENESIS

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Primary infections with dengue viruses (DENV) are usually asymptomatic or result in self-limiting dengue fever. However, a second infection with a different DENV serotype is much more likely to produce life-threatening dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS). Increased disease severity in secondary DENV infections is likely to result from immunopathogenic processes. One such mechanism, antibody-dependent enhancement (ADE), may occur when anti-DENV antibodies are present at levels high enough to bind to the virus, but too low to sufficiently neutralize it. Fcγ receptor-bearing immune cells could then take up these immune complexes, resulting in increased infection, enhanced viremia, and pathology. DENV immunopathogenesis remains poorly understood, however, due to the lack of an appropriate small animal model. We are using two approaches to study secondary DENV infection in mice. In the first model, interferon α/β x γ receptor-deficient (AG129) mice are sequentially infected with two different DENV serotypes. Sequences of infection were selected by infecting mice with each of the four DENV serotypes, then testing serum collected six weeks later for enhancing and neutralizing activity against the four DENV serotypes *in vitro*. Antisera neutralized heterologous viral serotypes in PRNT assays in most cases. However, a few combinations of primary and secondary viruses were identified that did not result in neutralization of the second virus in PRNT assays. Furthermore, *in vitro* ADE assays suggested that a D4-D1 sequence of infection may result in enhanced virus replication. This infection sequence is being pursued *in vivo*. In the second model, mouse antisera were tested via *in vitro* ADE assays to identify sera with the greatest enhancing effect on various challenge viruses. Serum with potent enhancing activity was passively transferred in varying amounts into naïve mice by passive transfer, followed by challenge with a heterologous serotype of DENV. Virus replication was measured to assess the effects of transferred antibody *in vivo*. These studies will provide important insights into the immunopathogenesis of secondary DENV infections.

7

DENGUE VIRAL DETERMINANTS OF SEVERE DISEASE IN MICE

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Dengue virus (DENV) causes dengue fever (DF) and dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS), the most prevalent mosquito-borne illnesses in humans worldwide. To investigate mechanisms underlying dengue disease pathogenesis, we have generated a new DENV-2 strain, D2S10, which causes a non-paralytic, TNF-α-mediated severe disease in AG129 mice lacking receptors for both type I and II interferons. Using this animal model, we sought to identify the viral determinants responsible for the D2S10 phenotype through reverse genetics. Specifically, full-genome-length infectious cDNA clones were constructed and analysis of AG129 mice infected with the various recombinant DENV strains revealed that two amino acid substitutions (N124D and K128E) in the envelope (E) protein were responsible for the D2S10 phenotype. Infection experiments using a variety of mouse and human cell culture models revealed that these two amino acid residues can positively or negatively modulate DEN infection depending on the cell type. In particular, preliminary results with mouse dendritic cell cultures suggest that these two mutations modulate cellular tropism of DENV. Experiments are in progress to identify cell types that are productively infected by each recombinant DENV strain in AG129 mice.

8

A FOUR-YEAR FOLLOW-UP OF THE SAFETY, IMMUNOGENICITY AND EFFICACY OF THE CANDIDATE MALARIA VACCINE RTS,S/AS02A IN CHILDREN VACCINATED AT AGED 1 TO 4 YEARS IN A MALARIA-ENDEMIC REGION OF MOZAMBIQUE

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A phase IIb trial of RTS,S/AS02A in children aged 1 to 4 years in southern Mozambique demonstrated a 30% (95% CI 11% to 45%) protection against clinical malaria disease, 58% (95% CI 16% to 81%) against severe malaria disease and 45.0% (31% to 56%) protection against infection, as reported previously. Follow-up investigation one year post immunization confirmed that protection induced by the vaccine was conserved and another two year period of follow-up was initiated. We will report the safety, immunogenicity and duration of protection induced by RTS,S/AS02A up to 45 months post first vaccination in children who were aged 1 to 4 years at the time of the first dose, and discuss the potential implications.

9

ASSESSMENT OF CELLULAR IMMUNE RESPONSES IN INFANTS PARTICIPATING IN A RTS,S/AS02D PHASE I/IIB TRIAL IN MOZAMBIQUE

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Protection for at least 18 months against *Plasmodium falciparum* malaria clinical and severe disease has been demonstrated in children aged 1 to 4 years, living in endemic areas of Southern Mozambique following immunization with the RTS,S/AS02A candidate malaria vaccine, as previously reported. The RTS,S/AS02A vaccine consists of the RTS,S antigen containing sequences of the circumsporozoite protein (CSP) of *P. falciparum* and the hepatitis B surface antigen (HBsAg) adjuvanted with AS02A, a proprietary GSK adjuvant system containing QS21 and MPL immunostimulants with an oil-in-water emulsion. Antibody responses (total IgG titers) to CSP have been investigated but correlation with protection of such humoral responses was not demonstrated in the context of the Mozambican children trial. Therefore, in a second trial, we assessed the role of cellular mediated immunity (CMI), which is reported as a crucial mechanism for protection induced by circumsporozoite antigen vaccines in experimental models. Cellular responses specific for antigen components of the RTS,S malaria vaccine candidate were evaluated in the first phase I/IIb, double blind, clinical trial of RTS,S/AS02D in infants aged at least 8 weeks at first dose. RTS,S/AS02D is the pediatric formulation of RTS,S/AS02A candidate vaccine, compatible with 0.5 mL syringes. Infants were randomly allocated to two groups and received immunizations of either RTS,S/AS02D or the comparator vaccine *Engerix-B*™, a hepatitis B vaccine at 10, 14 and 18 weeks of age. All participants also received the *TETRActHib*™ (DTPw/Hib) vaccine (Sanofi-Pasteur) at 8, 12 and 16 weeks of age. Peripheral blood samples were taken before the first immunization and 1 month and 3.5 months after the third vaccination. Fresh whole blood intracellular staining assay was performed on site to evaluate the expression of IL-2 and IFN-γ by CD4+ and CD8+ T cells in response to *in vitro* stimulation with peptide pools of either CSP or HBsAg. Determination of IFN-γ, IL-2, IL-4, and TNF-α was also done in culture supernatants using cytometric bead array system. Results and comparisons of cytokine responses to RTS,S components between the 2 study groups as well as over the different time points will be presented. Correlation of these CMI responses with antibody responses and protection will be discussed.

(ACMCIP Abstract)

10

A RANDOMIZED, OBSERVER-BLIND TRIAL TO COMPARE SAFETY AND IMMUNOGENICITY OF TWO ADJUVANTED RTS,S ANTI-MALARIA VACCINE CANDIDATES IN GABONESE CHILDREN

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An effective vaccine against *Plasmodium falciparum* would be an important public health measure in the fight against malaria. The vaccine currently furthest in development is the RTS,S vaccine. The antigen component contains a part of the *P. falciparum* circumsporozoite (CS) antigen, together with the hepatitis B surface antigen (HBsAg). These recombinant proteins are adjuvanted with either a liposomal formulation (AS01) or an oil-in-water emulsion (AS02), both containing the immunostimulants MPL and QS21. The RTS,S/AS02 vaccine has been shown to have a good safety profile and be efficacious against malaria in a series of studies throughout the past 10 years. In the largest trial, where approximately 2000 Mozambican children aged 1-4 years were vaccinated using a 3 dose schedule, the vaccine was well tolerated and an efficacy of 32% against clinical malaria and 49% against severe malaria was demonstrated over an 18-month follow-up period. Studies in adults have indicated stronger immunogenicity and suggested higher efficacy when RTS,S is formulated with the AS01 adjuvant system as compared to the AS02 adjuvant system. A randomized, double blind trial in Gabonese children aged 18 months to 4 years compared non-inferiority of RTS,S/AS01 versus RTS,S/AS02 in terms of safety and immunogenicity. From April to August 2006, 180 children received 3 doses of RTS,S with either the AS01 or AS02 adjuvant systems in a 0, 1, 2- month schedule. Reactogenicity was evaluated by active detection of solicited symptoms (pain, swelling, fever, irritability, drowsiness, loss of appetite) for 1 hour post administration at the vaccine center and at daily home visits for 6 days. Non-serious AEs were recorded for 1 month post-vaccination and serious AEs for 1 year post last vaccination. Safety laboratory tests were performed at screening and on days 6 and 90. For immunogenicity, anti-CS and anti-HbsAg antibodies were measured before vaccination and on days 60 and 90. The children will be followed up for 1 year post last vaccination. Results up to 1 month post dose 3 will be presented.

11

SAFETY AND TOLERABILITY OF A MULTI-STAGE, MULTI-ANTIGEN ADENOVIRUS-VECTORED *PLASMODIUM FALCIPARUM* MALARIA VACCINE, IN HEALTHY, MALARIA-NAÏVE ADULTS

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Malaria is a disease of global importance. Vaccine development has been challenging due to a complex parasite life cycle and the parasite's ability to evade the host immune response. Genetically-based vaccines have been shown to induce high levels of CD8 + T cells, which are believed capable of mediating sterile protection following exposure to malaria. Aiming to induce such cell-mediated as well as antibody responses to both pre-erythrocytic and erythrocytic stages of *Plasmodium falciparum*, Naval Medical Research Center, in partnership with GenVec, Inc, USAID and the US Army, is developing an adenovirus-vectored vaccine encoding the antigens PfCSP (expressed in sporozoite and liver stages) and PfAMA1 (expressed in sporozoite, liver and erythrocytic stages). The vaccine, called NMRC-M3V-Ad-PfCA, is currently undergoing clinical testing in the USA. The initial study involves malaria-naïve adults without evidence of pre-existing immunity to the Ad5 backbone, defined as an Ad5 neutralizing antibody titer of ≤ 500. We will present safety data from the first part of this study which involves two groups of volunteers (n=6/group) immunized by a single intramuscular injection using a dose escalation format with the first group receiving 1×10^{10} particle units (pu) per construct or 2×10^{10} pu total, followed four weeks later by the second group receiving, 5×10^{10} pu per construct or 1×10^{11} pu total dose (five-fold dose escalation). To date the NMRC-M3V-Ad-PfCA vaccine has shown to be safe and well tolerated with no vaccine-related Grade 3/4 adverse events reported.

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MEASUREMENT OF ANTIBODY FINE SPECIFICITIES INDUCED BY MALARIA VACCINE FMP1/AS02A FROM A PEDIATRIC PHASE 2B TRIAL IN WESTERN KENYA

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Previously, we reported the results of a pediatric Phase 2b trial in which the efficacy of the FMP1/AS02A malaria vaccine was evaluated in an area of intensive transmission in western Kenya. The FMP1 antigen corresponds to the MSP1-42 fragment of the 3D7 strain of *Plasmodium falciparum*. The target population was 12-48 month old children, and the primary clinical endpoint was fever greater than 37.5°C with parasite density greater than 50,000/µl. Even though the FMP1/AS02A vaccine did not protect children against *P. falciparum* malaria, we sought to define the antibody fine specificities induced by vaccination because the immunity developed in the context of several confounding factors including a pre-existing immunity that was caused by natural exposure; concurrent exposure that occurred during the trial; and allelic heterogeneity that was site prevalent. Primarily, we will report results for antibody fine specificities as measured by ELISA and multiplex flow cytometry and results of parasite growth inhibition assays. We will also report if there is a relationship between antibody specificity and other study related parameters such as clinical disease, parasite density, volunteer age, and bed-net use.

13

PURIFIED IGGS FROM UNVACCINATED MALIANS INTERFERE WITH THE BIOLOGICAL ACTIVITY OF APICAL MEMBRANE ANTIGEN 1-SPECIFIC IGGS AS JUDGED BY THE *IN VITRO* GROWTH INHIBITION ASSAY

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Apical Membrane Antigen 1 (AMA1) is a leading malaria vaccine candidate. Several phase 1 trials with AMA1 have been conducted in the US and Mali. In naïve American trials, an AMA1 vaccine formulation induced anti-AMA1 IgGs which showed biological activity as judged by an *in vitro* Growth Inhibition Assay (GIA). Although the AMA1 vaccine also induced a significant increase in anti-AMA1 antibodies in semi-immune Malian adults, there was no significant increase in GIA activity after immunization. These data suggested that antibodies elicited by the AMA1 vaccine in naïve people had different biological activity compared to antibodies induced by natural infection, or some fraction(s) of Malians' IgGs blocked GIA activity of AMA1 specific IgG. To test this hypothesis, total IgGs were purified from both US vaccinees' and unvaccinated Malians. AMA1-specific IgGs (AMA1-IgG) were separated from IgGs which not bind to AMA1 (nAMA1-IgG) by using AMA1 affinity chromatography. The amount of AMA1-specific antibodies of each sample was quantified by a standardized ELISA. When the percent inhibition of parasite growth in the GIA was plotted against antibody ELISA units, the US total IgG, US AMA1-IgG and Mali AMA1-IgG followed the same hyperbolic curve. No interference of GIA activity was observed when the US and Mali AMA1-IgGs were mixed in a GIA well. However, when Mali nAMA1-IgG was mixed with US AMA1-IgG, the GIA activity of the mixture was lower than that of US AMA1-IgG alone. This result suggests that some fraction of IgG (that did not bind to AMA1 protein) from people living in an endemic area might inhibit the biological activity of AMA1-IgG elicited by vaccination.

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DYNAMICS OF POLYMORPHISM IN *PLASMODIUM FALCIPARUM* APICAL MEMBRANE ANTIGEN-1 OVER THREE YEARS AT A VACCINE-TESTING SITE IN MALI

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Development of an effective malaria vaccine is threatened by extensive genetic diversity in vaccine antigens. It is important to understand the distribution and natural dynamics of vaccine antigen polymorphisms in endemic populations to guide vaccine design and permit distinction between natural fluctuations in genetic diversity and vaccine-induced selection. To understand the genetic variation in a blood-stage malaria vaccine antigen, apical membrane antigen-1 (AMA-1), at a vaccine-testing site in Bandiagara, Mali, where a malaria vaccine based on the 3D7 AMA-1 is presently in clinical trials, we genotyped samples collected from 100 children who participated in a malaria incidence study at this site from 1999-2001. Direct sequencing was used to genotype domain I of the *ama-1* gene from 682 infections. Sequencing of domains II and III is underway. The frequencies of single nucleotide polymorphisms (SNPs) were compared over three transmission seasons and in three age groups (≤5 years, 6-10 years, ≥11 years). To investigate the within-host dynamics of antigen polymorphisms, Cox-proportional hazards was used to model the time to next clinical episode in individuals' consecutive clinical infections as a function of change in predominant amino acid at each polymorphic site, year, and age. 118 haplotypes were observed (based on 34 SNPs), with the 3D7 haplotype having the highest frequency (10%). Allele frequencies remained relatively stable over time, consistent with balancing selection acting to maintain genetic diversity at this locus. Multiplicity of infection was higher at the beginning of the transmission season and in the oldest individuals (≥11 years). Analyses of the within-host dynamics of antigen polymorphisms suggest at least four polymorphisms in AMA-1 domain I (206, 207, 225, and 267) may be relevant in determining cross-protective immunity. These data show that the vaccine strain corresponds to the most prevalent AMA-1 haplotype at this site and identify specific amino acid residues that may be important to consider in designing vaccines that protect against genetically diverse malaria parasites.

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RISK FACTORS FOR DIARRHEAL DISEASE MORTALITY AMONG HOSPITALIZED CHILDREN IN RURAL WESTERN KENYA, 2005-2007

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Diarrhea is a major cause of childhood morbidity and mortality in Kenya. We conducted hospital-based surveillance to characterize the etiology of severe diarrheal illness, and identify risk factors for death among inpatient children in rural western Kenya. We enrolled all children <5 years old hospitalized with diarrhea (≥3 loose stools in 24 hours) at two

hospitals in rural western Kenya. Clinical and demographic information and stool samples were collected. Specimens were tested for enteric bacterial pathogens (culture) and rotavirus (EIA). From May 23, 2005 to February 22, 2007, we enrolled 963 children <5 years old hospitalized with diarrhea. We identified at least one pathogen in 331 (34%) stool samples. Eighty (8.3%) of the children enrolled died during hospital admission. Among children who died, non-Typhi *Salmonella* were detected in 15 (19%), *Shigella* in 7 (9%), and *Campylobacter* in 3 (4%). Rotavirus was detected in 5 (7%) of 71 whole stool samples collected from children who died. Four (5%) children who died were co-infected with two enteric pathogens. Case-fatality ratios by pathogen were: *Shigella* 19% (7 of 37), *Salmonella* 16% (15 of 95), co-infections 13% (4 of 32), *Campylobacter* 7% (3 of 44) and rotavirus 4% (5 of 133). Risk factors for death included a duration of diarrhea ≥5 days on admission [odds ratio (OR) 2.0; 95% confidence interval (CI) 1.1-2.9], having ≥7 stools per day before admission [OR = 2.1; CI 1.1-4.0], requiring intravenous rehydration [OR = 3.7; CI 1.1-15.1], and having a bacterial pathogen isolated from stool [OR = 3.7; CI 1.5-9.7]. Of a subset of 34 enrolled children who died, for whom additional information was available, 23 (68%) had a clinical diagnosis of malaria. Sixteen of these had an enteric pathogen [7 (21%) *Salmonella*, 5 (15%) *Shigella*, 3 (9%) rotavirus, 1 (3%) bacterial co-infection], but only three had malaria parasites on blood smear. Diarrheal disease is a major cause of mortality in Kenyan children. Early evaluation, and inpatient therapy may be critical in reducing inpatient mortality.

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FACTORS ASSOCIATED WITH KWASHIORKOR IN BOTSWANA DURING AN OUTBREAK OF DIARRHEA AND MALNUTRITION AMONG YOUNG CHILDREN

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Kwashiorkor, a form of severe acute malnutrition, is not commonly reported in Botswana. In early 2006, children with severe acute malnutrition, some with kwashiorkor, were admitted to the country's second-largest referral hospital during a diarrhea outbreak. We interviewed the caregivers of all medically stable pediatric inpatients March 22-24, 2006. Interviews conducted in the local language collected data on symptoms of diarrhea and malnutrition during the outbreak period (January-March 2006), feeding practices and beliefs. When possible, we weighed and measured children 6 months and older. We described the cohort and identified factors associated with kwashiorkor, defined as swelling of the feet, hands or face and/or skin peeling in 2006. Our sample of 53 children included 26 (49.1%) males, 49 (92.5%) children less than 2 years old and 29 (54.7%) less than 1 year old (Range: 1-31 months). During the outbreak period, 42 (79.2%) had diarrhea; 29 (54.7%) kwashiorkor; and 20 (38.5%) had at least one previous hospital admission. The median weight for age z-score, an indicator for underweight, was -2.97 (Range: -0.24, -4.74) and outpatient growth cards recorded at least 2 consecutive months of no weight gain for 35 (66.0%) children. Children with kwashiorkor were more likely to have had diarrhea (OR=4.3, 95% CI 1.0-18.8) or a prior hospitalization (OR=3.9, 95% CI 1.1-13.2) than those with other illnesses. Kwashiorkor was less likely among children whose parents believed Tsabana, a fortified soy-sorghum porridge distributed free of charge by the government as a general nutritional supplement, was good for children (OR=0.2, 95% CI 0.1-0.7). No associations were noted between kwashiorkor and the severity or duration of diarrhea, growth history, current feeding method (breastfeeding, feeding milk or formula), HIV status of mother or child, or household food security. In conclusion, kwashiorkor in young children admitted to hospital during a diarrhea outbreak period in Botswana was associated with recent prior diarrhea and hospitalization. Risk factors related to food intake were not identified in this small sample, but mothers who believed fortified porridge was good for children may have used it more, and their children were less likely to have kwashiorkor. Closer attention to growth monitoring, nutritional support and diarrhea management is needed, even in settings where kwashiorkor is not common.

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FLUID MANAGEMENT AMONG CHILDREN PRESENTING TO AN EMERGENCY DEPARTMENT DURING A DIARRHEA OUTBREAK IN BOTSWANA

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Oral rehydration therapy (ORT) was introduced in the 1970s to avert severe diarrheal morbidity and mortality. During a multi-pathogen diarrhea outbreak in Botswana from January - March 2006, 532 deaths occurred among >30,000 ill children. Compared with previous years, diarrhea cases quadrupled and deaths increased 25-fold during early 2006. We investigated use of rehydration therapies during the outbreak. We enrolled children <5 years old on presentation to the emergency department (ED) of a referral hospital in Botswana in March 2006. We defined a diarrhea case as ≥3 loose stools in 24h; cases were further classified by ED disposition. Guardians were interviewed about pre-hospital care; ED and related inpatient records were abstracted. We compared groups using chi-square or Fisher's exact test. Of 96 cases; 36 (38%) were admitted to hospital and 11 (11%) died. While ill but before presenting to the ED, 84 (88%) cases received ORT and 10 (10%) got intravenous fluids (IVF). Of 81 cases given ORT at home before visiting the ED, 20 (25%) purchased oral rehydration solution (ORS) sachets; 50 (62%) obtained free ORS sachets from clinics; and 24 (30%) prepared ORS at home. ORT was provided at home to 76 (90%) of 84 cases who had previously sought medical care, but to only 5 (56%) of 9 who had not sought medical care (odds ratio [OR] 7.6; 95% confidence interval [CI] 1.7 - 34). Seven (22%) of 32 cases admitted to hospital versus 20 (44%) of 45 cases discharged from ED were given ORT in the ED (OR 0.32; 95% CI 0.12 - 0.90); ORS was provided in sachets rather than as prepared solution. Of 36 cases admitted to hospital, 30 (86%) were treated with IVF, 12 (34%) with ORT ad lib, and 1 (3%) with ORT at defined intervals. In conclusion, during an outbreak in Botswana, children with diarrhea commonly received outpatient ORT and were more likely to be given home ORT if they had been evaluated at a clinic. The type of ORS families most frequently reported using was that provided by clinics at no cost. At a hospital with high diarrhea mortality, ORT was not used as first-line rehydration therapy. Educating families and medical workers about ORT and making appropriate ORS available could prevent future diarrheal morbidity and mortality.

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SUSCEPTIBILITY TO *VIBRIO CHOLERAE* INFECTION IN A COHORT OF HOUSEHOLD CONTACTS OF PATIENTS WITH CHOLERA IN BANGLADESH

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Vibrio cholerae causes a spectrum of infection in humans ranging from asymptomatic colonization to rapidly fatal secretory diarrhea. To evaluate factors associated with susceptibility to V. cholerae, we prospectively observed a cohort of household contacts of patients with cholera in Bangladesh. Contacts of patients presenting to the International Center for Diarrhoeal Disease Research, B hospital with acute watery diarrhea due to *V. cholerae* were selected for inclusion in this study. Within 4 to 6 hours of presentation of the index case, a field team discussed enrollment with household contacts of the index patient. Contacts were visited on each of the next six days and on days 14 and 21. During these visits, contacts were

questioned about diarrheal symptoms, and rectal swabs were obtained for culture. We compared the baseline characteristics of contacts that had a positive rectal swab for *V. cholerae* with contacts that had no evidence of *V. cholerae* infection. Of the 1077 household contacts that were enrolled, 938 completed 21 days of observation. Of the 938 household contacts that were evaluated, 202 had a positive rectal swab for *V. cholerae* and 422 had no evidence of *V. cholerae* infection. In the assessment of baseline immunologic markers among contacts, we found that *V. cholerae* antigen-specific serum IgA levels predicted individual's susceptibility to *V. cholerae* infection. Higher levels of IgA directed at the toxin coregulated pilus (TCP) and cholera toxin (CT) were associated with protection from infection with both the O1 and O139 serogroups V. cholerae. Higher serum levels of IgA specific to lipopolysaccharide (LPS) were associated with protection from infection with *V. cholerae* O1, but not with *V. cholerae* O139. Serum levels of these antibodies did not predict whether contacts developed symptoms if infected; a similar finding was observed for the vibriocidal titer. As previously described, we found individuals with blood group O were less likely to become infected with *V. cholerae* O1, but if infected had greater than twice the odds of developing symptoms. Because we hypothesized that additional genetic factors contribute to host susceptibility to cholera, pedigree analysis demonstrated that household contacts who were first degree relatives of the index case had increased odds of being infected with *V. cholerae* compared to non-related or less closely related household contacts. This suggests a possible additional component of susceptibility that merits further study.

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PROSPECTIVE STUDY OF DIARRHEA DUE TO PARASITES IN ADULT POPULATION AT A NAVAL BASE IN ANCÓN, LIMA, PERÚ

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Outbreaks of diarrhea are very common in developing countries, most of them due to bacterial diseases; protozoal diseases are increasingly recognized as an important cause of diarrhea. Diarrhea outbreaks due to *Cyclospora cayetanensis* have been reported twice at the Naval Base in Ancon, Peru, which indicates that these outbreaks do occur in developing countries among local populations. The aim of this study was to evaluate the incidence of acute diarrheal disease (ADD) due to parasitic infections, focused on cyclospora. A prospective study was carried out at the Ancon Navy Base. Recruits over 18 years old who entered into a 3 month training period were included. All participants completed a demographic, epidemiological and clinical questionnaire at the beginning of the study; and an interval questionnaire was applied every month and if an ADD episode occurred (defined as at least 3 stools per day). Stool samples were taken every month for identification of parasites using Ritchie and MIF methods, plus Kinyoun stain and autofluorescence for *Cyclospora*. Testing was performed at the Universidad Cayetano Heredia (UPCH) in Lima, Perú. 150 recruits entered the study in two three-month cohorts. 19 subjects were lost during the follow up because of voluntary withdrawal from recruitment training. 53 recruits developed diarrhea resulting in an incidence of 0.174 episodes per person per month. 12 of them were associated with a parasitic infection (20.69%) with an incidence of 0.036 episodes per person per month. The initial prevalence of parasites was *Cyclospora* 0.7% (1/149); *Giardia lamblia* 8% (12/149); *Hymenolepis nana* 1.34%(2/149); *Diphyllobothrium pacificum* 1.34%(2/149); *Trichuris trichuria* 0.7% (1/149). The overall incidence rates of parasitosis were: *Giardia lamblia* 9.01 episodes per 100 person per month; *Hymenolepis nana* 2.10; *Diphyllobothrium pacificum* 0.60; *Trichuris trichuria* 0.60 and *Ascaris lumbricoides* 0.30. The incidence of parasitic infections and their association with ADD was very high among this adult population; cyclosporiasis was rare whereas giardiasis was very common. Therefore in crowded and closed populations, parasitic agents should be considered as part of the ADD diagnostic working plan.

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PREDICTORS OF NON-TYPHOIDAL *SALMONELLA* BACTEREMIA IN FEBRILE CHILDREN PRESENTING AT HOSPITAL IN A *PLASMODIUM FALCIPARUM* HOLOENDEMIC AREA OF WESTERN KENYA

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Although non-typhoidal Salmonella (NTS) bacteremia is an important determinant of childhood malarial severity, the factors influencing susceptibility to NTS infection in children with malaria are poorly understood. As such, the pre-enrolment health history, and nutritional and clinical predictors of NTS bacteremia in febrile children presenting at Siaya District Hospital, western Kenya, with and without *Plasmodium falciparum* (Pf) infections were investigated [Pf(+), n=611; and Pf(-), n=129; aged 2-35 mos]. NTS isolation from blood and identification was performed by standard bacteriologic methods. NTS prevalence was 4.1% in Pf(+) and 13.2% in Pf(-) groups, with Salmonella typhimurium accounting for 88.0% and 76.5% of the NTS isolates in both groups, respectively. In both the Pf(+) and Pf(-) groups, NTS infection was associated with an elevated axillary temperature (p=0.001 and p<0.0001, respectively), increased granulocytes (p=0.046 and p=0.044, respectively), and decreased lymphocytes (p=0.050 and p=0.011, respectively) relative to non-bacteremic children. In addition, among Pf(+) children, NTS was associated with elevated plasma glucose levels (p=0.029), lower body weight (p=0.025), and height (p=0.038). Regression analyses controlling for age, gender, sickle-cell trait, and HIV infection identified predictors of NTS in Pf(+) children to include: fever (OR 3.5, 95% CI 1.3-9.5, p=0.015), underweight-for-age (OR 3.1, 95% CI 1.0-9.2, p=0.043), pre-enrolment diarrhea (>14 days; OR 15.0, 95% CI 1.1-20.6, p=0.043), pre-enrolment vomiting (1-3 days; OR 5.6, 95% CI 1.4-22.0, p=0.013), and pigment-containing neutrophils (OR 3.5, 95% CI 0.9-13.7, p=0.071). Conversely, susceptibility to NTS in the Pf(-) group was associated with fever (OR 5.3, 95% CI 1.4-20.2, p=0.014), wasting (OR 3.9, 95% CI 0.9-16.9, p=0.070), and pre-enrolment vomiting (4-14 days; OR 7.3, 95% CI 1.0-52.1, p=0.046). Taken together, these results demonstrate that, although elevated temperature is an important predictor of NTS bacteremia in children with and without malaria, there are distinct clinical and nutritional predictors associated with NTS infections in parasitemic versus non-parasitemic children.

(ACMCIP Abstract)

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IMPROVING MANAGEMENT OF SEVERE FEBRILE ILLNESS IN CHILDREN: INITIAL ASSESSMENT AND DESIGN OF AN INTERVENTION IN RURAL TANZANIA

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International guidelines for the integrated management of childhood illness (IMCI) include provisions for identifying children with potentially life-

threatening illnesses, providing pre-referral treatment, and comprehensive referral level care. Severe illness that presents with fever should almost always be managed with parenteral antimalarial and antibacterial drugs. Little is known about health provider or caretaker practices related to severe febrile illness in children. We conducted a rapid assessment of classification and management practices for children identified with severe illness at 62 peripheral health facilities in 4 districts between November 2006 and February 2007. A retrospective review of 297 severely ill children was completed using clinic registers and health worker interviews. A prospective follow-up study was completed on 168 severely ill children registered at a sub-sample of 18 health facilities. Findings informed the development of an intervention to improve management of severe febrile illness. Health workers assessed children for IMCI danger signs, but rarely employed IMCI terminology for illness classification. Severe malaria, severe pneumonia or severe malaria with pneumonia were identified in 96% of all children recognized as severely ill. Only 16.5% received both an antibacterial and an antimalarial drug for prereferral or definitive care. None received the nationally recommended combination of antibacterial and antimalarial treatments. Children enrolled in the follow-up study experienced a mortality rate of 4.8% within 7 to 14 days of initial presentation. In conclusion, health workers recognized a cadre of severely ill children with high risk for mortality but rarely classified or treated them according to national guidelines. A comprehensive intervention to address recognition, prereferral treatment and referral level care is currently underway in the study districts and will be described.

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DENGUE PATHOGENESIS; HOST AND VIRAL LESSONS FROM VIETNAMESE INFANTS AND CHILDREN

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The pathogenesis of dengue is poorly understood. Studies of infants with primary infections can provide information on humoral correlates of immunity and potentially, the clinical significance of antibody dependent enhancement. In 75 Vietnamese infants with primary dengue, we found significant heterogeneity in viraemia and NS1 antigenaemia at hospital presentation, and these factors were independent of disease grade or continuous measures of disease severity. Neutralising antibody titres, predicted in each infant at the time of their illness, suggested the majority of cases (65%) experienced DHF when the maternally-derived neutralising antibody titre had declined to less than 1/20. Cellular immune activation in $CD3^+CD4^+$, $CD3^+CD8^+$ and NK cells was evident in acute blood samples and correlated with infant disease severity. HLA-A*11-restricted $NS3_{133-142}$-specific $CD8^+$ T cell responses were detected in peripheral blood of infants and older children with DHF, but only after the major clinical events had resolved. Finally, we will describe the infection enhancing potential of neat plasma collected at 3 month intervals from a birth cohort of healthy Vietnamese infants and its relationship with the age related prevalence of DHF in Vietnamese infants.

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EVIDENCE FOR A CONSERVED T CELL RECEPTOR REPERTOIRE IN MEMORY CD8+ T CELLS SPECIFIC FOR AN IMMUNODOMINANT CTL EPITOPE IN DENGUE 1 NS5

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The antiviral cellular immune response to acute infection is characterized by activation and expansion of virus-specific CD8+ T cells. Clearance of the infection is accompanied by establishment of a stable pool of memory CD8+ T cells, which can persist for many years. The magnitude and kinetics of memory T cell activation in subsequent infection is determined by interaction of clonally distributed T cell receptors (TCRs) with the target cell viral epitope peptide/MHC class 1 complex. To examine the factors that govern the maintenance and nature of dengue-specific T cell memory, we assessed immunodominance and T cell receptor usage in individuals exposed to Den-1 in Hawaii in 2001, during the first known dengue epidemic in almost 60 years. We synthesized a peptide library based on the consensus deduced amino acid sequence of the Den-1 E, NS3, NS4A, NS4B, and NS5 proteins of the dominant epidemic virus strain circulating in Hawaii at the time and used this panel to identify an immunodominant NS5 329-337 epitope restricted by HLA-B*5502 in 3 of 12 subjects 5 years after infection. NS5 329-337-specific CD8+ T cells were highly cross-reactive, lysed target cells presenting variant epitope peptides representing heterologous serotypes, and secreted an array of proinflammatory cytokines. Spectratyping analysis showed that the NS5 329-337-specific T cell repertoire was skewed in each subject, with a major expansion of Vβ22+ T cells. Stimulation of PBMC from these Den-1-exposed individuals with heterologous dengue serotype peptides induced CD8+ T cells which efficiently lysed target cells presenting Den-1 epitope peptide and were cross-reactive for heterologous serotypes. The TCR repertoire of these T cells was highly restricted and distinct from the Den-1 NS5 329-337-specific T cell repertoire. We demonstrate the existence of a stable Den-1-specific memory CD8+ T cell pool in unrelated individuals in which the dominant T cell receptor, a major determinant for recognition of viral epitope antigens, is conserved. Current studies to determine public and private specificities of the Vβ22+ TCR are ongoing. In addition, we show that serotype cross-reactivity among memory CD8+ T cells is regulated by distinct T cell families. These findings have implications for pathogenesis and for vaccine design.

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MOLECULAR MARKERS IN SECONDARY DENGUE INFECTION: ELEVATED SOLUBLE ST2 PROTEIN (IL-33 RECEPTOR) IN SERA

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We have used global gene expression analysis to describe differential expression of mRNA of blood mononuclear cells during acute dengue virus infection. The mRNA of the interleukine-1 receptor like 1 (IL-1 RL-1 / ST2) also known as IL-33 receptor, has been found to be differencially elevated in dengue infections. Previous reports have found soluble form sST2 elevated in serum of patients with diseases characterized by an inflammatory response. Dengue virus infection induces the production of several inflammatory mediators like IL-1β, IFN-α, TNF-α, IL-6 and IL-8 *in vivo* during acute stage of the disease. The objective of this study was to evaluate the protein levels of soluble ST2 (sST2) in dengue infected patients to confirm the findings of mRNA expression. Twenty four patients with confirmed dengue infection and eleven patients with other febrile illness (OFI) were evaluated. Levels of sST2 in serum and clinical parameters were measured during the febrile, defervescence, post-febrile and convalescent stages of the disease. We found that at the end of the febrile stage and in defervescence, dengue infected patients have higher serum sST2 levels than OFI ($p=0.0088$ at day -1 and $p=0.0004$ at day 0). Furthermore, we found higher sST2 levels in patients with secondary dengue virus infections compared with patients with primary dengue virus infections ($p=0.047$ at day -1 and $p=0.030$ at day 0). This study suggests that sST2 could play a role in responses associated with secondary infections which should be further evaluated in future studies. Also, we confirmed predictions of molecular markers of disease that were generated by global gene expression analysis.

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SIGNIFICANT INCREASE IN DENGUE SEVERITY BETWEEN 2005 AND 2006 IN A HOSPITAL-BASED STUDY IN NICARAGUA

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In a hospital-based study of dengue in Nicaragua, a large increase in severity of cases was observed between the 2005-6 and the 2006-7 dengue seasons. Suspected dengue cases were enrolled upon presentation to the National Pediatric Reference Hospital between August 2005 and February 2006 and again between June 2006 and March 2007. Daily blood specimens were obtained from patients, along with a convalescent/discharge sample. Both serological and virological methods were used to confirm dengue-positive cases. In the first year, 15 cases (25%) were defined as primary dengue virus (DEN) infections and 45 cases (75%) as secondary DEN infections, with an average age of 9 years old (range 5 months to 14 years); in the second year, there were 8 (18%) primary DEN infections and 36 (82%) secondary DEN infections, with an average age of 8 (range 9 months to 14 years). In the 2005-6 dengue season, 9 DEN1 (18%) and 40 DEN2 (82%) serotypes were identified, compared to 2 DEN1 (6%) and 34 DEN2 (94%) serotypes in the 2006-7 dengue season. Of 60 cases in 2005-6, 48 (80%) were classified as dengue fever (DF), 8 (13%) as dengue hemorrhagic fever (DHF), and 4 (8%) as dengue shock syndrome (DSS); whereas in the 2006-7 season, of 44 cases, 18 (41%) were classified as DF, 15 (34%) as DHF, and 11 (25%) as DSS. Multivariate analysis was used to investigate the effect of age, sex, primary versus secondary DENV infection, dengue virus serotype, day of presentation, year of study, use of antibiotics, allergy, asthma, nutritional status, and route of admission (directly from home or via another healthcare unit) on disease severity. Immune status, year of study, age, and sex were significant factors and were therefore retained in the final model. When adjusted for age and sex, the odds ratio for secondary DENV infection was 15.7 (95%CI 2.93-84.0), and, interestingly, for the 2006-7 dengue season was 6.58 (2.32-18.6). Secondary infection is a well-known risk factor for severe dengue, and age-dependent differences in severity have also been noted previously. However, it is not clear why increased disease severity was observed in the second year of the study. To investigate potential factors contributing this phenomenon, full-length sequence of all the dengue viruses is being determined both directly from patient serum and from viral isolates. This should indicate whether a change in viral sequence may have contributed to the observed increase in dengue severity.

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ESTIMATING THE INCIDENCE OF DENGUE FEVER IN CAMBODIA: RESULTS OF A CAPTURE RECAPTURE ANALYSIS

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Dengue fever is a major public health problem in Cambodia. Since 2002, 10,000 - 16,000 hospitalized dengue cases have been reported annually among children <15 years of age; a reported annual incidence of 2/1000. To assess the National Dengue Surveillance System (NDSS) we conducted prospective, active surveillance for febrile illness and dengue virus infection among children 38ºC for ≥2 days. Of children with a documented fever, 89 had a confirmed dengue virus infection; an incidence of 13.4/1,000 children <16 years old. Comparison of names, age and gender in the 2 captures identified 20 matches; 69 confirmed dengue cases in the population under active surveillance were not found in NDSS and 9 dengue cases reported from NDSS were not captured by active surveillance as dengue cases. However, these 9 cases were captured as febrile cases, but did not have dengue virus infection. There was a 3-fold discrepancy between the number of dengue cases reported by NDSS and the actual number of dengue cases in the study population. In the study population, 41 children with dengue reported being hospitalized, however, only 20 were reported (captured) to NDSS. In conclusion, the incidence of dengue was about 6 times higher than the incidence derived from reported hospitalized cases, and there was a 3-fold underreporting of hospitalized cases to the NDSS. Further studies are needed to confirm these findings and determine whether they can be generalized to all of Cambodia.

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CHALLENGES FOR MEASURING GLOBAL DENGUE BURDEN: OVERCOMING SEVERE LIMITATIONS OF COUNTRY PASSIVE SURVEILLANCE SYSTEMS

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Throughout the tropics, dengue virus is believed to infect millions of people annually, causing substantial illness and thousands of deaths. However, country-specific official numbers of cases and deaths from dengue are just a very small fraction of what is believed to occur. For example, in the year 2004, 557,000 cases and 1,800 deaths were reported globally to WHO. For the same year, however, the projected global dengue burden was 8,269,087 cases and 19,524 deaths. Because the latter numbers are an order of magnitude greater, supportive evidence is needed to strengthen these projections. Estimation of disease burden is important for formulating policy and allocating resources on dengue prevention (vector control programs) and treatment. Additionally, burden estimations could help building an investment case for the introduction of new technologies such as prospective dengue vaccines. Policy makers also seek breakdowns by region, country, severity, outcome (i.e. fatal or not), treatment sector (public or private), and setting (hospital or ambulatory). Major challenges in measuring disease burden include: disagreement on aspects of the WHO case definition or its lack of uniform application, limited capabilities and standards of dengue laboratories, unsatisfactory sensitivity and specificity of diagnostic tests, misdiagnosis, inconsistent criteria for reporting dengue cases to WHO, poor surveillance and reporting systems, under-reporting of fatal and non-fatal dengue and misclassification in dengue reporting, limited public awareness in endemic regions, and incidence of infection among travelers. Perhaps the most important challenge is the poor surveillance and/or lack of reporting systems. Reporting of cases varies by country but is considered especially limited among ambulatory and private providers. Though population-based studies with active surveillance are the gold standard for burden estimations, their large cost and limited generalizability to other age groups, geographical settings and time periods, make alternative methods necessary. Several additional study designs may provide reliable estimates of disease burden in dengue.

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MULTI-COUNTRY STUDY OF COSTS OF DENGUE AMONG AMBULATORY AND HOSPITALIZED PATIENTS

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The direct and indirect costs of an episode of dengue have not been determined in multiple countries using comparable methods. During 2005-2006, a study was conducted in 8 countries: 5 in the Americas (Brazil, El Salvador, Guatemala, Panama, Venezuela) and 3 in Asia (Cambodia, Malaysia, and Thailand). Participants were recruited from suspected or confirmed dengue patients treated at hospitals and ambulatory facilities. Each patient (or parent), was interviewed on 1-2 occasions, medical records were abstracted, and medical cost data were obtained from facility budget and utilization statistics, and where applicable, insurance providers. Cost per treated case of dengue consisted of direct medical costs (public and private sector ambulatory and inpatient care), non-medical costs (e.g. transportation, extra food expense), indirect costs (e.g. days lost by patient and other household members from school, work, or other activities). Costs were expressed in 2005 international dollars (I$) to adjust for purchasing power parity. A total of 1695 eligible persons with dengue were enrolled in the study. The mean total cost per case was I$514 (SD $601) for 939 ambulatory patients and I$1491 (SD $1052) for 756 hospitalized patients, not adjusted for age and dengue diagnosis. Mean costs varied by country ranging from I$158 (Guatemala) to I$699 (Brazil) for ambulatory patients and from I$752 (Guatemala) to I$2182 (Thailand) for hospitalized cases. The shares of costs for direct medical, direct non-medical and indirect costs were 23%, 5% and 72%, for ambulatory patients and 68%, 9% and 23% for hospitalized patients, respectively. The distribution of these shares also varied by country and age group. However, total cost did not differ significantly within countries between unconfirmed and confirmed dengue cases, nor between adults (≥15 years) and children (<15 years). In conclusion, on average, a hospitalized case of dengue cost three times the cost of an ambulatory case. The total cost of an ambulatory dengue case was equivalent to 24 days of GDP per capita (ranging from 12 in Venezuela to 31 in Brazil) and a hospitalized case was 80 days (ranging from 46 in Venezuela to 111 in Cambodia). In conclusion, a dengue virus infection episode that requires outpatient or inpatient care imposes substantial costs to both the health sector and the overall economy.

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EFFICACY OF OXFENDAZOLE, ALBENDAZOLE AND PRAZIQUANTEL AGAINST CYSTIC ECHINOCOCCOSIS IN NATURALLY INFECTED SHEEP

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Cystic Echinococcosis (CE) is an endemic disease in sheep-raising regions of America, Africa, and Asia. Albendazole (ABZ) is the benzimidazole of choice; nevertheless prolonged and high doses are often necessary. Praziquantel (PZQ) is being used in combination with ALB while Oxfendazole (OXF) has demonstrated its efficacy when administered in doses of 30mg/Kg for 11 weeks. Antiparasitic drugs might be useful within control programs that target animal intermediate hosts. Animal clinical trials are required to establish practical dosages of OXF and its combination with other drugs. A randomized placebo-controlled trial was carried out to estimate the efficacy of OXF as single drug, and the combination of OXF/PZQ and ALB/PZQ in naturally infected sheep. We randomly assigned 25 ewes on each of the five treatment groups: 1)control; 2)OXF 60mg/Kg weekly for 4 weeks; 3)OXF 100mg/Kg biweekly 2 times; 4)ALB 30mg/Kg + PZQ 40mg/Kg weekly for 6 weeks; and 5)OXF 30mg/Kg + PZQ 40mg/Kg biweekly for 3 times. Animals were slaughtered 4 to 8 weeks after last treatment. We registered 1,294 cyst (689 pulmonary and 605 hepatic) from which 1,059 (549 pulmonary and 510 hepatic) were evaluated. A total of 363 (34.3%) fertile cysts were found. There was no difference of lung cyst fertility between groups, but groups 2, 3 and 4 had less hepatic fertile cysts than control group ($p<0.05$). For lung cysts, the lowest protoscolex viability was observed for ALB/PZQ and OXF/PZQ combinations (12.7% and 15.6%, respectively, $p<0.05$) as compared to control group (58.4%). Similarly, group 2 (OXF60) and OXF/PZQ had lower protoscolex viability for liver cysts (15.1% and 13.5%, respectively, $p<0.05$) than controls (45.9%). The highest efficacies were observed for OXF/PZQ for lung and liver cysts (73.3% and 70.6%, respectively), follow by the ALB/PZQ group with 78.3% and 59% for lung and hepatic cysts, respectively. This trial demonstrated that OXF/PZQ might be considered as an alternative for CE cases after OXF is licensed for human use. OXF/PZQ may be evaluated in control programs to reduce the infectivity of the cysts. More practical field dosages of OXF are required to investigate.

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CLUSTERS OF CONFIRMED SWINE CYSTICERCOSIS INFECTION SURROUNDING *TAENIA SOLIUM* TAPEWORM CARRIERS

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Hotspots of *Taenia solium* cysticercosis seropositivity surrounding human tapeworm carriers have been documented, although little evidence exists about clusters of infected pigs around carriers. We evaluated the association between necropsy-confirmed swine viable infection and the distance to tapeworm carriers in six rural communities in Peru. A total of 326 pigs were necropsied (coverage: ~87%), and 18 (5.5%) had viable *T. solium* cysts. Five tapeworm carriers were found by ELISA-coproantigen and confirmed as *T. solium* by serology (prevalence=1.1%). Four were also positive on direct microscopy. The prevalence of viable infection increased nearer carriers, from 0.5% at >500m to 10.6% at 2-500m ($p=0.003$), and then from 10.6% at 2-500m to 70.0% at the carriers' home ($p<0.001$). The median distance to the nearest carrier was 1769m for uninfected pigs, 1769m for pigs with degenerated cysts only, 154m in swine with both viable and degenerated cysts and 43m among pigs with viable cysts only ($p<0.001$). Eight of the pigs with viable infection (8/18=44.4%) were found at the carriers' homes and 17 (17/18=94.4%) within 500m. Swine *T. solium* cysticercosis infection clusters strongly around tapeworm carriers

and these hotspots should be targeted by control interventions to prevent further disease transmission

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EFFICACY OF NICLOSAMIDE GIVEN AS MASS OR TARGETED TREATMENT FOR *TAENIA SOLIUM* TAENIASIS

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Niclosamide is used for the treatment of human infections by *Diphyllobothrium spp., Hymenolepis spp.,* or *Taenia spp*. There is limited assessment of its efficacy on *Taenia solium* infections because of the low prevalence of the parasite and limited compliance with post-treatment follow up. We retrospectively compared the efficacy of niclosamide treatment administered as part of a community-wide control intervention (mass treatment) or after diagnosis (targeted treatment). As it can be understood, mass treatment does not involve diet or preparation of the individual, or the use of a purgative. Targeted treatment was accompanied by a previous diet and purge. Sixteen individuals were detected as *T. solium* carriers in field intervention/control studies carried by the Cysticercosis Working Group in Peru (CWGP). These were initially included with diagnosis of *Taenia spp*., based on demonstration of parasite material (proglottis and/or eggs) or strong positive coproantigen (CoAg) and recombinant rES33 positive in EITB. Another four patients who had *T. saginata* tapeworms (2 from the mass treatment arm and two from the targeted treatment arm) were not included in the analysis. All patients received niclosamide in a single oral dose of 2 g. Patients were asked for stool samples at days 15 and 30 after the treatment. We considered as criteria of cured absence of *Taenia spp* eggs and negative coproantigen at day 30 post-treatment. Niclosamide failed to cure five out of ten patients in the treatment arm, compared to one out of six in the targeted treatment group. In this field scenario, targeted niclosamide treatment of *T. solium* carriers had a better efficacy than did mass treatment.

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CONTEMPORARY NEUROSURGICAL APPROACHES TO NEUROCYSTICERCOSIS

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Neurocysticercosis includes a spectrum of illnesses, but most mortality and much of the morbidity stems from increased intracranial pressure. Surgery has been the traditional approach to increased intracranial pressure. Newer neurosurgical approaches are being applied to neurocysticercosis including increasing use of anti-parasitic and anti-inflammatory drugs and the availability of neuroendoscopy. While controlled clinical trials are leading to an evolving consensus on management of parenchymal neurocysticercosis, fewer data address contemporary neurosurgical approaches. To document the efficacy of these approaches, we reviewed all neurocysticercosis patients evaluated by the neurosurgery service at Ben Taub General Hospital, Houston, TX between 08/1997 and 12/2005. Overall, 32 patients underwent neurosurgical evaluation. Twelve underwent external ventricular drainage (EVD) initially and 2 required subsequent EVD. Ventriculoperitoneal shunts were placed initially in 18 patients and 3 required subsequent shunt placement; five patients required shunt revision or replacement. Fourteen subjects underwent surgical resection of cysticerci during their initial hospitalization. Ten underwent open approaches (2 suboccipital [IVth ventricle], 4 frontal [lateral ventricles], and 4 temporal [basilar cisterns]). Four underwent rigid endoscopic removal of cysticerci. Endoscopic removal was performed on a 5th patient who had previously experienced 2 episodes of shunt failure. None of the patients who underwent endoscopic procedures required a shunt or a second surgical procedure and all showed clinical improvement at discharge. Despite the availability of anti-parasitic and anti-inflammatory therapies, neurosurgery continues to play a major role in the management of neurocysticercosis. The rate of shunt failure is much improved compared to earlier series, but it remains high even with anti-parasitic and anti-inflammatory drugs. Endoscopic cysticercus removal is associated with less morbidity than was noted with other approaches and appears to be the neurosurgical approach of choice.

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ANTIGEN-DETECTION IN NEUROCYSTICERCOSIS: SENSITIVITY AND SPECIFICITY ACCORDING TO PARASITE STAGE AND NUMBER OF LESIONS

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Neurocysticercosis (NCC) is a pleomorphic parasitic disease of the human central nervous system. Clinical manifestations depend on the number, location and viability of lesions. Currently, serologic diagnosis of NCC is based on antibody detection by electroimmunotransfer blot (western blot). Antigen-detection ELISA assays have been described but no formal testing of sensitivity and specificity is yet available in the literature. Using known archive samples we assessed the sensitivity and specificity of a capture antigen-detection ELISA using two monoclonal antibodies (B158C11A10 and B60H8A4) developed against the related cestode *Taenia saginata*. Antigen levels were measured in samples from patients with subarachnoid cysticercosis (n=39), intraventricular NCC (n=11), viable parenchymal NCC (n=201) or healthy controls (n=81). Samples of patients with parenchymal NCC were further subanalyzed according to the number of brain parasites: one (n=41), two (n=44), three to five (n=37), six to ten (n=40), or more than 10 (n=39). We also analyzed 52 samples of patients with degenerating parasites only and 80 samples of patients with calcified parasites only. Sensitivity and specificity were very high for basal subarachnoid NCC were the highest (92% and 99% respectively) and patients with more than 10 viable brain parasites (100% and 96%). Sensitivity gradually decreased in parallel to the number of intraparenchymal brain cysts. There were obvious differences in antigen levels between samples of patients with active NCC and calcified NCC. Circulating antigen levels are high in subarachnoid NCC and are directly related to the number of brain cysts in intraparenchymal NCC.

(ACMCIP Abstract)

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COMPOSITION AND RELEASE PATTERN OF PARASITE GLYCOCONJUGATES DURING THE COURSE OF HUMAN AND EXPERIMENTAL NEUROCYSTICERCOSIS

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Neurocysticercosis (NCC) is an infection of the central nervous system (CNS) by larvae of the helminth *Taenia solium*. The severity of the symptoms is associated with the intensity of the immune response. After a long asymptomatic period in which host immunity is unable to resolve the infection, a chronic hypersensitivity reaction occurs and involves granuloma formation, fibrosis and angiogenesis. Since little is known about the initial response to this infection, a murine model using the cestode *Mesocestoides corti* was employed to analyze morphological changes microscopically in the parasite after 1 and 3 days of infection. *M. corti* coat or tegument is released from the parasite as indicated by a thinner layer in areas making close contact with the nervous tissue. These results were confirmed by infecting murine CNS with ex-vivo labeled parasites. Because more than 95% of NCC patients exhibit humoral response against carbohydrate based antigens, and the tegument in helminths is known to be rich in glycoconjugates (GCs), the expression of these molecules was analyzed in human, porcine and murine NCC. To determine the GCs present in the tegument, fluorochrome labeled lectins with specificity to different carbohydrates were used. GCs labeled by isolectinB4 were shed in the first few days of infection whereas GCs bound by wheat germ agglutinin and concavalinA were released continuously throughout the infectious process. Importantly, all of the labeled GCs were detected in host cells counterstained with Mac1 and CD68. Peanut lectin binding GCs, on the other hand, remained on the parasite and were not detected in host cells. The parasitic origin of the lectin-binding GCs found in host cells was confirmed using antibodies against *T. solium* and *M. corti*. Rapid release of tegument GCs could facilitate evasion of the early immune response whereas constant release of GCs provide a source of persistent antigen that may help the parasite to subsist in the adverse host environment resulting in the life-long sequelae seen in many NCC patients.

(ACMCIP Abstract)

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IDENTIFICATION OF A 38 KDA SPECIFIC ANTIGEN FOR THE DIAGNOSIS OF COENUROSIS

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Brain coenurosis is produced by the larval stage of *Taenia multiceps multiceps,* (*Coenurus cerebralis*). This zoonosis has dogs and other canids as definitive hosts and sheep and other ungulates as habitual intermediary hosts. Humans act as accidental intermediary host. Diagnosis is based on imagenological techniques (CT scan, MRI in human cases) and confirmed by histopathological examination. There is only one published reference on serological diagnosis in sheep using antigens extracted from the cyst fluid of *Taenia multiceps serialis* by electroimmunotransfer Blotting (EITB). Our objective was to determine specific antigen bands using crude membrane and cyst fluid antigens of *C. cerebralis* by EITB. We used a positive pool of sera from 7 sheep infected with coenurosis, confirmed by necropsy, and a negative pool of sera from 10 sheep from a commercial farm. Three reactive bands were identified using membrane antigen (38, 23.6 y 22 kDa) and twelve using cyst fluid (133.8, 112.2, 98.4, 92.1, 82.5, 78.9, 73.9, 70.7, 58, 55.5, 20.7 y 18.5 kDa). Cross reactions with cysticercosis and hydatid disease were examined using positive pools of human sera diagnosed with these two parasitosis. The only band which did not cross react was a membrane antigen of 38 kDa. This antigen may provide a tool for serological diagnosis of ovine coenurosis, and eventually differential diagnosis of coenurosis in humans with brain cystic lesions.

(ACMCIP Abstract)

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DEVELOPMENTAL ARREST OF MALARIA PARASITES IN MOSQUITOES FOLLOWING TREATMENT OF MICE WITH AS-I-145

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Given the rapid spread of drug-resistant malaria strains, the development of effective antimalarials that prevent transmission is an important strategy towards controlling malaria. We evaluated the transmission-blocking activity of the DNA-alkylating agent, AS-I-145, which gave virtually no toxicity to C57/BL6 mice at doses as high as 15 mg/kg. This compound exploits the A/T richness of the *Plasmodium* genome by binding to these nucleotides in the minor groove of DNA. The infectivity of *P. berghei* ANKA gametocytes following exposure to AS-I-145 *in vivo* was assessed in *Anopheles stephensi*. This compound was extremely effective at blocking differentiation of parasites in mosquitoes after treatment of mice. A single intraperitoneal injection of AS-I-145 (10mg/kg) in mice did not affect circulating gametocytes, but arrested parasite development within the midguts of mosquitoes fed on these mice 24 h after drug injection. Severe defects were observed by transmission electron microscopy of parasite oocysts on mosquito midguts at day 10 post-feeding. In addition, the number of sporozoites in both the midgut and the salivary glands were reduced by over 99% in these mosquitoes. DNA damage was detected in the midguts of mosquitoes using a real-time PCR assay showing that the damage persisted from the blood stages in the mammalian host to the mosquito. AS-I-145 could be an effective transmission-blocking drug by blocking the infectivity of *Plasmodium* within the mosquito.

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DIRECT MICROSCOPIC QUANTIFICATION OF TRANSMISSION DYNAMICS OF *PLASMODIUM* SPOROZOITES FROM MOSQUITOES TO MICE

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The numbers of malaria sporozoites delivered to a host by mosquitoes is thought to have a significant influence on the subsequent course of the infection in the mammalian host. We did studies with *Anopheles stephensi* mosquitoes with salivary gland infections of *Plasmodium berghei* sporozoites expressing a red fluorescent protein. After individual mosquitoes fed on an ear pinna or the ventral abdomen of a mouse, fluorescence microscopy was used to count numbers of sporozoites. Mosquitoes allowed to feed on the ear for periods of 3 vs 15 minutes delivered means of 281 vs 452 sporozoites, respectively; this may have epidemiological implications because mosquitoes can feed for longer periods of time on sleeping hosts. Mosquitoes feeding on the ventral abdomen, injected sporozoites not only into the skin, but also into the underlying peritoneal musculature. Although mosquitoes injected fewer sporozoites into the abdominal tissues, more of these were re-ingested back into the mosquito midgut, probably a consequence of easier access to blood intake from the abdominal area. Accordingly, we also calculated

the total number of sporozoites released from the proboscis (number deposited in the mouse tissue plus number re-ingested by the mosquito). The most consistent parameter of sporozoite transmission dynamics under all conditions of mosquito probing and feeding was the relatively slow total release rate of sporozoites (~ 1-2.5 per second) from the mosquito proboscis. Numbers of sporozoites introduced into the host by mosquitoes and the transmission efficiency of sporozoite delivery are multifactorial phenomena that vary with length of probing time, skin site being fed upon, and numbers of sporozoites within the salivary glands.

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PLASMODIUM FALCIPARUM GENETIC STRUCTURE IN THE FOUR MAJOR AFRICAN *ANOPHELES* VECTORS

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In African malaria endemic areas, major *Anopheles* vectors are often sympatric, infected with mixtures of *Plasmodium* species and genotypes. To investigate the vector host impact on *Plasmodium falciparum* population structure, we analysed oocyst infection and neutral diversity distribution within and among the four major species *Anopheles gambiae s.s., A. funestus, A. nili* and *A. moucheti* sampled at the scale of a village in a highly endemic area of Equatorial Africa. *Anopheles* females were collected in 2002, 2003 and 2004 in Simbock, Cameroon. Individually dissected oocysts were genotyped at 7 *P. falciparum* microsatellite loci. Patterns of parasite infection, genetic diversity and population structure were assessed within and among mosquitoes, vector species and sampling dates using FSTAT, HIERFSTAT and CANOCO softwares. A total of 6,848 midguts were checked for oocysts out of 11,148 mosquitoes collected from the four vectors, and 90 were found infected (1.3%, CI=1.1%-1.6%). *Anopheles funestus* was the most endophagic and most infected species while carrying the lowest oocyst load (Fischer and Bootstrap 2-sample t-tests, $P \leq 0.04$). Genetic analyses were based on 345 individually dissected oocysts from 85 mosquitoes. Allelic polymorphism (13.1±1.9 alleles per locus) and mean expected heterozygosity (0.79±0.04) were high. The heterozygote deficit within and among mosquitoes was significant (mean F_{IS}=0.17, $P<10^{-4}$ and mean F_{ST}=0.37, $P<10^{-4}$). A total of 22 mosquito guts out of 57 with >1 genotype contained a repeated oocyst genotype (39%). All significant patterns were consistent over time. Results of time (hierarchical analysis) and *Anopheles* species (CCA analysis) effects will be presented. Genetic structure of *P. falciparum* within and among mosquitoes displayed characteristic features of a low mixing parasite species, with significant overall F_{IS} within mosquitoes, high between-mosquito F_{ST}, and presence of repeated genotypes. The impact of the *Anopheles* species factor on *P. falciparum* infection and population structure is discussed.

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ANOPHELES GAMBIAE STAT PATHWAY PARTICIPATES IN MOSQUITO IMMUNITY

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Innate immune responses are mediated by the activation of various signaling processes. Here, we describe Janus kinase (JAK)/signal transducers and activators of transcription (STAT) signaling in the mosquito immune responses to bacteria. Unlike Drosophila and other mosquitoes, two member of STAT family (AgSTAT-A and AgSTAT-B) are present in *An. gambiae* mosquito. STAT-A and STAT-B genes and other component of this pathway SOCS (suppressor of cytokine signaling) and NOS (nitric oxide synthase) are expressed in the immuno-responsive *A. gambiae* Sua 5.1 cell line as well as mosquito midgut and body wall. NOS and SOCS are transcriptionally activated in response to bacterial challenge. Knockdown either STATs in Sua 5.1 cell, down regulates the mRNA expression of SOCS and NOS suggesting that STAT pathway regulates their expression. More over knockdown of STAT-A down regulate STAT-B expression suggested STAT-A is upstream of STAT-B. Further we tested whether STAT play a role in bacterial immunity in adult *An. gambaie* mosquito. STAT-B silencing does not affect mosquito survival, when adult were challenged with bacteria either by injection directly into hemocoel or by feeding bacterial culture. It suggested that this pathway might not be essential for bacterial infection.

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PRESENCE OF MALARIA ASEXUAL BLOOD STAGES SIGNIFICANTLY DECREASES THE BURDEN OF *PLASMODIUM FALCIPARUM* OOCYSTS IN *ANOPHELES* MOSQUITOES AFTER MEMBRANE FEEDING ASSAYS

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Anopheles mosquitoes normally feeding on blood during malaria transmission ingest not only gametocytes from the human host but also circulating asexual blood stages of the parasite. Upon ingestion, gametocytes undergo sexual development in the mosquito midgut leading to gamete fertilization, oocyst development and finally formation of transmission competent sporozoites. Previous studies have shown that antibodies directed against mosquito midgut stages of the parasites effectively suppress parasite development in the mosquito midgut. Any effect of the presence of asexual stages, homologous or heterologous to infecting strain gametocytes, has not been investigated. We analyzed, in 3 species of *Anopheles* (*A. gambiae, A. stephensi* and *A. freeborni*), the effect of the presence of *P. falciparum* (NF54) asexual blood stages on the infectivity of culture-derived gametocytes through membrane feeding assays (MFA). After feeding, mosquitoes were dissected and the midguts examined for the presence of various sporogonic stages of the parasite. We found that the number of oocysts (day 9) in the mosquitoes fed with only gametocytes was significantly higher than the number of oocysts in the mosquitoes fed with gametocytes plus asexual stages mixed at various ratios. To further examine possible developmental block due to reduced fertilization and ookinete formation, parasite densities were determined within the entire blood meal at 16 h and 27 h post-infection by IFA using monoclonal antibodies αPfs25 protein. These time points were chosen to differentiate between post fertilization parasite densities in the midgut before and after penetration of the midgut. No significant difference in the number of zygotes, retorts and ookinetes was observed between the two groups of mosquitoes. We also analyzed by real-time quantitative PCR three known innate immunity genes likely to have anti-*Plasmodium* functions. In these studies we did not find any significant difference in the expression of *Tep1, LRIM1* and *AgMDL1* between the two groups of mosquitoes, suggesting that the differences in infection levels was not due to a more potent up-regulation of these innate immune genes by asexual parasites. These studies suggest that the presence of asexual blood stages of the malaria parasite significantly down modulates the infectivity of gametocytes. We are now attempting to investigate possible mechanisms that could account for such biological findings.

(ACMCIP Abstract)

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SAMPLING TOOLS FOR ADULT MALARIA VECTORS IN URBAN DAR ES SALAAM, TANZANIA

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Effective and sensitive mosquito sampling tools are essential to any vector control program so as to monitor and evaluate the impact of interventions. However, in urban Dar es salaam, of all tested collection techniques, human landing catch (HLC) is the only method currently sufficient sensitive for routine monitoring of *Anopheles*. HLC is labour-intensive and cumbersome, requiring intense supervision to the extent that is impossible to sustain on large scales. HLC is also ethically difficult to justify because it increases exposure of participants to malaria, making the study and suppression of transmission difficult and prohibitively expensive in urban Dar es salaam malaria control program. New sampling tools were initially developed in Kilombero Valley, south- east Tanzania where very high densities of Anopheles gambiae enable rapid assessment multiple trap designs. The most promising designs of canvas tent trap do not require electricity and require no human exposure. These designs were compared with CDC light traps and HLC in both rural Kilombero and urban Dar es Salaam using a Latin-square randomized experimental design. Sensetivity varied by time of year and location between approximately 22 and 62% of that of HLC but practicalilty ensured it was at least as sesentive per dollar spent in practical programmatic application.

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HEROIC FAILURES? THE FIRST SOUTH ASIAN MALARIA CONTROL PROJECTS AFTER THE DISCOVERY OF MOSQUITO TRANSMISSION

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After Ross discovered the mosquito transmission of malaria, two field studies in South Asia were initiated to test the practicality of malaria control. In the military barracks of Mian Mir near Lahore, mosquito brigades of laborers were sent to drain puddles and oil canals. In the penal colony of the Andaman Islands in the Bay of Bengal, prisoners were used to drain swamps and oil puddles. Intense physical activity was initially interpreted as indicative of malaria control using unsophisticated methods. Within a short period of time, however, it was clear that mosquito control would not be a simple or cheap way to control malaria transmission. The periodic epidemiology of malaria at Mian Mir associated with excessive rainfall triggering massive breeding of *Anopheles culicifacies* was not understood. Neither was the fact appreciated that malaria in the Andaman Islands was inversely proportional to the distance from the sea, due to the brackish-water breeding *An. sundaicus*. The intended demonstration project in Mian Mir resulted in a poorly executed field trial whose interpretation has been disputed ever since its conclusion in 1909. Installation of wire screening in the barracks was much more successful than any direct anti-mosquito measures. Failure of malaria control in the Andaman Islands Penal Colony using mosquito brigades resulted in a variety of adjunctive field trials using bed nets and prophylactic drugs. Major engineering works to drain the mangrove swamps surrounding the penal colony triggered a massive malaria epidemic and quintupling of the all-cause mortality rate. It was estimated that 5000 pounds sterling and 400,000 rupees (then considered vast sums by the colonial government) were expended in Mian Mir and the Andaman Islands Penal Colony respectively without considering the huge human labor contributions. Both control efforts were interpreted as failures discrediting the "mosquito hypothesis" of Ross who continued to declare that the correct procedures had not been pushed to a successful conclusion. Malaria control in the Indian subcontinent was dogged for over four decades by the specter of public health failure. Adequate understanding of the local epidemiology remains critical to the design and execution of malaria control field trials.

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REPRODUCIBILITY OF A SPOROZOITE CHALLENGE MODEL FOR *PLASMODIUM VIVAX* IN HUMAN VOLUNTEERS

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Several *Plasmodium. vivax* vaccine candidates are currently being evaluated in Phase I clinical trials, but cannot be assessed for protective efficacy in the absence of a reliable human challenge model. Investigators at MVDC have developed methods for reproducibly generating *P. vivax* sporozoites in *A. albimanus* and have now tested the model in two clinical trials. In the first, 18 volunteers confirmed by PCR to be Duffy positive (Fy+) were exposed to 3, 6 or 9 infected mosquito bites. 17/18 developed parasitemia with a mean prepatent period of 11 days. In a second trial, completed recently, we demonstrated that volunteers can be reproducibly infected by as few as 2 infected mosquito bites: 24 healthy adult, malaria-naïve volunteers were randomly assigned to 3 groups of 6 Fy+ and 2 groups of 3 Fy- volunteers. Each group was exposed to 3 ± 1 infective bites, The batch of mosquitoes used to infect each group had been membrane-fed on gametocytemia blood derived from individual donors presenting at a local clinic with *P. vivax* infections. Challenge volunteers were monitored by blood smear (TBS) and for signs and symptoms of malaria beginning on day 7. All Fy+ volunteers developed positive TBS, accompanied by signs and symptoms consistent with malaria, with a mean pre-patent period of 14 days for group 1 and 10.8 for groups 2 and 3 (group 1 statistically significantly delayed relative to groups 2 and 3, Kruskal-Wallis, p=0.02). All infected volunteers received treatment the day of positive TBS. No Fy- individuals developed malaria symptoms and all remained TBS negative through day 30 post-challenge, when they where treated. No differences in parasite density were observed among the Fy+ groups and all volunteers recovered after treatment without any severe or serious adverse events recorded. In conclusion, we have developed a safe, reliable and reproducible *P. vivax* challenge system for humans, using, to date, 4 batches of mosquitoes each infected from a different gametocytemic donor, and as few as 2 infective bites, with prepatent periods ranging from 9 to 16 days.

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COMPARISON OF THE IMMUNOGENICITY OF ADENOVIRUS 35-PFCS ALONE AND IN HETEROLOGOUS COMBINATION WITH AN ADENOVIRUS 5-PFCS CONSTRUCT

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The RTS,S antigen-based *Plasmodium falciparum* circumsporozoite protein (CS) is the only vaccine candidate thus far with proven efficacy in humans. We have made two adenoviral constructs using replication-incompetent PER.C6-dependent Adenovirus 35 and Adenovirus 5 with an optimized CS sequence, terminated at the C-terminal end prior to the GPI anchor sequence and prior to a putative glycosylation site (Ad35CS and Ad5CS). Using rhesus macaques, we compared two doses of Ad35CS given at a three month interval with a six month interval, and with a prime of Ad5CS followed six months later by an heterologous Ad35CS boost. The adenoviral constructs produced good Th1 responses to both the C- and N-term of CS by both IFNγ ELISpot and multiparameter intracellular staining, and the responses were clearly detectable after just one vaccination. The heterologous vector strategy produced better responses that also persisted better at three and six month post vaccination. Serologic responses were equivalent across all groups.

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A NON-ADJUVANTED SELF-ASSEMBLING POLYPEPTIDE NANOPARTICLE (SAPN) MALARIA VACCINE CONFERS STERILE PROTECTION TO LETHAL SPOROZOITE CHALLENGE

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Most protein-based vaccines require formulation with an adjuvant to achieve protective immunogenicity. We report here the development of a malaria vaccine based on a new vaccine platform technology, Self-Assembling Polypeptide Nanoparticles (SAPN), which are small particles (diameter of 20 nm) formed from 60 linear polypeptide building blocks consisting of pentameric and trimeric coiled-coil domains separated by a linker, with epitope antigens displayed on the N- or C-terminus or both. Each nanoparticle displays the peptide epitopes in repetitive arrays on its surface. A SAPN displaying the *Plasmodium berghei* CSP epitope ($(DPPPPNPN)_2$) and designated SAPN-PbCSP, induced a protective immune response against a lethal sporozoite challenge in the *P. berghei* mouse malaria model without the need for an adjuvant. Balb/c and C57BL/6 mice immunized with three doses of 10ug SAPN-PbCSP per dose produced a long-lasting (> 6 months) high titer antigen-specific antibody response with maximum ELISA titer values obtained after administration of the third dose. The use an adjuvant (Montinade ISA 720) increased the rate of immune response to achieve maximum ELISA titer values after two doses but no significant difference ($p<0.05$) was observed after the third. Anti-SAPN-PbCSP specific antibody titers strongly correlated with protection. Serum but not cells conferred protection to mice against lethal sporozoite challenge following a passive transfer. Our results suggests that the SAPN platform is potentially superior to other vaccine technologies used to date as these have all required adjuvants to be protective.

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ENHANCED IMMUNOGENICITY OF MALARIA CS PEPTIDE VACCINES USING A TOPICAL ADJUVANT CONTAINING A POTENT SYNTHETIC TLR LIGAND, IMIQUIMOD

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A primary goal of pre-erythrocytic vaccines is to elicit antibodies that effectively target extracellular sporozoites as they transverse to the host hepatocyte. In addition, cellular immunity, mediated by the inhibitory cytokine IFN, is required to target residual EEF that develop from sporozoites that escape antibody neutralization. In previous preclinical and clinical studies, we demonstrated that synthetic peptides containing minimal T and B cell epitopes of the *Plasmodium falciparum* CS protein can elicit sporozoite neutralizing antibodies and IFN producing CD4+ T cells. However, synthetic peptides require strong adjuvants, as they lack the endogenous "danger" signals found in virus or bacterial vaccines that stimulate dendritic cell maturation, primarily through Toll-like receptors (TLR), required for induction of strong adaptive immunity. In recent studies, we explored a topically applied synthetic TLR 7/8 ligand, Imiquimod, as adjuvant for *Plasmodium falciparum* CS synthetic peptides. Imiquimod cream is approved for topical treatment of dermatologic conditions in humans and has been shown to function as a topical adjuvant for model protein antigens. Mice immunized s.c with falciparum CS peptides, followed by topical application of the TLR adjuvant, developed anti-repeat antibody titers 1-2 logs higher than mice receiving peptide alone. Antibodies included a broad range of IgG isotypes indicating a mixed Th1/Th2 response. These antibodies were biologically active and neutralized sporozoite infectivity of a transgenic rodent parasite that expresses *P. falciparum* CS repeats. Studies of cellular responses elicited by this completely synthetic immunogen and adjuvant formulation are in progress. In addition to enhancing immunogenicity of peptide vaccines, the use of a topical adjuvant, rather than co-formulation in adjuvant, can eliminate potential problems associated with protein modifications and instability of the immunogen, as reported for malaria recombinant proteins and virus-like particle vaccines formulated in oil emulsion adjuvants.

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ANIMAL IMMUNOGENICITY STUDIES OF A BLOOD-STAGE MALARIA VACCINE BASED ON A COMBINATION OF AMA1 AND $MSP1_{42}$

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A blood-stage antigen mix vaccine, BSAM-1, with two allelic forms each of AMA1 and $MSP1_{42}$ is being developed. Combining functionally independent antigens could induce immune responses that complement one another and generate a more effective immune response against a single parasite. BSAM-1/Alhydrogel is composed of AMA1-FVO, AMA1-3D7, $MSP1_{42}$-FVO and $MSP1_{42}$-3D7 in a 1:1:2:2 ratio adsorbed to Alhydrogel. In animals, AMA1, $MSP1_{42}$ and BSAM-1 formulated on Alhydrogel or Alhydrogel+CPG 7909 are safe and immunogenic. Within BSAM-1/Alhydrogel formulations, no immunologic inhibition was detected. When CPG 7909 was added to BSAM-1/Alhydrogel, a significant increase in overall antibody production to both AMA1 and $MSP1_{42}$ ($P< 0.016$) was seen, but decreased anti-AMA1 or anti-$MSP1_{42}$ antibody responses were detected relative to the individual component Alhydrogel+CPG 7909 formulations at comparable doses ($P < 0.0367$). This immunologic inhibition could have resulted from high antibody production following vaccination with both a large protein load and a potent adjuvant that may have overwhelmed the immune system. IgG purified from rats and rabbits vaccinated with BSAM-1/Alhydrogel or BSAM-1/Alhydrogel+CPG 7909 exhibited high levels of *in vitro* parasite growth inhibition activity. Nearly all of the IgG responsible for this biological effect was found to be anti-AMA1 specific and could be reversed by addition of recombinant AMA1 proteins. There was no apparent detriment to the biologic function of the antibody in BSAM-1/Alhydrogel+CPG 7909 groups even when decreased anti-AMA1 responses were observed. The ability to reverse the parasite growth inhibition activity with AMA1 but not $MSP1_{42}$ protein is not surprising given that anti-AMA1 antibodies have a lower threshold for inhibiting parasite growth than anti-$MSP1_{42}$ antibodies. GIA may not be the only mechanism of immunity (e.g. CD4+ T cell immunity and ADCC); as MSP1 vaccines protect against rodent malaria, the combination vaccine may protect by a combination

of immune mechanisms. Thus, we will determine whether anti-$MSP1_{42}$ immunity may show additional effects in the presence of anti-AMA1 antibodies. As no animal model can be used as a surrogate model of human vaccine efficacy, clinical evaluation of BSAM-1/Alhydrogel+CPG 7909 is planned.

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MONOVALENT AND BIVALENT ADENOVECTORED VACCINES EXPRESSING THE *PLASMODIUM FALCIPARUM* ANTIGENS AMA-1 AND MSP1-42 (3D7) ELICIT FUNCTIONAL ANTIBODIES IN NZW RABBITS

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NMRC and GenVec, Inc, in partnership with MVI and United States Agency for International Development, are developing adenovirus-vectored vaccines to protect against *Plasmodium falciparum* (Pf) malaria, due to the demonstrated ability of this vaccine platform to induce strong cell-mediated immunity. Although adenovectors also induce antibody responses, titers are generally less than those induced by recombinant proteins in adjuvant and it has been unclear whether these responses will afford protection against blood-stage malaria where antibody appears to mediate protection. To test this, we performed growth inhibition assays using immunoglobulin purified from rabbits immunized with first and second generation adenovectored malaria vaccine candidates. Our first generation vaccine, termed NMRC-M3V-Ad-PfCA, is a mixture of two monovalent Ad5 serotype adenovirus vectors expressing either PfCSP or PfAMA1, and is currently being evaluated in the clinic. Our second generation vaccine is a bivalent adenovectored malaria vaccine, Ad-PfAMA1/PfMSP1-42, comprising a single Ad5 serotype adenovirus vector that co-expresses both PfAMA1 and PfMSP1-42. Both first and second generation adenovectored vaccines were administered to NZW rabbits intramuscularly at $1x10^{10}$ per construct in a two or three-dose regimen. Study endpoints were antibody titers (ELISA) and functional antibodies (GIA). Antibody titers were evaluated after each dose, by ELISA. Functional antibody responses were assessed prior to immunization and 2 or 8 weeks after the final dose, by GIA. Both malaria vaccine candidates demonstrated robust antibody responses and GIA activity predominantly associated with PfAMA1. The vaccine-induced GIA activity was effective against the homologous *Pf* 3D7 parasite strain, but not against the heterologous *Pf* FVO strain. Overall, data demonstrate that both monovalent and bivalent adenovectored malaria vaccine candidates can induce robust functional antibody responses, supporting clinical evaluation of these first and second generation adenovectored malaria vaccines.

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INDUCTION OF ANTIBODIES IN RABBITS AGAINST THE PREGNANCY MALARIA VACCINE CANDIDATE VAR2CSA USING *PICHIA PASTORIS* YEAST AND PLASMID DNA IMMUNIZATION

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Pregnancy-associated malaria (PAM) is associated with the sequestration in the placenta of *Plasmodium falciparum*-infected erythrocytes (IEs) that bind to chondroitin sulphate A (CSA). VAR2CSA is the main candidate for a pregnancy malaria vaccine, but its large size (~350 kDa) and extensive polymorphism may pose a challenge to vaccine development. Here we used *Pichia pastoris* to produce four of the six DBL domains from FCR3VAR2CSA to immunize rabbits. Separately, we immunized with plasmid DNA encoding the same six DBL domains. Rabbit antibodies generated by protein or DNA immunization were highly specific to the immunizing domain and cross-reacted to only a limited extent between different DBL domains by ELISA or Western Blot. Rabbit antibodies raised against three recombinant proteins (rDBL1x, rDBL3x, rDBL6ε) and four plasmid DNAs (DBL1x, DBL3x, DBL5ε and DBL6ε) were able to detect the native protein on the surface of FCR3-CSA infected erythrocytes (from ~20% of IEs positives to ~90%). However, only limited cross-reactivity was observed for two of those sera with 3D7-CSA and none of the sera cross-reacted with 7G8-CSA infected erythrocytes, both expressing the *var2csa* transcript. In contrast, antibodies to the VAR2CSA DBL4ε domain reacted strongly with recombinant proteins, but did not label CSA-binding infected erythrocytes. To examine whether steric interactions prevented DBL4 recognition in the native protein, FCR3-CSA IEs were treated with protease. Trypsin digestion removed the N-terminal DBL domains and revealed strong binding of DBL4ε anti-sera. This study demonstrates that *P. pastoris* is suitable for production of recombinant VAR2CSA proteins for vaccine development and indicates that individual DBL domains may not be sufficient to overcome inaccessibility or polymorphism in VAR2CSA DBL domains.

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SURVIVAL OF *FRANCISELLA TULARENSIS* TYPE A IN BRACKISH WATER

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Martha's Vineyard, Massachusetts (MV) has been the location of two outbreaks of pneumonic tularemia. Landscaping activities are associated with risk, suggesting inhalational exposure to environmental fomites. PCR-testing of water and soil samples collected in a MV site with known enzootic tularemia activity paradoxically failed to demonstrate the presence of *Francisella tularensis* Type A; however, other *Francisella* spp. were detected. Although *F. tularensis* Type B persists in freshwater and mud for several weeks, no published report describes the environmental stability of Type A, which is responsible for the tularemia cases on MV. Accordingly, we sought to determine whether Type B (LVS strain) or Type A (B38 strain) could survive in brackish water collected on MV and from freshwater collected from a mainland stream. *F. novicida* and *F. philomiragia* were compared as well because these organisms are water-associated. Filter-sterilized water samples were inoculated with 10^4 cfu/mL of each test strain and incubated at room temperature. The suspensions were tested for growth by colony forming unit counts (CFUC) at various time points after inoculation. CFUC for all strains that were incubated in brackish water remained stable for the first day with a steady decline of CFUC thereafter. A five-fold decrease in CFUC was observed with strain B38 by day 2. With the exception of *F. philomiragia*, all strains showed few to no CFU by day 7. CFUC of all strains inoculated into freshwater declined rapidly during the first day, with no colonies observed for strain B38. Only *F. novicida* continued to form colonies to day 7. In contrast, dense suspensions (10^8 - 10^9) persisted longer; all strains suspended in brackish water persisted for at least 22 days with the exception of B38 which persisted to 15 days. Freshwater failed to support strain B38 viability past 3 days. Brackish water may maintain the viability of concentrated *F. tularensis* Type A for at least 3 days in a laboratory setting without extrinsic nutritional supplementation. It may be that brackish water

sources, common in the southern portion of MV, may serve as a basis for prolonged environmental persistence of *F. tularensis* Type A and thus the unusual prevalence of pneumonic tularemia there.

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DRIVERS OF VARIABILITY IN WATER QUALITY AND DIARRHEAL DISEASE IN NORTHERN COASTAL ECUADOR

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The safety of recreational and drinking waters is commonly measured using indicators of fecal contamination. However, spatiotemporal variability of these indicator organisms makes interpretation of data difficult. We explore sources of variability in water quality (WQ) as measured by E.coli concentrations in source and household water samples at varying timescales over the course of 1 year in a rural Ecuadorian village that suffers from high rates of diarrheal disease. For surface source waters, we observe just as much hourly variability as daily or weekly variability (range: 0-45,000 CFU/100 mL). In the wet season, week-to-week variability can be explained by rainfall patterns. A 1" increase in rainfall is associated with an 8% increase in E.coli counts ($p<0.0001$). In the dry season, peaks in contamination appear to occur mostly independently of rainfall pulses. Number of people in the river can also explain hour-to-hour variability in both seasons. This suggests that a "runoff effect," influenced by peak rainfall events, operates at a seasonal time scale, whereas a "concentration effect," influenced by local contamination events, operates at a daily time scale. For household water samples, both seasonality and household level factors such as water source, container type, and capping of container affect variability in WQ. Interestingly, rainfall protects against contamination in the household, perhaps due to seasonal changes in hygiene, water replacement rate in containers, or reliance on rain and well water. Uncertainty in WQ measurements drive the remaining sources of variability, suggesting that geometric means of indicator counts from multiple samples taken at different times would be a more appropriate measure of WQ. This analysis suggests that, in addition to established household-level factors, climatic variables interact with contamination sources, such as poor sanitation, in explaining variability in WQ. We discuss these findings in the context of factors that can increase variability in hydrological patterns, such as climate change and deforestation.

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RECONTAMINATION OF HOUSEHOLD DRINKING WATER: A CONTROLLED EXPERIMENT IN NORTHERN COASTAL ECUADOR

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Waterborne diseases are still responsible for approximately 2.5 million deaths each year. We describe the results of a controlled experiment to assess contamination of drinking water between the source and point-of-use in northern coastal Ecuador. Samples of source waters were taken at the time that household members filled their drinking water containers, and a control container was filled at the same time and kept in controlled conditions to avoid recontamination. Household and control containers were then resampled daily until the household water was finished to evaluate water quality as measured by E.Coli and enterococci. This experimental design allows for a controlled assessment of die-off and recontamination events, comparing source waters to both control and household samples, and to our knowledge is the first study to use paired samples or controls in assessing recontamination between source and point-of-use drinking water quality. We observed on average a more than half-log reduction of indicator organisms between the source of drinking water to its point-of-use, followed by an average 0.2-log increase during storage. While this confirms that recontamination is occurring, the overall reduction of contamination between the source and the point-of-use seen here contradicts the trend seen in the literature showing that water in the home is generally more contaminated than at the source. We suggest that this may be due to the poor initial source water quality stemming from the reliance of these villages on untreated surface water and simple piped water systems for their drinking water. In most other studies documenting contamination between source and point-of-use improved source waters are tested. The results of this study argue for the importance of paying attention to source water quality and the factors that affect it such as sanitation.

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HOUSEHOLD-SCALE DRINKING WATER TREATMENT IN CAMBODIA: A RANDOMIZED, CONTROLLED TRIAL OF LOCALLY MADE CERAMIC FILTERS

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Household drinking water treatment has been shown to be an effective intervention to reduce diarrheal diseases in developing countries. Improvements in household drinking water quality and associated health impacts of low-cost ceramic water filters, one promising technology for point-of-use water treatment, have not been adequately characterized. A randomized, controlled intervention trial of two locally produced ceramic drinking water filters was conducted in the rural/peri-urban village of Prek Thmey, Cambodia. Outcomes of interest were diarrheal disease and dysentery in all individuals and particularly in children under 5 years of age during the 22 week trial (18 weeks post-randomization). Interventions were a locally-produced porous clay pot filter, called the ceramic water purifier (CWP), manufactured and implemented by the NGO Resource Development International (the CWP1) and a modified version of it employing an alternative base clay mixture (the CWP2). Major findings were that: (i), use of either filter resulted in a significant decrease (>40%) in diarrheal disease, an effect that was observed in all age groups and both sexes after controlling for clustering within households and within individuals over time; (ii), CWP1 filter was associated with a substantial reduction in dysentery (61%), an effect not observed with the CWP2; and (iii), both filters reduced *E. coli* in water up to 99.9999%, with mean reductions of 99% to 99.9%. These results suggest that locally produced ceramic filters as implemented in Cambodia are effective interventions for the improvement of household drinking water quality and they significantly reduce the risk of diarrheal diseases in users.

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HEALTH IMPACT STUDY OF THE BIOSAND FILTER IN BONAO, DOMINICAN REPUBLIC

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More than 1 billion people in the developing world lack access to improved sources of drinking water; even more lack microbiologically safe drinking water. Annually, 3-5 billion cases of diarrhea result in 1.6 million deaths. A number of household water treatment and safe storage technologies such as chlorine disinfection, solar disinfection and ceramic filtration have been documented for their ability to reduce diarrheal disease and improve microbial water quality. A promising technology is the biosand filter (BSF), a household-scale, intermittently operated slow sand filter. While an estimated 80,000 BSFs are in use globally, there is little scientific evidence of their ability to improve microbiological water quality in the field and reduce diarrheal disease in users. The purpose of this research was to document the ability of BSFs to reduce diarrheal disease in user as compared to non-user households. A randomized controlled trial of the biosand filter was performed in two areas of the city of Bonao, Dominican Republic in 2005-2006. Approximately 150 households were

enrolled in the study and from September 2005 to February 2006, they were asked to report cases of diarrheal disease each week as baseline (pre-intervention) illness rates. In February 2006, 50% of the households were randomized to receive the BSF intervention and all intervention and control households were visited weekly for diarrheal disease surveillance from February 2006 to August 2006. Initial results indicate 45% less diarrheal disease in filter households compared to non-filter households. This observed reduction in diarrheal disease is within the range reported for other effective household water treatment processes such as chlorine disinfection or ceramic filtration. This is the first study to rigorously document the ability of the biosand filter to reduce diarrheal disease and it provides critical evidence to support the continued implementation of the biosand filter in the developing world.

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A LONG-LIFE, POINT-OF-USE HOUSEHOLD DRINKING WATER PURIFICATION DEVICE BASED ON HALOGEN CHARGING OF POLYSTYRENEHYDANTOIN BEADS (HALOPURE)

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HaloPure®, a hydantoin-based, insoluble, derivatized polystyrene disinfection medium, can be brominated or chlorinated to generate contact biocidal activity against microbial contaminants in a single pass flow of water. The halogen content can be maintained by constant exposure to a low level of free halogen provided by a replaceable activator tablet, extending biocidal effectiveness in routine use for long periods, probably years. This unique feature has been designed into an in-home, gravity-feed, point-of-use (POU) water purification device consisting of a collection basin, integral activator tablet slot, tablet life indicator, and disinfection chamber containing HaloPure® beads. Inserted between canisters of a typical ceramic candle water filter system, the device adds potent disinfecting functionality to basic suspended particle filtration systems that have been used in many tropical countries for over 100 years. Appropriately configured, the device achieves high performance against bacterial and viral contaminants, sufficient to raise the prospect of compliance with the USEPA purifier guide standard for drinking water when using cyst-reducing ceramic candles in the upper canister prefilter. Devices are challenged with rotavirus, poliovirus and *Klebsiella* terrigena. Disinfection byproducts measured in the first 300 L of water were below USEPA MCLs, with subsequent decreasing levels. TTHM were 0.062 mg/L. HAA5 were 0.0028 mg/L. In a simulated home use pattern, the activator tablet eluted 1.9 mg/L bromine into the water, subsequently captured by the HaloPure® beads. Mean residual bromine was 0.49 mg/L (range 0.24-0.76) in > 300 L treated water from one tablet. The tablet alone does not effectively disinfect, but as a slow-eluting halogen source to repopulate binding sites on the HaloPure®, device efficacy is enhanced and prolonged. By upgrading commonplace water clarifying filters to disinfecting/purifying status, this device enables the provision of convenient, clean safe water for longer periods and lower cost than previously possible with the POU approach.

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EFFICACY OF ONE DROP POINT-OF-USE CHEMICAL DISINFECTANT TO INACTIVATE WATERBORNE MICROORGANISMS

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In many regions of the world, microbial contamination of drinking water requires point-of-use treatment in order to be made safe to drink. Of the chemical treatments, chlorine remains the most widely used to disinfect drinking water. Although its strong oxidizing properties make chlorine effective at inactivating waterborne microorganisms, it is often rejected by users due to taste and odor problems and toxic disinfection by-products. An alternative is One Drop, an aqueous solution of natural ionic minerals, including silver, gold, aluminum, zinc and copper believed to have microbiocidal properties when added in small quantities to contaminated water. In small quantities, One Drop is harmless to humans and does not affect taste or odor, providing an advantage over chlorine and other chemical disinfectants. The purpose of this study was to test One Drop for its ability to inactivate both indicator and pathogenic microorganisms in raw surface water. Different volumes of One Drop (0, 1, 2, or 4 drops) were added to one-liter volumes of raw surface water spiked with *E. coli* B, *Klebsiella terrigena*, MS-2 coliphage, *Salmonella typhimurium* WG-45, and *Vibrio cholerae*. Concentrations of each microbe were measured both initially and after 10, 30, 90, and 240 minutes at ambient (room) temperatures of 23-25°C. Reductions of >6 $\log_{10}$ (>99.9999%) were achieved for *E. coli* B and *Klebsiella terrigena*. Reductions of both *S. typhimurium* WG-45 and *V. cholerae* also were great, at >4.5 and >4.8 $\log_{10}$, respectively. Reductions generally increased over time and with increasing number of drops added. Microbe reductions with One Drop were more rapid and extensive than natural reductions in the same test water without One Drop. Initial evidence suggests that virus reductions are also achieved, based on >3.7 $\log_{10}$ inactivation of coliphage MS2. We conclude that One Drop reduces concentrations of important pathogenic bacteria and perhaps viruses in raw water and may serve as an effective and low-cost means of household or other point-of-use water treatment.

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THE INSIGHTS OF MANAGING INSECTICIDE RESISTANCE IN MALARIA VECTORS WITH THE PLANT EXTRACTS IN TROPICAL AFRICA

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This study aimed at evaluating the repellency and feeding inhibition of *Ocimum suave* (OS) and *O. kilimandscharicum* (OK) extracts against mosquitoes in the field and laboratory. In the laboratory, the comparison was done with standard natural product (Citronella) while in the field they were compared to standard synthetic repellent 20% (DEET). In the field, human landing catches were done and results showed that *O. suave* had highest repellency (89.3%) against *Anopheles arabiensis* and 83.9% against *Culex quinquefasciatus*. *O. kilimandscharicum* had 86.3% efficacy against *An. arabiensis* and 86.6% against *Cx. quinquefasciatus.* In the feeding inhibition tests, OS had a range between 83.5%-88.9% and OK repelled between 71.2% and 85.3% in the two mosquitoes species. The results are discussed with reference to the other related studies conducted on *Ocimum* species essential oils. It is recommended that these products to be produced on commercial scale and adopted as complementary control method against malaria vectors.

(ACMCIP Abstract)

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CLIMATE CHANGE AND VECTOR BORNE DISEASE IN THE UNITED STATES: QUO VADIS

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Ongoing climate warming has the potential to impact spatial patterns of vectors, vector-borne pathogens, or incidence of vector-borne disease. Although well-designed empirical studies determining the effect of climate variables on vector life history quickly was identified as a priority area, the fledgling research field focusing on the effect of climate change on vectors and vector-borne pathogens instead has been characterized by the development of a plethora of models predicting future change based

on empirical data of highly variable quality. We also note a surprising lack of studies in the United States either: 1) empirically determining correlations between climate variables and vector presence or abundance or pathogen presence at the warm or cool ends of their vector ranges; 2) experimentally manipulating temperature to examine how projected realistic future temperature increases will affect life history traits of vectors; 3) using epidemiological data to study climate-associated change over time in spatial patterns of incidence of vector-borne disease; or 4) initiating long-term empirical field studies capable of demonstrating that future climate change resulted in changes in spatial patterns of vector distribution/abundance or pathogen presence/prevalence. We have started to address some of these issues in field studies targeting the Colorado Front Range and will present baseline data for spatial patterns of key vectors (*Culex tarsalis*, mosquito vector of West Nile virus; *Demacentor andersoni*, tick vector of *Francisella tularensis* and *Rickettsia rickettsii*) along altitude gradients including climatic conditions ranging from suitable to prohibitive for vector establishment. Efforts also are underway to use epidemiological data from regions of the United States sensitive to climate warming to develop knowledge of fine-scale current baseline incidences for use in retrospective and prospective long-term studies for vector-borne diseases such as plague and West Nile virus disease.

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TICK BITE PREVENTION BEHAVIOR AMONG PARTICIPANTS IN THE GEORGIA TICK ATTACH STUDY

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Tick bite prevention measures are recommended by the Georgia Division of Public Health to prevent tickborne illness. To better understand what prevention measures Georgians are taking, we examined the prevention behaviors of participants in the Georgia Tick Attach Study. The study was conducted in partnership with the University of Georgia and the Georgia Poison Center between April 2005 and December 2006. Georgia residents and non-residents who were bitten by a tick were asked to submit their tick for identification and testing for tickborne pathogens. Following receipt of the tick, a telephone questionnaire was administered to assess risk factors and determine if the participant became sick after his/her tick bite. During the study period, 442 people submitted 575 ticks. In May 2006, questions were added regarding what participants were doing at the time of their tick bite and what prevention measures were taken, if any. Of 72 participants surveyed, 32 (44.4%) took at least one precaution intentionally to prevent tick bites. Twelve (16.7%) took primary prevention measures to prevent tick bites, such as wearing long pants or light colored clothing. Twenty-eight (38.9%) took secondary prevention measures such as performing tick checks or taking a shower to remove ticks before they were attached long enough to transmit pathogens. Only 3 participants wore repellent to prevent tick bites. Participants were not more or less likely to practice precautions if they were away from home, living in a rural area, or if they spent more than the median amount of time outside. In this study, people with tick bites were more likely to look for attached ticks soon after an outdoor activity than to take primary precautions to prevent ticks from attaching.

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COMPARISON OF IRRITANT EFFECTS OF DDT AND α-CYPERMETHRIN AGAINST RESISTANT AND SUSCEPTIBLE STRAINS OF *AEDES AEGYPTI* (DIPTERA: CULICIDAE)

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Insecticidal activity is commonly measured as the level of toxicity produced against insects. However, other properties such as irritant and repellent effects are of major interest in vector control because these behaviors are also involved in the disruption of the host-vector contact. Experimental huts used in conjunction with a recently developed High-Throughput Screening System (HITSS) allowed us to evaluate the toxicant, contact irritant and spatial repellent effects of α-cypermethrin and DDT against *Aedes aegypti* populations. Initial laboratory and field tests were performed against a DDT resistant population obtained from Pu Teuy, Kanchanaburi province, Thailand. Data from the experimental hut studies showed that α-cypermethrin exhibited a strong contact irritant effect resulting in a 50% increase of exiting mosquitoes 2 hours earlier compared to the control hut. DDT produced a weaker irritant effect than α-cypermethrin (resulting in a 30% increase in exiting 1 hour prematurely compared to the control). In order to determine if the resistance status of the test population altered the behavioral response of the insect, these insecticides were tested in the lab against a colonized resistant population and also against a susceptible strain colonized from Houston, TX. These effects were confirmed in the lab with both populations and demonstrated that the resistant population is as irritated by α-cypermethrin and DDT as the susceptible one. This is the first study to demonstrate that the resistance status of a vector population does not significantly impact the behavioral response of the insects to irritant and repellent chemicals. In conclusion, we confirmed the behavioral effects of these insecticides, showed congruence between field and lab results and most importantly we showed that DDT still elicits behavioral action on a DDT resistant population.

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EVALUATION OF THE FAT-TAILED JIRD, *PACHYUROMYS DUPRASI NATRONENSIS* (RODENTIA: GERBILLIDAE), AS A NEW ANIMAL MODEL FOR STUDIES OF *LEISHMANIA TROPICA* INFECTION AND TRANSMISSION

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The fat-tailed jird, *Pachyuromys duprasi natronensis* De Winton, 1903, is a burrowing gerbilline rodent found in the Western Desert of Egypt and across North Africa. While there is no record of this rodent serving as a natural reservoir of *Leishmania*, we conducted experiments to determine its potential for use in the laboratory as an animal model for Old World leishmaniasis. Jirds captured in the Marsa Matrouh governorate, Egypt, adapted readily to captivity and a diet of laboratory animal (rabbit) feed, supplemented with fresh vegetables. Two colony-born jirds were inoculated subcutaneously in the hind foot with cultured metacyclic promastigotes of *Leishmania tropica* EP119, a new human isolate from Aydin, Turkey. Visible lesions developed within one month at the inoculation sites. Tissue from these active lesions was homogenized in PBS and inoculated into the hind footpads of another ten colony-born jirds. We thereafter monitored the persistence, dissemination, and visceralization of the parasites in these animals. Skin lesions, spleen, liver,

and bone marrow of jirds sacrificed at 2, 3, and 5 months post-inoculation (PI) were screened by culture and Polymerase Chain Reaction (PCR). By one month PI, all jirds had developed footpad lesions. Parasites were detected in bone marrow two months PI and from spleen and liver three months PI. Only 62.5%, 50.0%, and 37.5 % of the tested animals were parasite-positive for bone marrow, spleen, and liver, respectively. We then tested the ability of the sand fly *Phlebotomus sergenti* to transmit *L. tropica* to jirds. Sand flies were infected by membrane feeding of a suspension of homogenized skin-lesion *L. tropica* amastigotes and after 8-13 days allowed to re-feed on uninfected jirds. Although the jirds did not develop lesions after being bitten by infected sand flies, their hind and fore feet, livers and bone marrow were PCR positive for *L. tropica* DNA three months post feeding. Transmission of *L. tropica* from infected jirds to uninfected laboratory-reared sand flies was also demonstrated. These collective results show that the fat-tailed jird can be used as an animal model for *L. tropica* studies and could serve as a natural rodent reservoir for *Leishmania* infection.

(ACMCIP Abstract)

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MOLECULAR CLONING AND CHARACTERIZATION OF A NOVEL SPHINGOMYELINASE-LIKE PROTEIN FROM THE TICK *IXODES SCAPULARIS*

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Tick saliva contains an array of pharmacological compounds that include immunomodulators, inhibitors of pain/itch responses, anticoagulants, inhibitors of platelet aggregation, and vasodilators and modulation of wound repair, all of which contribute to the evasion of host immune and haemostatic defenses. In this study, a novel sphingomyelinase-like protein encoded by a cDNA clone from an *Ixodes scapularis* cDNA expression library derived from 18-24 hour fed salivary glands was cloned and characterized. The full-length sequence of this tick protein (1,277 bp) showed a predicted open reading frame (ORF) of 427 amino acids (48.5 kDa) with an isoelectric point (pI) of 8.74 and a predicted signal peptide cleavage site indicating a secreted protein. The predicted amino acid sequence from this gene revealed a significant degree of similarity to the sphingomyelinase P1 precursor (SMase P1) found in the venom of the spider *Loxosceles intermedia*, including a conserved glycerophosphoryl diester phosphodiesterase motif which characterizes these families. The tick protein was expressed in an insect-cell expression system and the purified protein was shown to hydrolyze sphingomyelin to ceramide and phosphorylcholine. In order to determine if the *I. scapularis* sphingomyelinase-like protein can cause Th2 polarization of helper T-lymphocytes, the protein was assayed using a clonotypic CD4 T-cell adoptive transfer model system. CFSE-labeled T-cell receptor clonotypic CD4 T-lymphocytes with a Th1 bias upon exposure to cognate antigen, Influenza Hemagglutinin (HA), were adoptively transferred into BALB/c mice injected intradermally with tick recombinant protein followed by injection of 200 ug of HA peptide after 1 hr on day 0. Four days later, cells were immunophenotyped, their division assessed by CFSE dilution, and intracellular cytokine staining performed. Administration of the tick protein caused a significant increase in the percentage of clonotypic CD4+T-cells producing the Th2 cytokine, IL-4. These results suggest that the *I. scapularis* sphingomyelinase-like protein is capable of promoting polarization of the immune response towards a Th2 profile.

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ANALYSIS OF *IN SILICO* STEREOELECTRONIC PROPERTIES OF PMD (P-MENTHANE-3-8-DIOLS) AND ITS DERIVATIVES TO DEVELOP A PHARMACOPHORE FOR INSECT REPELLENT ACTIVITY

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PMD (p-menthane-3-8-diol) is an insect repellent that can be either synthesized chemically or derived directly from the steam distillate residue of the leaves of lemon eucalyptus, *Corymbia citriodora citriodora*. It is one of the few natural products endorsed by the CDC for topical application to protect against mosquitoes. However, no analytical or quantitative structure activity studies or toxicological evaluations of PMD have been reported in the open literature. In our ongoing efforts to understand the mode of action of various insect repellents, we report here the results of detailed quantum chemical based analysis of stereoelectronic properties of PMD and eight synthetic derivatives. Our studies with calculated and experimental observations indicate that lower aqueous stabilization (favorable lipophilicity) and larger separation of electrostatic potential energy together with a large localized negative electrostatic potential region by the oxygen atom play a definite role in the repellent activity of these compounds. These stereoelectronic profiles may aid in designing more effective compounds with insect repellent activity.

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DIFFERENTIAL EXPRESSION OF SALIVARY GLAND CDNAS IN LABORATORY AND FIELD POPULATIONS OF *PHLEBOTOMUS PAPATASI*

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During blood feeding, hematophagous insects, including sand flies, inject saliva into the skin of the host. Vector saliva contains molecules that interfere with the host's coagulation cascade, induce vasodilation and cause inflammation. Sand fly saliva exacerbates *Leishmania* infection in animal models; however, repeated exposure of animals to either bites or salivary gland homogenates of *Phlebotomus papatasi* protects against *L. major*. PpSP15 has been shown to be the molecule responsible for protecting mice against disease progression and lesion size. However, preliminary data also suggest that different *P. papatasi* salivary proteins produce distinct protection responses in different animals. Furthermore, for SP15 seven variants have been previously characterized but our data suggest that this number may be greater with as many as 14 variants. Currently, 10 *P. papatasi* cDNAs have been identified but no information pertaining to their relative abundance amongst different individuals within a same population of between individuals of distinct populations is known. Here we analyzed the relative expression of SP15 from field and laboratory populations of *P. papatasi*. Relative expression was calculated

using ΔΔCt and the *P. papatasi* Israeli strain (PPIS) as the calibrator population, as this colony has undergone several bottlenecks since being established and is the strain currently used in the sequencing project. Our results suggest a certain level of variation in the abundance of SP15 is detected between different populations. Furthermore, a difference between individuals within sand flies from a colonized Turkey strain (PPTK) and from a field population from southern Jordan (PPJS) also was detected. Currently we are investigating a possible correlation between levels of cDNA expression and different haplotypes.

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OVERVIEW OF DEPLOYED WARFIGHTER PROTECTION PROGRAM ACTIVITIES AT THE USDAS CENTER FOR MEDICAL, AGRICULTURAL AND VETERINARY ENTOMOLOGY

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In 2004, the US Department of Defense (DOD) established a new research initiative to rapidly identify and develop novel methods to protect US military personnel from insects that transmit pathogens that cause many important human diseases. The focus of this program, known as the Deployed War-Fighter Protection (DWFP) Research Program, is to find new tools for combating pest and vector species that impact deployed war-fighters. The program places emphasis on discovery, evaluation, development, and enhancement of: (1) new pesticides that are effective against mosquitoes and flies; (2) new personal protection products that prevent mosquito and fly bites; and (3) new application and personal protection methodologies and strategies. Because of the historical and productive relationship between the DOD and the US Department of Agriculture (USDA) in solving vector-related problems among military personnel, the DOD is providing $3 million annually to the USDA. These funds are being utilized in the Agricultural Research Service, the USDA's in-house research program, to conduct research in support of this new initiative. This presentation provides an overview of the DWFP research conducted at the Center for Medical, Agricultural and Veterinary Entomology (CMAVE) in Gainesville, Florida and includes results of progress made to date.

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THE EFFECT OF WEST NILE VIRUS PERCEPTIONS AND KNOWLEDGE ON HUMAN PREVENTION PRACTICES AND VECTOR BREEDING IN RESIDENTIAL YARDS IN UPSTATE NEW YORK

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A knowledge, attitudes, and practices (KAP) questionnaire and an entomological survey on larval breeding places of West Nile Virus (WNV) vectors in residential yards were conducted in two neighborhoods in upstate New York, to test the hypothesis that residents' WNV knowledge and perceptions would relate to practices that prevent mosquitoes from biting and breeding. Results from multivariate modeling showed that perceptions of WNV were related to the number of positive containers in a respondent's yard and their use of mosquito preventive measures, whereas knowledge about WNV was not. The most abundant vector found in positive containers (buckets, flower pots and bird baths) was the invasive species Ochlerotatus japonicus (77%). Because this species is a potential vector of WNV as well as many other arboviruses (Eastern Equine Encephalitis, La Crosse, and Saint Louis Encephalitis), more research should focus on possibilities to control its breeding in residential yards. To our knowledge, this is the first study that directly investigated the relationship between knowledge, perceptions, and breeding of WNV vectors in residential yards.

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MICROGEOGRAPHICAL ANALYSIS OF GENETIC STRUCTURE IN *TRIATOMA INFESTANS* POPULATIONS FROM NORTHERN ARGENTINA

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The control of *Triatoma infestans*, the main vector of Chagas disease in South America, has had limited success in the Gran Chaco region due to high recolonization rates. A better understanding of the genetic structure, dispersal dynamics and phylogeographical relationships among *T. infestans* populations is needed in order to determine the source of reinfesting bugs and help design improved vector control strategies. We conducted a micro-geographical population structure study, analyzing the multilocus genotype of six microsatellites, from 337 *T. infestans* collected in 21 houses of 11 rural villages in Santiago del Estero province, northern Argentina. Genic and genotype diversity were assessed for populations at each capture site and pair-wise comparisons between sites were performed. Significant differences were detected among population pairs, among and within villages, and sub-structure was detected even within a capture site. The genetic differentiation among sample pairs was not consistent with a model of isolation by distance. The absence of significantly differentiated population pairs within a village, confirmed the occurrence of gene-flow by active migration, whereas events of local isolation were also detected. Reinfestation from independent sources might have occurred simultaneously in a village, and migration from different sources can explain the admixed origins populations established at a single site. Genetic structure of *T. infestans* populations varied among villages, which confirms differences in population history and dispersion dynamics reported previously for these neighboring villages. Evidence of gene-flow occurrence among villages reinforces the need of establishing vector control efforts beyond the village level.

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FAST-GAS: A FIELD-DEPLOYABLE SOURCE OF CARBON DIOXIDE FOR USE IN VECTOR SURVEILLANCE

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The (CDC) miniature light trap is a standard tool for conducting biting arthropod surveillance when baited with carbon dioxide (CO_2). Currently, CO_2 is only available as dry ice, a compressed gas, or as the byproduct of propane combustion. Significant issues (cost, shipping hazards, weight, etc.) preclude the use of these forms of CO_2 during military and many public health operations. A field-deployable source of CO_2 is urgently needed. This research team has been working on development of a CO_2 generator since 1999. We have developed field-deployable sources of CO_2 and are working on adaption of these prototypes to deliver CO_2 to a CDC miniature light trap. The APTIV systems provide CO_2 output in the range of 200 - 400 mls/minute for 12-16 hours using 1.5-3 kg of starting material. The reaction is somewhat temperature dependent, but does not produce pressures above 5 psi. Starting materials are generally recognized as safe (GRAS) and suitable for storage for long intervals at ambient conditions. These materials are not considered a hazardous material for shipping purposes. APTIV's delivery device is less than 1 cubic foot in size and weighs less than 3 kg. Further product development is planned to ruggedize this system for extended use under harsh environmental

conditions (hot, dusty desert environments, tropical jungles, etc.). The device is very user friendly, easy to operate, and can be be set-up in less than 5 minutes.

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EVALUATION OF EARLY INFLAMMATORY RESPONSE EXPRESSION IN RESPONSE TO *PHLEBOTOMUS DUBOSCQI* BITES

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The saliva of bloodfeeding arthropods contains a wide variety of molecules that modulate their host's hemostatic, inflammatory and immune responses. The immune response against saliva was shown to protect mice against Leihsmania infection. To further explore the saliva-induced immune and inflammatory response in the skin that occur after *Phlebotomus duboscqi* bites, we used a macroarray approach to compare the expression profile of cytokines, chemokines and receptors involved. Two groups of C57BL/6 mice were used. One group was pre-exposed to *P. duboscqi* bites and the second were non-exposed. After challenge with bites of non-infected *P. duboscqi*, we followed the kinetics of the skin immune response in these animals. RNA was extracted from the skin, processed to labeled cRNA and then hybridized to a macroarray containing genes from a great variety of cytokines, chemokines and receptors that could be involved in inflammatory and immune responses. The results show that two hours after challenge with *P.duboscqi* bites a greater expression of CCL1, CCL19, CXCL14 and IL-15 was observed in the naïve mice when compared to the pre-exposed group. These differences were also observed in other time points. These results suggest that the saliva from *P.duboscqi* is able to modulate the expression of a great variety of cytokines and chemokines and the early immune response in the skin. Understanding the molecules important in this process is essential to elucidate the mechanisms involved in saliva-induced protection.

(ACMCIP Abstract)

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SERO-PREVALENCE OF CYSTICERCOSIS IN CHILDREN, ADOLESCENTS AND ADULTS LIVING IN A SCHISTOSOMIASIS ENDEMIC COMMUNITY IN LEYTE, THE PHILIPPINES

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This study was undertaken to estimate the sero-prevalence of cysticercosis in a *Schistosoma japonicum* and geohelminth infections endemic area of the Philippines. Cysticercosis remains an important cause of neurological disease worldwide. Previous studies have shown that cerebral cysticercosis is a risk factor for stroke in young and middle-aged people. However, accurate prevalence data of cysticercosis for the Philippines are unavailable. There were only case reports once in a while in the Philippine literature with two latest cases reported in 2002 and 2004 correspondingly. As part of a longitudinal treatment-reinfection study of schistosomiasis, we enrolled N=617 *S. japonicum* infected individuals aged 7 to 30 and N=103 individuals aged 7 to 18 who were not infected with *S. japonicum.* All subjects were residents of 4 contiguous rice-farming villages in Leyte, The Philippines. We evaluated N=509 subjects (*S. j* prevalence in sub-sample was 85.7%) for the presence of antibodies to *Taenia solium* cyst fluid using a commercial ELISA assay. The reported sensitivity and specificity of this assay kit are greater than 96% with no reported false positives occurring in samples from patients with trichinosis, facioliasis and schistosomiasis. The overall sero-prevalence of cysticercosis in this study sample was 24.6% with no significant difference based on *S. j* infection status (P=0.699). Sero-prevalence did not differ between females and males (27.2% (52/191) vs. 23.0% (73/318), respectively, (P=0.279) or by age groups. We detected no aggregation of sero-prevalence within household (P=0.144). In conclusion, the sero-prevalence of cysticercosis in this community appears considerably higher than expected and surprisingly is unrelated to host age. These results suggest that cysticercosis may be a significant, but under-recognized public health concern and warrants re-examination of this cohort with an FDA-licensed diagnostic kit for cysticercosis.

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CHILDREN SEROLOGY OF ECHINOCOCCOSIS INFECTION AS AN ENVIRONMENTAL HEALTH INDICATOR TO GUIDE PREVENTIVE ACTIVITIES IN NINGXIA, PR CHINA

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This study was undertaken to develop an environmental health indicator for use as a basis for developing preventive measures against cystic (CE) and alveolar (AE) echinococcosis infection in children from rural communities in Xiji County, Ningxia Hui Autonomous Region, P.R. of China. A cross-sectional survey was conducted in 2002 among 861 children under 18 years old. After ultrasound (US) abdominal examination, a questionnaire to identify risk factors for infection (socioeconomic, sanitation and hygiene variables) and collected filter-paper blood samples from each child for specific antibody detections using EmP and EgB antigens. The overall prevalence of echinococcosis by US was 0.1% for CE; no detectable AE was found. The results showed the dog/fox faeces environmental contamination and age-bias behaviour were significant risk factors for both human AE and CE. There was a statistically significant association between both infections and child domicile indicating that infection risk also included geographic independent factors, with the eco-geographic environment being a risk factor for AE and the socio-geographic environment increasing risk for CE. In conclusion, the environmental health indicator, which incorporated the most significant biological, environmental and social factors associated with the risk of echinococcosis infection, can provide clear "warning signals" to decision-makers for the institution of specific control measures in this Chinese population.

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COMPARISON OF RECOMBINANT AGB ELISA WITH COMMERCIALLY AVAILABLE ELISA IGG IN THE DIAGNOSIS OF CYSTIC ECHINOCOCCOSIS

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An experimental ELISA test for detection of anti-*Echinococcus granulosus* antibodies was set up using a new recombinant antigen (rAgB) from *E.granulosus.* The aim of this study was to assess the sensitivity of this new ELISA test compared to a commercially available ELISA test routinely used in our diagnostic laboratory. 148 patients (70 females and 78 males) were included in the trial. The WHO IWGE standardized ultrasound classification was used to assess cyst stages. Nine patients had active cysts (CE1 and CE2), 14 had transitional cysts type CE3a, 36 had transitional cysts type CE3b, 49 had inactive cysts (CE4 and CE5), 20 patients had undergone surgical treatment for echinococcosis, and 20 had non-parasitic cysts (negative controls). Sera from all patients were assessed for positivity to anti-*E granulosus* antibodies with a commercial ELISA kit routinely used in our diagnostic laboratory (Echinococcus Ab-Cypress Diagnostic-Langdorp-Belgium) and with an experimental ELISA test using recombinant *E.granulosus* antigen B8/1 (rAgB8/1). Assessment of between-test concordance was carried out by Kappa test of concordance on total sera from CE1, CE2, CE3a, CE4, CE5 and negative controls, and separately in groups: active cysts (CE1, CE2 and CE3a), inactive cysts (CE4 and CE5), and negative controls. Sera from patients having type CE3b cysts were not included in the statistical analysis because of the very variable serological results in this type of transitional cysts. Sera from follow-up patients after surgery were not included in the statistical analysis because of the difference in surgical techniques used. All sera from negative controls were assessed as negative by both ELISA tests, showing a 100% concordance. The two ELISA tests showed a concordance of 49.4% among sera from patients having active cysts (CE1,CE2 and CE3a); among those having inactive cysts (CE4 and CE5) concordance was 54.9%. The overall concordance between tests was 67.7%.The two ELISA tests for detection of anti-*E.granulosus* antibodies showed a good level of concordance. In conclusion, our findings indicate that no real advantage can be gained from the use of rAgB8/1 over commercially available ELISA kits. Details of serology values in transitional stages and in post-surgical patients will be discussed.

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PAIR V CONSERVATIVE SURGERY FOR UNCOMPLICATED ECHINOCOCCAL CYSTS: EVALUATION OF COSTS IN ITALY

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There is no consensus about the best treatment (surgery, chemotherapy and percutaneous treatments) for hepatic cystic echinococcosis (CE). Retrospective studies have addressed the pros and cons of each option, but costs of treatments have never been studied extensively. The aim of this paper is to compare the cost of PAIR and conservative surgery for non-complicated echinococcal cysts of the liver in Italy. Costs of hospital stay, operating theatre, disposable items, salaries for health personnel and sonograms, were obtained from the S.Matteo Hospital Administration and pharmacy. The institution is a large tertiary care teaching hospital in Lombardy, a region in Northern Italy. Costs of 1-year follow-up were included. The costs were calculated for a three-day hospital stay for PAIR procedure and 4-day hospital stay for surgery, both without complications. The 2006 mean specific cost of a PAIR treatment for a 3-day hospital stay and 1 year follow-up was 2,072 EUR (average exchange rate: 1 EUR = 1.26 USD). Main cost entries were the following: a) hospital stay (net cost): € 1,500 (72.4% of the total cost); b) disposable items (needles, catheters) € 239 (11.5%); c) personnel: € 156 (7.5%); d) drugs (30-days albendazole administration as prophylaxis of secondary echinococcosis): € 72 (3.5%); e) ultrasound scans (paid by patient): € 105 (5.1%). In the same year and same hospital, the specific cost of a surgical conservative intervention and 1-year follow-up was 3,267 EUR. Main cost entries were the following: a) hospital stay (net cost): € 2,000 (61,2% of the total cost); b) operating theatre (2 hours): € 140 (4,3%); c) disposable items: e.g. :Fibrin Glue € 500 (15,3%); d) personnel: € 300 (9,2%); e) pre-operative tests (Labs, ECG, Chest X-ray, anesthesiological evaluation): € 150 (4,6%); f) drugs (30-days albendazole administration as prophylaxis of secondary echinococcosis): € 72 (2,2%); g) ultrasound scans (paid by patient): € 105 (3,2%). In conclusion, PAIR is significantly less expensive than conservative surgery for uncomplicated echinococcal cysts of the liver.

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PRELIMINARY RESULTS FROM A SURVEY ON KNOWLEDGE, ATTITUDES AND PRACTICES REGARDING CLINICAL MANAGEMENT OF CYSTIC ECHINOCOCCOSIS IN EUROPEAN, NORTH AFRICAN AND MIDDLE EASTERN COUNTRIES

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The clinical management of cystic echinococcosis (CE) is notoriously difficult: competences are scattered across several specialties, three different treatments are available, but they have never been properly compared, and evidence of their efficacy, especially long-term, is lacking. Further, there are setting-related problems: in most developed countries, CE is an imported disease of low incidence and prevalence and is found almost exclusively in migrants from endemic regions. In the latter, many patients do not have access to treatment because of poor infrastructure. Thus, studies carried out in either of the two different environments lack external validity, i.e., results obtained in one setting may be different from those in the other and practices that can work in one may not be applicable to the other. Clinical practices regarding CE vary widely, but no systematic study of their distribution by clinicians' experience, type of training or institution, and country of residence has been carried out so far. This study was undertaken to investigate the knowledge, attitude and practices of clinicians dealing with CE in primary, secondary and tertiary care hospitals in different countries across Europe, North Africa and Middle East. All 9 countries are partners in a 3-year project funded by the European Union on coordinated approach to control CE (FP 6 - INCO 509102.) A two-page questionnaire was prepared with questions on the recipients' training (surgery, medicine, radiology, other), on experience, if any, with clinical management of CE, on interdisciplinary management, if any, in their hospital. Questions regarding how the recipients would classify (WHO and Gharbi's classification) cysts types shown in photographs and which treatment modalities they would choose for each of them, were included. Questionnaires were sent to clinicians working in hospitals that were randomly sampled from regions in the partner countries. Data collected from the preliminary analysis of 900 returned questionnaires from the partner countries will be presented. These findings confirm a wide variety of practices within the same region and prove the need for a more coordinated clinical approach if the scarce resources available for this condition are to be employed more rationally.

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IN VITRO AND *IN VIVO* ACTIVITY OF THE ANTI-CANCER AGENT 2-METHOXYESTRADIOL (2ME2), EITHER ALONE OR IN COMBINATION WITH ALBENDAZOLE, AGAINST *ECHINOCOCCUS MULTILOCULARIS* METACESTODES

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The metacestode (larval) stage of the tapeworm *Echinococcus multilocularis* causes alveolar echinococcosis (AE), a mainly hepatic disease characterized by continuous asexual proliferation of metacestodes by exogenuous budding, resulting in the tumor-like, infiltrative growth of the parasite lesion. Current chemotherapeutical treatment of AE relies on the use of benzimidazoles (albendazole, mebendazole), but these drugs act parasitostatic rather than parasitocidal, and in case of side effects such as liver toxicity, patients are left without valuable alternatives. 2-ME2 is a natural metabolite of estradiol, with a documented anti-angiogenic and anti-tumour activity, most notably against breast and prostate cancer. Treatments of *in vitro* cultured *E. multilocularis* metacestodes with 2-

ME2 (2-10μM) showed that the drug has an adverse effect on parasite viability. First, addition of 2-ME2 (5 and 10 μM) resulted in increased alkaline phosphatase activity in medium supernatants within 10 days of treatment. Second, scanning and transmission electron microscopy showed that the germinal layer of *E. multilocularis* metacestodes was dramatically damaged following 2-ME2 treatment, and the effect was dose dependent. Third, 2-ME2 *in vitro* treatment downscaled the 14-3-3-pro-tumorogenic zeta-isoform expression in *E. multilocularis* metacestodes. Bioassays were performed in mice injected with 2-ME2-treated and albendazole-treated metacestodes, or parasites treated with both 2-ME and albendazole. These assays indicated that, *in vitro*, a parasiticidal effect is achieved by treatment with a combination of both compounds, while both albendazole and 2-ME alone do not kill the parasite completely. Currently, *in vivo* treatment experiments in *E. multilocularis*-infected mice are being performed. Mice are applied either 2-ME, albendazole, or a 2-ME/albendazole combination by daily intra-gastric inoculation for a period of 6 weeks. These investigations will show, whether either 2-ME, or a 2-ME/albendazole based treatment, is also effective *in vivo*.

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PLASMODIUM VIVAX ASSOCIATED ACUTE RESPIRATORY DISTRESS SYNDROME AFTER EXTENDED TRAVEL IN AFGHANISTAN

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A 21-year-old soldier developed anorexia, vomiting, diarrhea and fever 10 days after returning to the United States from an 8 month deployment in Afghanistan. His symptoms persisted over the next 5 days until he presented in respiratory failure with a PaO_2:FiO_2 ratio of 63, requiring urgent intubation and ventilator support. Chest X-ray revealed diffuse bilateral alveolar opacities consistent with Acute Respiratory Distress Syndrome (ARDS). Although sputum and blood cultures did not reveal a causative agent, Giemsa stained blood smears were positive for *Plasmodium vivax* alone, which was later confirmed by small subunit ribosomal RNA (ssrRNA) polymerase chain reaction amplification. After a tenuous course marked by splenic rupture and prolonged requirement for ventilator support, the patient ultimately recovered. Although generally considered benign, this and other recent reports of vivax malaria associated lung injury emphasize the need for persistent pursuit of the diagnosis in febrile travelers returning from vivax endemic locations as well as aggressive monitoring for and management of life threatening complications.

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DON'T PICK THE WILD MUSHROOMS! A RARE CASE OF LIVER FAILURE DUE TO MUSHROOM POISONING IN NEW YORK STATE

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Mycetismus is rare in the US. 100 of 5000 species of mushrooms are toxic. Less than 10% are potentially lethal. Best known is the deadly "deathcap" belonging to the genus Amanita. Ingestion can be fatal. North American Mycological Association reported 148 poisonings in humans, including 3 deaths and 51 poisonings in dogs during 2004. The majority of these occurred in the western US. Cases in the Northeast are uncommon. In New York, 5 cases were reported in 1999. We report a case of a man who developed liver toxicity after eating mushrooms at Bear Mountain State Park in Upstate New York. A 59 yo Korean man presented to Flushing Hospital Medical Center in New York with abdominal pain, nausea, vomiting, diarrhea and atrial fibrillation. His wife developed similar GI symptoms a day later and was admitted to another facility. Physical examination revealed anicteric sclera, irregular cardiac rhythm and a tender abdomen with hyperactive bowel sounds. He was treated for atrial fibrillation while gastroenterology and critical care consultations were obtained. He and his wife ate wild mushrooms one day earlier at Bear Mountain State Park. Over 24 hours liver enzymes and coagulation indices worsened. He was treated with hydration, activated charcoal, N-acetylcysteine, and penicillin G. The transplant center (NYU) was contacted and transfer arranged. After a course of plasmapheresis, liver function improved and was discharged in good condition several days later. Mushroom toxicities are usually caused by *Amanita phalloides*. The amatoxins lead to hepatocellular injury by interrupting protein synthesis. Cooking or drying cannot deactivate the toxins. A significant percentage of cases occur among immigrants who mistake a toxic variety of mushroom with a non-toxic type present in their native country. Liver toxicity progresses in 4 stages: 1) Quiescent 6-12 hours. 2) Severe abdominal pain, vomiting, diarrhea 12-24 hours 3) Improvement in GI symptoms with development of hepatic and renal toxicity 24-36 hours 4) jaundice, fulminant hepatic failure, coagulopathy, encephalopathy, GI hemorrhage. Early recognition is essential. IV hydration, gastric lavage, and activated charcoal help eliminate the toxins. High dose penicillin, cimetidine and N-acetylcysteine are commonly used but their benefit is unproven. Hemodialysis, hemoperfusion and plasmapheresis are used to eliminate toxin from the body. Consideration for liver transplant is essential.

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LEPROSY IN AGUA DE DIOS LEPROSARIUM - COLOMBIA, 2006: PATIENT CHARACTERISTICS AND APPLICATION OF MOLECULAR METHODS FOR DRUG RESISTANCE SURVEILLANCE AND STRAIN TYPING

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During 2006 a study for detection of leprosy patients and contacts was carried out at Agua de Dios Hospital, Colombia. Volunteers were tested for acid fast stain in slit skin smears (SSS) and skin biopsy. The contacts of confirmed patients were included. DNA from SSS and biopsies samples positive for leprosy, was obtained with the aim to carry out molecular strain typing at 15 short tandem repeat loci using PCR, and drug resistance by detection of characteristic mutations in target *rpoB* and *folP1* genes. DNA of SSS samples from contacts was analyzed by PCR of four loci with the initial aim to detect *Mycobacterium leprae* DNA. One hundred and eight (108) volunteers were enrolled, 73 suspected patients and 35 contacts. At present 36 volunteers had been diagnosed as leprosy patients. Twelve (34%) of them had received leprosy treatment before, and they visited the hospital due to relapse of clinical symptoms. BI was positive (>0) in 22/36 patients (61%). Clinical inspection, bacteriological, and hystopathological studies for 37 enrolled volunteers were not conclusive of leprosy. Clinical and histopathology study from one patient, shows signs, symptoms of neurological compromise of tissues, compatible with pure neural leprosy (PNL). *Mycobacterium leprae* typing from samples of 35 leprosy patients showed that some specific markers are not variable, while others vary in every one of the strains tested; no particular relationships among strains tested can be concluded. Samples from 2 patients that consulted for leprosy relapse, show mutations by substitution at *rpoB*, one of them also shows a mutation in *folp1* gene, constitute the first report of rifampicin and dapsone resistance in leprosy patients from Colombia. The substitution mutation Asp-516-Tyr found in *rpoB* in one relapse patient has not reported previously, so, the strain's resistance level to rifampicin is not determined yet. Multi-resistance found in 1 patient, and the identification of novel *rpoB* mutations is an alert that urges for the continued searching of this phenomenon in Colombian leprosy patients that consult for relapses.

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MYIASIS EVEN IN A DESERT ENVIRONMENT? SARCOPHAGIDAE AND OTHER LARVAL INFECTIONS IN KUWAIT

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Myiasis is defined as the infestation of live vertebrates by Dipteran larvae which feed on the host's dead or living tissue, liquid body substances, or ingested food. Such infestations of humans are primarily observed in tropical climates where there is the greatest diversity of species causing myiasis, especially obligate species. We now report autochthonous myiasis in the desert environment of Kuwait. We first describe myiasis complicating a decubitus ulcer in an 85-year-old Kuwaiti female. On admission, she was semi-comatose, malnourished, debilitated and in a poor physical condition. There were numerous bed sores on her back and three ulcers had to be debrided. One on the right trochanter was foul-smelling with a hemorrhagic discharge. The cavity was 10 cm deep and 7 cm wide. Three fly larvae crawled out of this ulcer, were collected, placed in saline and preserved in 70% ethanol. The larvae were up to 10 mm long and 4 mm wide. The posterior spiracles were deeply sunken in an invagination hidden by overlapping surrounding tissue, had an open peritreme and slits that were almost vertical. These are characteristic of the family Sarcophagidae. As no larvae were reared to adulthood, a species identification was not possible. However, *Bercaea africa* (= *Sarcophaga cruentata*) is a potential candidate being the most common synanthropic sarcophagid species that has been recorded in Kuwait and identified in cases of human myiasis elsewhere. We also review previously reported cases to assess the clinical importance of myiasis in this desert climate. Ophthalmomyiasis due to the larvae of *Oestrus ovis;* intestinal myiasis due to *Megaselia* sp. and *O. ovis* and urinary myiasis due to *Psychoda* sp. have all been reported as community-acquired. The larvae of *Lucilia sericata* and *M. scalaris* were a cause of nosocomial infections. Thus even in a desert environment, where the insect fauna may be considered to be limited, diligent investigations have shown myiasis to be clinically important. We emphasize the need to document such infections to enhance patient-care and contribute to the pool of knowledge on the subject.

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BASELINE STUDY ON MALARIA DISEASE WITH ETHNIC MINORITY GROUP IN RATTANAKIRI PROVINCE

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Malaria poses a great problem in Cambodia because it exacts a heavy toll in terms of illness and deaths among the country's poor. The appropriate and effectiveness on malaria control relies mainly on insecticide treated net (ITNs). The aim of our baseline study are finding, the key factors that influence bed nets/ ITNs use, and the information on seeking an advice and treatment for malaria among the Kreung minority group, who live in remote malaria high risk area in Ouchum district, Rattanakiri Province along the Vietnam border. The study was focus on their socio-economic status in relation with malaria transmission and prevention, the level of knowledge about malaria etiology, signs and symptoms, cause of malaria and malaria prevention, the use of bed nets and ITNs, and the access to other health facilities. The exploratory descriptive research design will use in our experiment. The key factors for our experiment are conducted through the Focus Group Discussion (FGD) and Household Questionnaires. The baseline finding that, knowledge in preventing malaria of household respondents appeared almost in correct tolls around 80%, and knowledge in malaria transmission indicated correct cause of transmission more than 90%. Notably, only around one third of the people who got fever within 48 hours had seek their advice/ treatment for fever, but interestingly this activity was high for pregnant women (6 out of 7) and children under five 50%. More than this, we also noted that the majority of them have sought their advice/treatment at health center. Data on bed nets reveal that most of households owned at least one net (86%) but only around 30% owned ITN. And notably, only 62% and 24% of them had slept under nets and ITNs last night, respectively. We also found that the key issue for the low net re-treatment was the delay, cancelled and too early time by the re-treatment net teams. Importantly, the baseline study also fount the association between a socio-economic status with the malaria transmission and prevention, while the malaria knowledge was decreasing mostly among people who their socio-economic was lower.

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LARGE SCALE FOLLOW-UP AND MANAGEMENT OF HUMAN VACCINATIONS BY WEB-BASED HIGH PERFORMANCE DATABASE SOFTWARE IPGVAX: CONCEPT AND FIELD EVALUATION IN GUADELOUPE (FRENCH CARIBES)

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The widespread implementation of childhood vaccination programs has considerably reduced the occurrence of many vaccine-preventable diseases. However, a substantial proportion of the remaining morbidity and mortality from vaccine-preventable diseases presently occurs among older adolescents and adults. Persons who escaped natural infection or were not vaccinated with toxoids or vaccines against diphtheria, tetanus, measles, mumps, rubella, and poliomyelitis may be at risk of these diseases and their complications. Contrary to the efficient vaccination coverage during childhood (>85%), a systematic approach is necessary to ensure that every adult is appropriately protected. To increase the variable level of adults coverage, physicians should maintain detailed records containing information about each persons previous vaccinations and call back in time for secondary boosting to avoid a full primary series of doses. In line with health authorities requiring, we developed a web-based database suitable for region-scaled vaccination management named IPGvAx. This easily web-accessible data manager handles protocols of vaccination in a clear and simple approach, and appropriate identification data on patients ie no medical data except vaccinations and physicians hand written information on potential side effects. Written in webdev language, crypted data from the protected server database are instantly accessible through password accesses depending upon users echelon. IPGvAx can easily manage over 500,000 patients simultaneously, depending upon web speed access, opening a very large sharing possibility for records containing information about each persons previous vaccinations. We describe characteristics of this running application presently used in Guadeloupe, it greatly improves the follow-up of vaccinated individuals and can improve vaccination coverage by health system (public and private), particularly: (i) real-time analysis of vaccine consumptions; (ii) automatic recall of consultants; (iii) help for Vaccine Adverse Event Reporting System traceability (iv) instant statistics.. Such integrated processing would be of substantial help in vaccination programs for developing countries, as well as for other advanced northern countries.

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SCHOOL-BASE DENGUE CONTROL PILOT PROJECT IN CAMBODIA

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Dengue haemorrhagic fever (DHF) is the number one cause of mortality in pediatric wards during the dengue transmission season. Several ecological and socio-economic factors enable the mosquito vector - *Aedes aegypti* - to flourish. The disease itself and the efforts to control it bring a significant economic and social burden. This abstract describes a pilot project of school-based dengue education which has taken place in two dengue hyper-endemic districts of Cambodia between 2004 and 2006. The project aimed to strengthen and increase the inter-governmental partnership in dengue prevention and control by involving schoolchildren and encouraging them to take part in dengue control activities. Coordination meetings took place between different stakeholders following conception of ideas and advocacy. Educational classroom and extra-curricular activities were conceived, materials designed and produced and teaching staff were educated about their use and how to implement the project. This education programme is designed to contribute to a longer-term, sustainable and cost-effective community-based strategy for dengue prevention and control. Assessments of students' and parents' dengue knowledge and entomological assessments were conducted both prior to and following the education period. Significant improvements were recorded in both parents' and students' dengue knowledge and practices following education. There was an increase in the reported weekly washing of water jars, improvements in the method of washing jars and in seeking medical treatment at government hospitals when a child was suspected of being infected with dengue. The entomological surveys also show a marked improvement. This programme has demonstrated both a significant increase in understanding of dengue prevention activities and a significant decrease in important entomological indexes and may be associated with a decreased risk of dengue outbreaks in these communities.

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GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G6PD) MUTATIONS IN CAMBODIA: G6PD VIANGCHAN (871G>A) IS THE MOST COMMON VARIANT IN THE CAMBODIAN POPULATION

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We conducted a survey of malaria diagnoses and glucose-6-phosphate dehydrogenase (G6PD) testing in remote areas of Cambodia. Blood specimens from 670 people were collected by the finger-prick method. Of these people, 24.9% were found to have malaria, and 7.0% of people were G6PD deficient. In the Khmer, the largest ethnical population in Cambodia, the G6PD deficiency rate of males was 12.6% (25/199) whereas the rates in the minorities of the Tum Pun and the Cha Ray were 1.1% (1/93) and 3.2% (2/63), respectively. Of the G6PD-deficient subjects, 97.9% (46/47) were G6PD Viangchan (871G>A), and only one case (2.1%) was G6PD Union (1360C>T). Since G6PD Mahidol (487G>A) is common in Myanmar according to our previous study, the current finding suggests that the Cambodian population is derived from homogeneous ancestries and is different from the Myanmar population. All G6PD Viangchan cases were linked to two other mutations of 1311C>T and IVS-11 nt93T>C in the *G6PD* gene.

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POLYMERASE CHAIN REACTION WITH TWO MOLECULAR TARGETS IN MUCOSAL LEISHMANIASIS´ DIAGNOSIS: A VALIDATION STUDY

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We validated the Polymerase Chain Reaction (PCR) with a composite reference standard in 61 patients clinically suspected of having Mucosal Leishmaniasis, 35 of which were cases and 25 were non-cases according to this reference standard. Patient classification and test application were carried out independently by two blind observers. One pair of primers was used to amplify a fragment of 120 bp in the conserved region of kDNA and another pair was used to amplify the ITS rDNA. PCR showed 68.6% (95% CI 59.2-72.6) sensitivity and 92% (95% CI 78.9-97.7) specificity; positive likelihood ratio: 8.6 (95% CI 2.8 - 31.3) and negative likelihood ratio: 0.3 (95% CI 0.3 - 0.5), when kDNA molecular target was amplified. The test performed better on sensitivity using this target compared to the Internal Transcript Spacers (ITS) rDNA molecular target which showed 40% (95% CI 31.5-4.3) sensitivity and 96% (95% CI 84.1-99.3) specificity; positive likelihood ratio: 10 (95%CI 2.0 - 58.8) and negative likelihood ratio: 0.6 (95% CI 0.6 - 0.8). The inter-observer agreement was excellent for both tests. Based upon results obtained and due to low performance of conventional methods for diagnosing mucosal leishmaniasis, we consider PCR with kDNA as molecular target is a useful diagnostic test for and the ITS rDNA molecular target is useful when the aim is to identify species.

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ZINC, COPPER AND IRON IMBALANCE IN INDIAN KALA-AZAR

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Visceral leishmaniasis (VL) or Indian kala-azar is a protozoan disease caused by the parasite *Leishmania donovani*. The disease is pandemic in eastern part of India and is a major public health problem for Bihar state, India. The disease predominantly affects the people of low income group in whom the nutritional status is very poor. The essential role of micronutrient is widely accepted. In recent years, a protective immune response against intracellular pathogens, such as *Leishmania* has been defined as Th1 response and zinc deficiency has been shown to lead a selective Th1 deficiency in human volunteers and no data is available about the role of Zinc and other important trace elements in Indian Kala-azar. We investigated zinc, copper and iron in the blood samples of Indian kala-azar patients before the start of anti-leishmanial chemotherapy and after their initial cure. The preliminary observations reflects the imbalance in zinc/copper ratio which might serve as a marker for decreased Th1 response or immunodeficiency in Indian kala-azar, particularly in severe form of the disease. The study suggests that zinc therapy along with chemotherapeutic agent may reduce the severity of the disease and unresponsiveness to the drug in the long run.

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INTEGRATION OF INFORMATION TECHNOLOGIES IN CLINICAL STUDIES IN NICARAGUA

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Clinical studies and trials require accessibility of large amounts of information in a timely manner with a high level of quality control. Information technologies can greatly facilitate the necessary processes and compliance with established standards such as Good Clinical Practice (GCP) and Good Laboratory Practice (GLP). In Managua, Nicaragua, the use of personal digital assistants (PDAs), bar codes, fingerprint scanners, and geographic information system (GIS) technology has been integrated to facilitate the implementation of a prospective cohort study of dengue transmission among ~3,800 children. Specifically, sample reception and handling was greatly improved through the use of PDAs with attached barcode scanners to monitor control of temperature, transport and processing time, and specimen quality. GCP and patient care benefited from PDAs that streamline documentation of clinical histories and consults and barcodes that allow tracking of medical files, consent documents, and patient flow through the health center. Real-time analysis of data

on all levels augmented quality control, logistics, and planning. Studies were undertaken to quantitatively assess the performance of PDAs versus paper and pencil questionnaires in terms of accuracy; time savings obtained using barcode and fingerprint scans for identification of study children and location of medical charts; and the specific advantage of GIS in directing field visit strategies, localization of study childrens' homes, and spatiotemporal tracking of confirmed dengue cases. Further benefits were derived from interdigitation of numerous databases as well as automation of laboratory procedures. Finally, the integration of the various technologies in the annual blood sample collection resulted in reduction in personnel from 23 employees to 12, in workday from 15 to 8 hours, and in data entry personnel from 5 to one; allowed stricter control of supplies; and permitted daily data analysis with graphical and geographic output. Importantly, the design and implementation of this system was driven by user needs in a developing country. We have demonstrated that the combined use of information technologies in clinical studies has numerous advantages, such as improved quality control, streamlined laboratory and fieldwork procedures, increased efficiency and reduced costs, real-time analysis of data, local capacity building, and better quality of patient care.

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TREATMENT PATTERNS AND THE COST IMPLICATIONS OF CLINICAL, MICROSCOPY AND RAPID DIAGNOSTIC TESTS FOR MALARIA DIAGNOSIS AT HEALTH FACILITIES IN ZAMBIA

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Zambia is one of the first countries in Africa to adopt artemisinin-based combination therapies (ACTs) as first line treatment for malaria. The ACTs have been proven to be effective in treating uncomplicated malaria. However, for malaria cases to be treated successfully, prompt and accurate diagnosis is required. This also helps to ensure that non-malaria cases are managed correctly. Improving malaria diagnostic capacity has been intensified so as to ensure that patients seeking care are correctly managed. However, microscopy capacity among government health facilities is estimated at about 27% (MIS 2006). Therefore, health centres without malaria microscopy services are being provided with rapid diagnostic tests (RDTs). Where neither microscopy nor RDTs are available, patients are treated based on clinical diagnosis. A prospective evaluation was carried out at selected health facilities in 6 districts to assess prescription patterns and cost implications of using clinical, microscopy or RDTs for malaria diagnosis. The providers' perspective of costing was undertaken. The study was conducted over 8 months covering both the high and low transmission seasons. All age groups were included in the analysis. Parasite prevalence ranged from 10 to 26%. The diagnostic tests results had a role in determining which antimalarial was prescribed to patients (cheaper Sulphadoxine-pyrimethamime or expensive Artemether-lumefantrine). Clinical diagnosis did not lead to potential savings on antimalarial treatment. Using microscopy and RDTs could lead to 56% and 60% savings on antimalarial treatment respectively. The average cost of treatment per visit was USD1.44 for clinical, USD1.21 for microscopy and USD1.18 for RDT strategy. The unit cost per false positive visit was highest in the clinical strategy (USD 1.17) and lowest in the RDT strategy (USD 0.16).

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ACTIVITIES OF ARTEMETHER-LUMEFANTRINE AND AMODIAQUINE-SULFALENE-PYRIMETHAMINE AGAINST SEXUAL STAGE PARASITES IN FALCIPARUM MALARIA IN CHILDREN

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The activities of artemether-lumefantrine and amodiaquine-sulfalene-pyrimethamine on sexual- stage parasites were evaluated in 42 of 181 Nigerian children with uncomplicated *Plasmodium falciparum* malaria who had gametocytaemia before, during or after treatment with the two combination therapies. The children were randomized to the standard dose regimens of the two combination therapies. Clinical recovery from illness occurred in all children who carried gametocytes. Gametocytaemia was detected in 20 patients (11%) before treatment and in another 22 patients (12.2%) after treatment. Gametocyte carriage rates were similar in both combination treatment groups but the area under the curve of gametocyte-time plot was 8-folds higher in amodiaquine-sulfalene-pyrimethamine-treated than in artemether-lumefantrine-treated children. The pretreatment gametocyte sex ratio was female biased in both treatment groups. There was a short-lived but significant increase in gametocyte sex ratio in children treated with amodiaquine-sulfalene-pyrimethamine but not in those treated with artemether-lumefantrine. These results indicate both combination therapies had moderate effects on gametocyte carriage but artemether-lumefantrine may be more potent at reducing transmissibility in *P. falciparum* malaria by exerting greater effects on post-treatment gametocyte density and gametocyte sex ratio.

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AZITHROMYCIN FOR THE TREATMENT OF AMERICAN CUTANEOUS LEISHMANIASIS. PRE-CLINICAL AND CLINICAL DATA

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Azithromycin was shown to have activity against *Leishmania major in vitro* and in a mouse model and therefore is a potential oral alternative for the treatment of leishmaniasis. The current animal and human study evaluated the activity of azithromycin against species of *Leishmania* causing cutaneous disease in Argentina. Golden hamsters, infected through footpad injections with metacyclic promastigotes of *Leishmania (V) braziliensis* or *Leishmania (L) amazonensis,* were treated at the start of infection with azithromycin at doses of 450mg/Kg orally and compared to untreated controls and animals treated with meglumine antimoniate (MA). Measurement of footpad thickness, lesion cultures and analysis of disease dissemination were performed. Patients from Northwestern Argentina with confirmed diagnoses of cutaneous leishmaniasis were randomized into groups receiving oral azithromycin 500mg/day (22 patients) or intramuscular MA, 10mgSb/Kg/day (23 patients), both for 28 days and followed for one year. Efficacy was defined as complete re-epithelization without relapse for 12 months after completing therapy. Identification of infecting species from the lesions was done by PCR analysis. Treatment of Golden hamsters with oral azithromycin had no activity against infections with *L. (L) amazonensis*. Azithromycin demonstrated significant activity at controlling lesion size caused by *L. (V) braziliensis* relative to untreated controls but was inferior to meglumine antimoniate,. Neither drug was able to totally eliminate parasites from the lesions in hamsters. In the clinical trial azithromycin, which was well tolerated, cured 45.5% of the patients whereas meglumine antimoniate cured 82.6% of the patients,. In 17 patients, species identification was obtained and demonstrated *L (V) braziliensis* in all cases. It was concluded from both the animal and the human studies that oral azithromycin had moderate activity as monotherapy against *L (V) braziliensis*, with no activity against *L (L) amazonensis*.

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THE PREVALENCE OF SUBSTANDARD AND COUNTERFEIT PRESCRIPTION DRUGS IN CHENNAI (FORMER MADRAS), INDIA

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Poor drug quality in developing countries is a common and often under-appreciated problem. Causes include poor manufacturing, poor storage, repackaging, dilution with fillers and, increasingly over the last decade, criminal counterfeiting. Since there have been no published studies of drug standards in India, we examined the quality of three commonly prescribed drugs, supplied by a broad range of public pharmaceutical outlets in the city of Chennai. Three commonly prescribed drugs, considered expensive enough to attract the attention of counterfeiters, (artesunate, ciprofloxacin, rifampicin) were purchased from each of 100 pharmacies chosen around the city. The 300 samples were analysed in Canada by high pressure liquid chromatography (Cantest BioPharma Services). Compared to accepted industry standards (mean ± 10%), ciprofloxacin levels were within manufacturing limits (99.2 ± 7.1%, NS). However, artesunate tablets contained roughly 20% less than the stated content (80.1± 9.1%, p<.0001) while rifampicin was almost 10% below stated dose (91.6 ± 5.7%, p<.001). No tablet from any sample contained less than 50% of the stated dose. Although Indian manufacturers are thought to be an important source of counterfeit drugs, our study found no evidence of counterfeiting. However, two of the three sampled drugs were significantly below their stated tablet content. Whether this was due to poor manufacturing standards or inadequate storage and handling cannot be defined with this study but the levels found represent a substantial risk of treatment failure for some treated patients. Our results, plus the limited available literature, suggest that substandard drugs are an important clinical problem, recently compounded by a growing counterfeit market involving more expensive prescription drugs. At present, there is surprisingly little quantifiable data concerning drug standards in developing countries. Our study is designed to improve that level of knowledge and has the potential to add important information for planners in the field.

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GRAM NEGATIVE BACILLARY INFECTIONS AMONG CANCER PATIENTS AT AL-AMAL HOSPITAL IN DOHA/QATAR

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Gram Negative Bacillary Infections Among Cancer Patients at Al-Amal Cancer Hospital

Infection is a common complication in cancer patients that can prolong their hospitalization, lead to acute organ dysfunction and eventually death. The current work, focuses on infections encountered among cancer pattients aiming to determine the frequency of isolation of gram negative bacilli and their antibiogram patterns among in-patients and out-patient clinics at Al-Amal Hospital in the state of Qatar. A total of 128 different clinical specimens (blood, urine, sputum, stool, swabs from wounds and others) were collected from cancer patients (in-patients and out-patients) at Al-Amal Hospital and were routinely and fully investigated including antibiotics sensitivity using disc diffusion method according to the NCCLS standards at the microbiology laboratories (Department of Laboratory Medicine and Pathology /Hamad Medical Corporation Doha - Qatar). Data analysis was performed using Microsoft Office Excel 2003 and Minitab Student Release 12 programs. Data showed that most patients (87.5%) were over thirty years, where 58.59% males, 69.53 % Arabs, 70.31% underlying solid cancers, and 74.22 % non-neutropenic. Highest rate of infection was among blood samples compared to other specimens as urine, sputum, stool, wounds and others. *Pseudomonas aeruginosa* isolation rate (28.91%) exceeds the rate of other gram negative bacilli, however members of *Acinobacters baumannii/ calcoaceicus* complex which showed the least percentage (0.78%) The antibiogram pattern for all the isolated (18 different species) gram negative bacilli revealed that *Pseudomonas aeruginosa* was sensitive to only two antibiotics; namely nitrofurantoin and colistin, compared to other gram negative bacilli that showed variable results towards the antibiotics tested (twenty two antibiotics discs employed). All organisms tested for colistin were sensitive, whereas, all organisms tested for ampicillin / sulbactam, cefazolin, and cefixime in this study showed were resistant to them. In conclusion, data in the present work indicates that most gram negative bacilli among cancer patients are nosocomial and hospital-acquired since most of those patients are immunocompromised and many of them are hospitalized. Gram negative bacilli pathogens showed variable degrees of resistance towards the antimicrobial agents tested.

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TANNINS, IONS, CATIONS AND MALARIASIS: OBSERVATIONS AND THEORY

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Tropical fruit, Punica grantum Linn's Sun dried dermis powder has been in continuous use by Indian Red Cross, Koraput, from 06/1998. Thousands of non confounding complaint cases. Prophylactic at 1gm/dose. Therapeutic at 2gm/dose. Contradiction and side effect Free. Effective against resistant Pf, vivax, in all stages, ages, pregnancy, lactation. Effective against Measles, Chicken Pox, Cytomegalo viruses, URTI. Punicalin, Punicalgin, Punicafolin, are 3 physio compatible organic acid moieties (tannin), of OH Phenolic gr., have low pH and pKa values coexpressed with high K+, on hydrolysis. Dermis of immature fruit is more effective, has higher % of tannins and K+. Alone, Punica powder @ 2gms/dose, or soak-extract in deionised, demineralised H2O, ~10ml/2gm at ambient, when ingested, delivers parasite clearance (ii) Sans K+, clearance period widens, broad spectrum efficacy is retained (iii) recharged with saturated KCL 10-20% v/v, potency is regained (iv) 0.01-0.05mM, MgCl2, adjuvants, better shelf (v) Oral via feeding apparatus @ 6 hourly of 5gm Punica + 2gm NaCl + 250mg KCL + 5 mg MgCl2 in 100-150ml H2O is helpful in 'semi morbid' Cerebral status sans deleterious effect (vi) In known (Chloroquinn, and Mefloquinn, etc.) resistant case, conjoint administration of identical mono and divalent cations, suggests efficacy regain (vii) Reduced side effects and contradictions in high potency MDTs noted (viii) Arrests Fugacity (ix) 4-Acetamedophenol @500mg down regulates spiraling systemic inflammation [4] (x) Acetamedophenol + N. Arbortritis Linn precipitously reverses catchexia symptoms, Arbor. downturns Neutrophilia [1] (xi) Ingestion of cations, acetamedophenol or arbortritis, jointly, severally, do not prevent nor clear resistant parasites, as do tannin trio. Tannin-Ion combination suggest 'efficient uptake', 'prophylaxis' and 'kill', in all parasite stages. Parasites have affinity for ions (for biological development Plasmesin - ion catabol pathway). K+ is candidate engine to target the aspartic protease vacuoles. Tannin trio are plasmocidals. Para-magentism is viable Bio-physics. In-vitro quantitative validation warranted.

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CASE MANAGEMENT AND OUTCOMES FOR CHILDREN PRESENTING WITH FEVER AND NEGATIVE BLOOD SMEARS AT GOVERNMENT HEALTH CENTERS IN UGANDA

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Uganda national guidelines recommend that febrile patients without evidence of other diseases be treated for malaria regardless of blood smear results. With the introduction of newer, more costly combination regimens, this strategy may be less advisable. This observational study investigated the clinical management and outcomes of children aged 6 months to 10 years presenting with fever and negative smears at three government health centers in Uganda: two in high transmission areas, and one in a medium transmission setting. All participants were managed according to the usual standard of care by health center clinicians. Information on diagnosis and treatment recommendations was collected in patient exit interviews. A repeat blood smear was done on day 3 to evaluate for interval development of parasitemia, and children's caregivers reported on symptom resolution on day 7. Of the 430 children enrolled, 199 (46%) were prescribed an antimalarial. Of the 414 children who returned for the day 3 visit, 50 (12%) had a newly positive smear. Antimalarials were prescribed more often at the high transmission sites than at the lower transmission site (63% vs 17%, p<0.001). Children who received an antimalarial were no more or less likely to develop patent parasitemia by day 3 than those who did not (13% vs 11%, p=0.438). Of the 408 children who returned for the day 7 visit, 389 (96%) had resolution of symptoms. Children who received an antimalarial were no more or less likely to experience symptom resolution than those who did not (97% vs 95%, p=0.365). Antimalarials were prescribed for nearly half of the symptomatic children with negative smears, but we did not observe any parasitological or clinical benefit to this management strategy. In order to reduce the costs and possible long-term adverse effects of over-treating malaria, clinicians need not prescribe antimalarials to all patients with negative smears. Where possible, a follow-up visit is recommended for further assessment and management. Results of this study emphasize the need for improved diagnostic strategies for fever cases in Africa.

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EFFICACY AND PHARMACOKINETICS OF ARTEKIN (DIHYDROARTEMISININ AND PIPERAQUINE) FOR THE TREATMENT OF UNCOMPLICATED FALCIPARUM MALARIA IN VIETNAM

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The World Health Organization (WHO) recommends that antimalarial drugs are not to be used as monosubstances for the treatment of *Plasmodium falciparum* malaria. To combat and delay the spread of multiple-drug resistant *P. falciparum*, WHO recommends the use of artemisinin based combination therapies such as artekin (dihydroartemisinin and piperaquine). Although recent studies in Southeast Asia have shown artekin to be highly efficacious and well tolerated for the treatment of malaria infections, detailed efficacy, pharmacokinetic, and safety data from Phase 1-IV studies, which are required by regulatory bodies in developed nations, are limited. Also the therapeutic index of piperaquine remains poorly defined and dosage recommendations for piperaquine are empirically. We characterised the pharmacokinetics of piperaquine in Vietnamese subjects with uncomplicated *P. falciparum* malaria using conventional pharmacokinetic approach. Twelve Vietnamese patients were treated with a 3-day course of artekin and serial blood samples were collected for 35 days after commencement of treatment. Plasma piperaquine concentrations were measured by HPLC. The treatment course was well tolerated and all patients were cured of their infections. Efficacy parameters of artekin and the pharmacokinetics of piperaquine will be presented. Artekin is a highly effective and affordable new artemisinin combination therapy that has favourable pharmacokinetics-pharmacodynamics for rapidly reducing the biomass of malaria infections and for preventing recrudescences.

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EFFECTIVENESS OF EXISTING NET DISTRIBUTION STRATEGIES FOR ACHIEVING COMMUNITY-WIDE COVERAGE AND PROTECTION IN RURAL TANZANIA

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

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PERFORMANCE OF A MALARIA RAPID DIAGNOSTIC TEST VERSUS TRADITIONAL MICROSCOPY AMONG RURAL UGANDAN OUTPATIENTS

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There is urgent need for accurate, inexpensive, non-technical malaria diagnostic tests for use in resource-poor settings. We report the performance of a BD malaria rapid diagnostic test (BD-RDT) in detecting falciparum malaria in adult Ugandan subjects (HIV+ and HIV-) seeking care in rural mobile clinics. BD-RDT is a rapid chromatographic immunoassay for qualitative detection of a *Plasmodium falciparum* antigen. Adult subjects attending rural outpatient clinics Nov 1 2006-April 11 2007 underwent venipuncture for whole blood. Subjects were consented participants in ongoing studies which allowed for use of samples in testing new diagnostic assays. BD-RDT was performed by trained technicians in a research laboratory on fresh whole blood samples. Results were compared

to malaria blood smear (BS). Most HIV+ subjects were on trimethoprim-sulfamethoxazole prophylaxis. 149 subjects were tested;127 (85.8%) were HIV+. 22 (14.8%) samples were BD-RDT+ and BS+, 123 (82.6%) were BD-RDT- and BS-, 4 (2.7%) were BD-RDT+ but BS-; 0 were BD-RDT- but BS+. Among BS-positive samples, 0 (0%) had <100 parasites/microliter, 6 (27.2%) had 100-500 para/µL and 16 (72.7%) had >500 para/µL. There were no non-*falciparum* infections detected on BS. In conclusion, sensitivity of BD-RDT compared to BS was 100%, specificity was 97%, positive and negative predictive values were 85% and 100% respectively. BD-RDT performed excellently in detecting *falciparum* malaria in our patient population. Apparent false-positives may represent resolved or partially treated parasitemias with residual antigenemia. Further testing should focus on asymptomatic subjects, children, and performance under field (rather than laboratory) conditions. BD-RDT will be of high utility in malaria-endemic resource-poor settings where timely microscopy is not available.

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ECONOMIC ASSESSMENT OF PUTATIVE CONTROL STRATEGIES AGAINST *NEOSPORA CANINUM*

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The protozoan abortifacient *Neospora caninum* accounts for important economic losses to the livestock industry. In the present study, a Monte Carlo simulation model was developed to estimate the direct costs caused by N. caninum with and without different control strategies and to estimate the costs of selected control strategies. In addition, the costs of each individual control strategy and the prevented losses were compared by calculating the benefit-cost ratio (BCR) and the net present value (NPV). The control strategies considered were "testing and culling of all seropositive female cattle", "discontinued breeding with offspring from seropositive cows", and "vaccination of susceptible and infected female cattle". As substances active against *N. caninum* are currently being developed, an additional scenario considering chemotherapeutical treatment of female offspring was also included. Each parameter in the model that was considered to be uncertain, was described using probability distributions. The models were run with 20,000 iterations over a time period of 25 years. All sub-scenarios that required yearly serological testing of all cattle in the population produced high costs and thus were not economically justifiable. Among the sub-scenarios where all animals were tested in the first year and only a specified subset of the population in subsequent years, two control strategies revealed BCRs >1 and positive NPVs: "Discontinued breeding with offspring from seropositive cows" (BCR=1.15, NPV= CHF 20 million) and "medical treatment of all female calves" (BCR=2.62, NPV= CHF 77 million). In economic terms, the best control strategy at present would therefore be "discontinued breeding with offspring from seropositive cows". However, medication - once available in the future - was shown to express the highest benefit-cost ratio (2.62). Although less promising, vaccination may be remodeled as soon as evidence about ist efficacy in preventing endogenous and exogenous transmission is specified.

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IMMUNIZATION WITH LEPTOSPIRAL IMMUNOGLOBULIN-LIKE (LIG) PROTEIN WITH ALUMINIUM HYDROXIDE ADJUVANT CONFERS STERILIZING IMMUNITY IN THE HAMSTER MODEL FOR LEPTOSPIROSIS

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Subunit vaccines are a potential intervention strategy to prevent leptospirosis, an important neglected disease in developing countries. Lig proteins are a putative virulence factor which has bacterial Ig-like repeat domains and is expressed on the surface of *Leptospira*. We previously reported that immunization of recombinant Lig protein fragments in Freund's adjuvant conferred protection against lethal challenge but not against sub-lethal infection in the Golden Syrian hamster model of leptospirosis. Methods were modified to obtain purified *Escherichia coli* derived preparations with enhanced solubility for three repeat domain fragments (LigANI, LigBNI and LigBrep) of LigA and LigB proteins from *L. interrogans* serovar Copenhageni strain Fiocruz L1-130. Hamsters (n = 10 per experiment) were immunized with two doses (10-80 µg) of purified fragments in aluminium hydroxide adjuvant and challenged two weeks afterwards with a lethal dose (2.5× LD_{50}) of *L. interrogans* strain Fiocruz L1-130. Immunization with doses as low as 20 µg of LigBrep fragments conferred sterilizing immunity (100% in 3 experiments, $P<0.0001$). In contrast, immunization with LigANI conferred protection against mortality (90-100% in 5 experiments, $P<0.0001$) but did not confer protection against sub-lethal infection, while immunization with LigBNI did not confer protection against mortality (0-40% in two experiments, $P>0.08$). However, immunization with the combination of LigANI and LigBNI conferred sterile immunity against lethal challenge (100% in two experiments, $P<0.0001$). Immunofluorescence studies of pre-challenge sera found that immunized hamsters produced surface-binding antibodies. Passive transfer of rabbit hyperimmune sera to the three Lig fragments conferred sterilizing immunity (90-100%, in two experiments, P<0.0007) in hamsters.Together these findings indicate that immunization with recombinant Lig proteins in aluminium hydroxide confers sterilizing immunity in the standard hamster model of leptospirosis and that the mechanism of immunity is antibody-dependent. Lig proteins may therefore serve as a sub-unit vaccine candidate for human leptospirosis.

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MORBIDITY AND MORTALITY PATTERNS OF MEDICAL ADMISSIONS IN A NIGERIAN SECONDARY HEALTH CARE HOSPITAL

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This is to determine the prevalence and pattern of disease in a secondary health care facility and to compare this with previous report from a similar setting. A retrospective study of the morbidity and mortality data using the medical admission register at the Adeoyo State hospital, Ring road, Ibadan, over a 5-year period (1996 - 2001). A total of 2609 patients were admitted with a mean age of 45.1 ± 19.5 years (range 10 - 98 years). Females with a mean of 46.3 ±19.4 years were older than males with a mean of 44.1± 19.5 years. There was a slight male preponderance (53%). Causes of admission included cardiovascular diseases (36.8%) and infections (24.9%) including tuberculosis (1.3%) and Human

Immunodeficiency Virus (HIV) infection / Acquired Immunodeficiency syndrome (AIDS) (2%). Cardiovascular diseases had increased by 150% compared with previous report from similar setting. Non-communicable diseases accounted for 57.9% of all the medical admissions, and progressively increased over time. The mortality rate was 18.9%. Cerebrovascular accident was the leading cause of death, 97 of 494 (20%), tetanus 61 (12%), Meningitis 55 (11%) and congestive cardiac failure 49 (10%). The age specific mortality rate was highest at 65 years of age and above. In conclusion, cardiovascular diseases and infections are prevalent, though the former more commonly; there is a rising profile of non-communicable diseases. This may reflect changing demographic, social and lifestyle attributes and indicates a need for significant health policy initiation.

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MALARIA TRANSMISSION INTENSITY AND MORBIDITY PATTERNS IN PARTS OF THE IMO RIVER BASIN, SOUTH EASTERN NIGERIA

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This study was conducted to analyze the relationship between malaria transmission intensity and morbidity in six communities in the Imo River Basin of Nigeria located at different altitudes. Blood samples were collected from 502 children (aged <5 years) and examined microscopically for malaria parasites. Indoor resting mosquitoes were collected in ten houses in each community using the pyrethrium spray catch method, female *Anopheles gambiae* complex *sensu stricto* species were identified and examined for parity and infectivity rates. Community survey using structured questionnaires and key informant discussions was employed to determine malaria specific signs and symptoms in children. In addition, out patient attendance records from 2003 to 2006 were analyzed for annual malaria morbidity rates. Malaria parasiteamia among children ranged from 0-36.7%,with the lower altitude areas(>100m) recording higher prevalences than the higher altitude areas(<100m). 205 of the total mosquitoes caught were female *An. gambiae complex sensu stricto* out of which 183 were infected with malaria parasite. Transmission rates were higher in the low altitude communities where Itu (20.5%) scored highest than at high altitude communities where Nguru (6%) had the lowest score. Data from both the community surveys and out patient attendance records showed higher malaria morbidity in the higher altitude than in the lower altitude communities where there were higher densities of indoor resting anopheles mosquitoes.Splenomegally, anaemia and cerebral malaria were the most severe morbidity indicators reported and were more prevalent at the higher altitudes than at the lower altitudes. This study suggests that there is higher malaria morbidity at lower transmission intensities. Altitude as an important determinant of malaria transmission is also indicated.

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TOXOPLASMA GONDII AND *TOXOCARA* SPP. CO-INFECTION (NOTE: REMOVE ITAL)

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Toxoplasma gondii and *Toxocara* spp. (*canis* and *cati*) infections, which can cause systemic and ocular disease, share soil ingestion as a mode of exposure. In order to estimate the prevalence of infection with one or both of these organisms, we used enzyme immunoassays to test serum samples from persons ≥ 12 years old obtained in the Third National Health and Nutrition Examination Survey (1988-94), a representative sample of the U.S. population. Among persons tested for both *T. gondii* and *Toxocara* spp. (N=16,646), the age-adjusted *T. gondii* antibody prevalence was 23.6% (95% confidence interval [CI] 22.1%-25.1%) and the age-adjusted *Toxocara* spp. antibody prevalence was 14.0% (95% CI 12.7%-15.4%). Of those *T. gondii* antibody positive, children 12-19 years old had the highest *Toxocara* spp. antibody rate (28.9%, 95% CI 21.6%-37.6%). In multivariate analysis controlling for age, race/ethnicity, sex, urbanicity, birth in the U.S., census region, poverty, crowding, head of household education, cat ownership (*T. gondii* model), dog ownership (*Toxocara* spp. model), blood lead level as a pica indicator (*Toxocara* spp. model), persons infected with *Toxocara* spp. were much more likely to be infected with *T. gondii* (OR 1.93, 95% CI 1.61-2.31), and similarly, persons infected with *T. gondii* were much more likely to be infected with *Toxocara* spp. (OR 1.91, 95% CI 1.59-2.29). Prevention of both *T. gondii* and *Toxocara* spp. infections share common interventions and should include hand washing after soil exposure or gardening, washing of vegetables and fruits eaten raw that may have been contaminated with soil, and prevention of soil contamination in public areas by dog and cat feces.

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A QUANTITATIVE ALGORITHM FOR PRIORITIZATION OF NATURALLY OCCURRING INFECTIOUS DISEASE THREATS TO THE U.S. MILITARY

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Identifying which infectious disease pathogens pose significant threats to the U.S. military and organizing an optimal portfolio of research efforts to develop countermeasures for such threats are ongoing challenges for the Military Infectious Diseases Research Program. A quantitative algorithmic method (Infectious Diseases Investment Decision Evaluation Algorithm or ID-IDEAL) that utilizes Armed Forces Medical Intelligence Center information to determine which naturally occurring infectious disease pathogens pose the most substantial threat to U.S. deployed forces in the absence of specific mitigating countermeasures is a useful tool. ID-IDEAL scores the relative importance of various diseases by taking into account both threat likelihood on a country-by-country basis and disease severity. According to this analysis, the top three endemic disease threats to U.S. deployed forces are bacteria-caused diarrhea, malaria, and dengue fever.

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MORTALITY RATES IN COHORTS OF CHILDREN TWO YEARS AFTER SEVERE OR MILD MALARIA IN RURAL NORTHERN GHANA

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Malaria is a major cause of morbidity and mortality in children in Ghana and accounts for 25% of deaths in children under the age of five years. The contribution of previous malaria episodes to child mortality in Ghana is not known. We investigated the long term mortality risk following severe malaria in a cohort of children treated for severe malaria or uncomplicated malaria between 2002 and 2004 using the Navrongo demographic surveillance system (NDSS). Clinical and laboratory factors

that place young children (age, 6-59 months) at increased risk of developing severe malaria in northern Ghana were evaluated during the period. For each child with severe malaria enrolled, two other patients with mild malaria seen at an outpatient department (OPD), and two healthy community controls matched for age, sex and geographical location were recruited. Out of 868 children admitted for severe malaria, 3.6% died during admission. We linked 93% (777/837) of those that were treated and discharged to the NDSS database. Most of the cases (56.9%) were males. Thirty (21.0/1000 child years, 95% *CI: 14.2-29.9*) of the severe malaria cases died within 22 months post admission. Forty-two (14.0/1000 child years, *95% CI: 10.1-18.8*) of the 1,642 and 38 out of 1,674 (12.4/1000 child years, 95% *CI: 8.8-17.0*) mild cases and community controls respectively died within the follow-up period. The risk of dying 22 months post severe malaria was 1.7 times higher than other children in the general population and 1.5 times that of children who had mild malaria. Severe malaria may have sequalae effect that could impact on the survival of its victims.

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INFORMATICS FOR DISEASE SURVEILLANCE IN DEVELOPING COUNTRIES: EVALUATION OF THE EARLY WARNING OUTBREAK RECOGNITION SYSTEM (EWORS)

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Health surveillance in developing countries contends with limitations in infrastructure, health investment, laboratory equipment, information systems, and public health workforce. Improving surveillance could prevent costly (in human and economic terms) delays in epidemic response, and increase compliance with the International Health Regulations (2005). Computer and information technology developed for "syndromic surveillance" systems, which use pre-diagnostic data for early outbreak detection and are common in wealthy countries, might improve surveillance in resource-poor settings. To guide syndromic surveillance adaptation to resource-poor settings, we evaluated EWORS in Lao PDR (September 2006) and Peru (March 2007). Initiated in Indonesia in 1999, EWORS was implemented in Lao PDR in 2003 and Peru in 2005 through Ministry of Health (MoH) partnerships with the US Department of Defense. EWORS monitors daily counts of syndromic groupings for infectious disease-related patient records, and includes an MoH-based hub, sentinel sites (public hospitals or community health centers), and computer-based data entry and analysis. Evaluation included interviews with EWORS operators and stakeholders, inspection of facilities and equipment, and data analysis. Host-country stakeholders found EWORS useful for identifying outbreaks, guiding outbreak responses with scarce resources, and improving communication. Where available, performance data indicated improvement since EWORS implementation (e.g. in reporting timeliness). EWORS informatics-based enhancements should include automated detection algorithms, map-based displays and other visualization tools, integration with other MoH systems for data collection and information sharing, and communication technologies to link remote sites. EWORS should undergo regular system monitoring and evaluation, including exercises and simulated outbreaks. In conclusion, syndromic surveillance can be adapted effectively in resource-poor settings. Partnerships linking surveillance experts with developing country MoHs might extend advanced informatics capabilities to populations in the most epidemic-prone settings.

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STUDY ON THE CORRELATIONS AMONG OF CLIMATE FACTORS, MOSQUITO INDICES AND EPIDEMICS OF DENGUE IN KAOHSIUNG, TAIWAN

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Although mosquito larval indices have been used for dengue surveillance over 30 years, their relation to dengue epidemics is inconsistent and questionable. This study tended to clarify the possible effect that climate factors (rainfall, temperature, and relative humidity) may have on mosquito density and also epidemics of dengue. Epidemiological information of dengue epidemic, daily meteorological data plus vector density in Kaohsiung in 2002 were used to investigate possible relationships among climatic factors, mosquito density shown by different indices and number of dengue cases. Compared with the previous year 2001 before the large-scale of epidemic, the Kaohsiung's temperature data in 2002 were significantly higher (daily mean temperature: 25.11 vs. 25.71°C, p=0.028; maximal temperature: 28.95 vs. 29.69°C, p=0.004; minimal temperature: 22.14 vs. 22.75 °C, p=0.046) whereas Kaohsiung's relative humidity (RH) accompanied with rainfall in 2002 were less (RH: 75.38 vs. 74.24%, p=0.03; mean of daily accumulated precipitation: 3.73 vs. 1.79 mm, p=0.02). The hot and rainy season of 2002 was from June to August, while the daily mean temperature was from 28.99 to 29.25°C, and yearly accumulated precipitation was 1037.5 mm with peaks at mid-May and early August. Interestingly, the means of the levelsof Breteau index, House index and Container index increased from March (level of BI: 0.73, HI: 1.86%, CI: 3.31%) and reached to the peak in June (level of BI: 1.61, HI: 5.07%, CI: 9.37%), and then decreased rapidly in July (level of BI: 1.39, HI: 4.61%, CI: 7.11%). The 2002 epidemic of dengue/DHF started from May, and case number increased dramatically from mid-June, reached to the peak (1,299 dengue fever plus 77 DHF cases) in September. Cases decreased from October and the epidemic ended up till January 2003. The time interval between the peaks of vector density and dengue cases onset was about 3 months. Using general linear model, we found that density of residents was significantly associated with the epidemic of dengue. Additionally, the peak of rainfall in Taiwan was followed by high population density of mosquito, but this correlation could be interrupted by different strategies of vector control. More detailed verification with other years/areas in Taiwan is in progress. Future efforts will focus on possible mechanisms of climate factors involved in different entomological and epidemiological characteristics of dengue in Taiwan.

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SEROTYPE DETERMINATION AND ELUCIDATION OF NEW DENGUE GENOTYPE MARKERS VIRUS THROUGH THE STUDY OF THE NON-STRUCTURAL NS5 GENE

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Dengue (DEN) is an infectious disease caused by DENV from the genus *Flavivirus* of the family *Flaviviridae*. It has a (+) sense RNA genome and replicates in the cytoplasm of host cells. This virus is mainly transmitted to humans by the vector mosquito *Aedes aegypti*. Based on serologic responses, DEN viruses were defined as DEN serotypes 1, 2, 3 and 4. Determination of serotype and genotype of DEN virus circulating in Mexico may be relevant to treat DENV infection and in designing new

control strategies. To provide more information on DEN epidemiology and virulence, we characterized a number of DEN viruses isolated in Oaxaca and Veracruz States of Mexico in 2005-2006; (1) We obtained 72 isolated of DENV, 24 from Oaxaca and 48 from Veracruz. The cytopathic effect observed in the syncytia formation and lysis of the C6/36 cell monolayer of *Aedes albopictus* was variable for the 72 isolates and a relationship was observed between isolates from Dengue (DF) and those from dengue hemorrhagic fever (DHF); (2) From the 72 isolates, 9 were serotype one; 54 serotype 2; 7 serotype 3, and 2 serotype 4 by reverse transcriptase polymerase chain reaction (RT-PCR); (3) The nucleotide sequence of the 3' end of protein E and NS5 RNA was obtained from 8 isolates from Oaxaca State obtained in 2005; (4) The phylogenetic analysis displayed the 8 isolated in the branch of DNV-2 Asian-American genotype. In conclusion, our results suggest that DENV-2 was the predominant serotype in the outbreak of DEN and DHF in 2005 to 2006 in Oaxaca State as well as in the outbreak of Veracruz State in 2006, with the Asian-American genotype being the most prevalent in Oaxaca State. All these studies widen our knowledge of the virus and facilitate developing DEN control strategies

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VIROLOGICAL AND SEROLOGICAL SURVEILLANCE OF DENGUE FEVER/DENGUE HEMORRHAGIC FEVER IN THAILAND, 2003 TO 2006

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The incidence of dengue fever/dengue hemorrhagic fever (DF/DHF) in Thailand has continuously increased since the first recognized outbreak in 1958. From 2003 to 2006, the number of reported dengue cases were 39,135 to 63,657 per year. From 2003 to 2006, National Institute of Health, Department of Medical Sciences received blood specimens from 7,630 dengue suspected cases. According to the results of IgM and IgG antibody capture ELISA and/or virus isolation and/or RT-PCR, 86.1% of the total cases were confirmed to be infected by dengue virus. Confirmed dengue cases were found all year round, but the most prevalent between June to August. The majority of confirmed dengue cases were in the age group 10 to 14 years.Of the total, 92.6% of confirmed dengue cases experienced secondary infection. All four dengue serotypes were identified, of which DENV-1 was the most predominant. Almost all of the DHF cases caused by DENV-2 and DENV-4 were in secondary infection, while 14.0% and 9.8% of DHF cases caused by DENV-1 and DENV-3 were in primary infection. These results indicate that DENV-1 and DENV-3 induce DHF in both primary and secondary infections, and suggest that DENV-2 and DENV-4 in Thailand are less likely to cause DHF in primary infections.

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LONG-TERM CLIMATE AND ENDEMIC DENGUE TRANSMISSION

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Dengue is the most common arboviral disease in the world with hundreds of thousands of cases reported annually. In endemic areas, major epidemics occur intermittently, with smaller seasonal outbreaks occurring in between. While the timing and location of major outbreaks remains unpredictable, substantial research suggests that climate and the immunological status of the host population may be key drivers. We analyzed the correlation between dengue incidence and El Niño Southern Oscillation (ENSO), temperature, and precipitation using surveillance data from Puerto Rico, Mexico, and Thailand. In order to study the effect of climate on the occurrence of major outbreaks independent of the normal seasonal variation of dengue and climate data, we used wavelet filtering to remove background noise and the seasonal periodic from all time series. ENSO correlated significantly but weakly with dengue incidence in all countries. Temperature and precipitation correlated with both ENSO and dengue incidence, but their association with dengue transmission on the long-term scale was less than that of ENSO. Thus, while climate may have an impact on the occurrence of major dengue epidemics, this study suggests that it is not the main determinant. These findings highlight the fact that climate may be important but is not sufficient for predicting dengue transmission.

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COMMUNITY PARTICIPATION PROJECT FOR DENGUE PREVENTION AND CONTROL IN PUERTO RICO: ENTOMOLOGIC SURVEY RESULTS IN 2005-2006

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Dengue is a mosquito-borne viral disease that is endemic throughout the tropics. *Aedes aegypti* is the most important vector worldwide. In the absence of a vaccine, dengue prevention efforts often focus on the use of insecticides to control the adult mosquito population. Because of the limited efficacy of current control programs, new approaches to dengue prevention involving community participation are needed. The goal of this project was to implement a novel approach to community-based dengue prevention by developing a model that involves the active participation of residents in planning and conducting activities to reduce *Ae. aegypti* in their community. The specific entomologic objective for a two-year period (2005 to 2007) was to reduce larval indices by 50% in the selected community. We selected two comparable communities in terms of population size, baseline mosquito indices, and level of infrastructure. We designated one community as the intervention community (IC) and the other as the control community (CC). The intervention consisted of house to house visits to eliminate breeding containers and distribution of targeted educational materials for dengue prevention. Entomologic surveys were conducted in all houses in both communities before and after implementation of dengue prevention activities. We used the Breteau Index [(BI); number of containers positive for *Ae. aegypti* larvae or pupae per 100 houses] and the House Index [(HI), number of breeding sites per 100 houses] as our outcome measures. There was no difference between the 2005 pre-intervention HI in IC (42%) and CC (46%). There was a significant difference between HI in the IC (35%) and CC (48%) by the 2006 post-intervention survey (p-value=0.02). However, there was no significant difference in decline in HI in IC (42% to 35%, p-value=0.19). The 2005 pre-intervention BI for IC was 76 and 98 for CC. After the intervention, the BI for CC increased by 18% (98 to 116). There was a 26% decrease in the BI for IC (76 to 56). In conclusion, for the first year of the project, larvae indices increased in CC and decreased in IC but the 50% reduction goal was not reached. In 2007, dengue prevention activities continued with the development of a dengue prevention video and a second post-intervention evaluation. Results from this project will aid in determining the utility and limitations of community participation strategies for dengue prevention in Puerto Rico.

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CHANGES IN PATTERNS OF DENGUE TRANSMISSION IN A PEDIATRIC COHORT STUDY IN NICARAGUA

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A prospective cohort study was established in Managua, Nicaragua, in August of 2004 and currently consists of ~3,800 children 2-11 years of age in the catchment area of the Health Center Socrates Flores Vivas (HCSFV). Annual samples are collected at the beginning of the dengue season (July-August) each year, and detection of dengue occurs by enhanced passive surveillance through attendance at the HCSFV. Study compliance has been very high, with 93% of active participants having attended the HCSFV at least once when ill during the study period, 97% of possible dengue cases reporting to the HCSFV within the first 72 hours since onset of fever, 96% of possible dengue cases with convalescent samples, and case detection rates ~20-fold higher at the HCSFV than at all other health centers in Managua. From 2004 to 2006, the seroprevalence of anti-dengue virus (DEN) antibodies rose from 24% to 32% in the 2-year-old cohort and 90% to 94% in the 9-year-old cohort, with a rise in average positivity from 60% to 66%. A number of differences were noted between the first two study years. First, the incidence of primary and secondary DEN infection were both 7.3/100 persons in Year 1 (Y01) compared to 8.4 and 13.8/100 persons, respectively, in Y02. Second, the incidence of dengue cases based on the participants' barrio varied greatly between the two study years, not surprisingly, since dengue is a focal disease. Third, there were 2.7 times more symptomatic cases per DEN infection in Y02 than in Y01, and more confirmed dengue cases presented with undifferentiated fever as opposed to classic dengue symptomatology in Y02 as compared to Y01. Lastly, different DEN serotypes predominated, with more DEN2 in Y02, more secondary infections, and a higher mean age of symptomatic dengue cases. Interestingly, preliminary PRNT results reveal a large amount of heterotypic antibodies after primary DEN infection; these findings are being further investigated. New initiatives include screening of febrile cases with an identified focus other than dengue to unveil potential "masked" dengue cases and a dengue index-cluster study to capture acute asymptomatic cases. Thus, in addition to providing biological samples for vaccine safety research and a potential site for vaccine testing, this cohort study reveals changes in patterns of dengue transmission over time.

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VERY EFFECTIVE DENGUE VACCINES INCREASE THE INCIDENCE OF DENGUE HEMORRHAGIC FEVER IN NON-VACCINATED POPULATION: AN ISSUE OF MEDICAL ETHICS AND SOCIAL EQUITY

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Cross-immunity and/or clinical age-dependency generate a partially "negative" correlation between DHF incidence and transmission intensity (i.e. viral inoculation rate, mosquito abundance), as reported previously. This paradox materialized for dengue fever (DF): incidence of DF increased "because" mosquito control took effect. Since mosquito control is indispensable to protect the naive, vaccine is an emergent necessity. However, vaccination could also increase incidence. We simulated vaccination programs by Individual-based model. Four epitopes were hypothesized. Epitope 1 elicits life-long sterile immunity against homo-serotype and transient cross-immunity. Epitope 2 elicits life-long weak immunity against homo-serotype but is not cross-reactive. Epitope 3 elicits enhancing immunity. Hypothetical Epitope 4 elicits immunity which blocks enhancement. From these, four vaccines were developed. Strong "clean" vaccine (from epitope 1); strong but "dirty" vaccine (epitopes 1+3); weak vaccine (epitope 2), and enhancement-blocking vaccine (epitope 4). Dirty/clean denote whether the vaccine is enhancing or not. Mono-, bi-, tri- and tetra-valencies were also compared. If the pre-vaccination transmission intensity was very high, strong tri- or tetra-valent vaccines, clean or dirty, increased DHF incidence in non-vaccinated population, depending upon the coverage. This was because transmission intensity decreased. Weak vaccine did not cause this adverse increase much, although efficacy was limited. Enhancement-blocking vaccine was ideal, both in efficacy and adverse effects. In conclusion, our results raise a concern of medical ethics and social equity. Endpoint of a vaccine may not be reduction of viremia, but reduction of clinical manifestation of severe illness.

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PREDICTIVE VALUE OF CLINICAL FINDINGS FOR THE EARLY DIAGNOSIS OF DENGUE INFECTION

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Dengue, a mosquito-borne viral infection, is a leading cause of pediatric hospitalizations in the tropics. It is a growing public health threat throughout the Americas, where 500,000 dengue cases were reported in 2006. Timely diagnosis and supportive care can be life-saving, but may be challenging as initial symptoms are nonspecific and serological tests confirm dengue late in the course of illness. Methods: In the first phase of analysis, we reviewed surveillance data from all suspected dengue cases among children 5-15 years old that were evaluated in Patillas, Puerto Rico between June 2005 and May 2006. We analyzed demographic and clinical data from all laboratory-positive and laboratory-negative cases to identify distinguishing characteristics of dengue infection at initial presentation. Laboratory-positive cases were defined as patients with anti-dengue IgM positivity, IgM seroconversion, or dengue virus identified by polymerase chain reaction (PCR). Of the 341 suspected dengue cases, 38 (11%) were laboratory-positive and 121 (36%) laboratory-negative. Excluded from analysis were 182 (53%) laboratory-indeterminate patients. In univariate analysis, laboratory-positive patients were more likely to have rash ($p<0.01$), and were older than laboratory-negative patients ($p<0.05$). Laboratory-negative patients were more likely to report cough ($p<0.01$). The presence of rash in the absence of cough had a positive predictive value (PPV) of 100% (95% confidence interval [CI] 100-100) and a negative predictive value (NPV) of 82% (95% CI 71-87) in correctly identifying laboratory-positive dengue patients. In conclusion, these clinical markers may help identify dengue infection among children with suspected dengue in settings where rapid diagnostic tests are not available. Further study is needed to determine the utility of these markers when applied to populations with moderate dengue prevalence and where other febrile illnesses with rash may be more common. We plan to conduct similar analysis in adults with suspected dengue.

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DENGUE INFECTION IN BHUTAN

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No cases of dengue infection have been previously reported in Bhutan. Between July 24 and August 5, 2006, acute illness samples were collected during an outbreak of dengue-like illness from Phuntsholing in the south of the country. Thirty samples tested in country by the Dengue Duo IgM and IgG Rapid Strip (Panbio, Queensland, Australia) were positive. An additional sample did not undergo testing in country. These 31 samples underwent comfirmatory dengue testing at the Armed Forces Research Institute of Medical Sciences (Armed Forces Research Institute of the Medical Sciences) in Bangkok, Thailand. Of these 31 samples, 26 were positive for dengue by either RT-PCR/nested PCR or the Armed Forces Research Institute of the Medical Sciences in-house dengue IgM/IgG capture EIA. Twenty-three samples were positive by RT-PCR, all of which were dengue serotype 3. Eighteen samples were positive by EIA, of which 12 were determined to be primary infection and 6 were secondary infection. Mean age of the positive cases was 26.1 years old with a range of 7 to 50 years old. This is the first report of dengue infection in Bhutan. The number of primary infections and the advanced mean age suggest that dengue infection has only recently begun to circulate in Bhutan.

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DEVELOPMENT OF A DEN-2 PDK-53-BASED CHIMERIC TETRAVALENT VACCINE

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The live-attenuated candidate dengue type 2 (D2) PDK-53 vaccine has been shown to be safe and immunogenic, generating long-lasting neutralizing antibodies and cellular immune responses in human clinical trials. We are using D2 PDK-53 as the genetic backbone to engineer candidate chimeric vaccine viruses that express the structural genes of D1, D3 and D4. Previous work demonstrated that these viruses retained the distinctive attenuation markers of D2 PDK-53, and the tetravalent mixture of D2 and chimeric D2/1, D2/3 and D2/4 generated neutralizing antibody responses to all four dengue serotypes in mice and non-human primates. To complete development and clinical testing of these vaccine viruses, we have formed an international consortium consisting of scientists at InViragen, the Centers for Disease Control and Prevention, the University of Wisconsin and Shantha Biotechnics, an Indian vaccine manufacturer. GMP-quality seed stocks for each of the four vaccine viruses were rederived via transfection of certified Vero cells with viral genomic RNA transcribed from the original infectious cDNA clones. Following amplification of these seeds, all four rederived viruses contained the expected genomic sequences. The seed viruses were sequentially plaque-purified, and 24 isolates were spot sequenced to ensure retention of the three D2 PDK-53-specific attenuating mutations. Following complete genomic sequence analyses of 16 potentially suitable premaster viruses, a single premaster seed virus for each dengue serotype was chosen. The final, formulated D2-based tetravalent vaccine is being produced and tested for toxicity and efficacy in pivotal animal models. Human clinical testing of the D2-based vaccine will assess its safety, its ability to generate neutralizing antibody responses and ultimately its ability to protect against dengue fever. Development of an affordable, easily delivered, safe, and effective dengue vaccine will protect those most at risk of dengue, DSS, and DHF.

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A PHASE 1 CLINICAL TRIAL OF A DENGUE-1 DNA VACCINE: PRELIMINARY RESULTS

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DNA vaccines incorporating the dengue (DEN) pre-membrane and envelope genes were constructed, tested in non-human primates and shown to protect against live DEN virus challenge. Based on these studies, a Phase 1 clinical trial was initiated using a monovalent DEN-1 DNA vaccine candidate (D1ME100). The trial was designed as an open-label, dose-escalation, safety, and immunogenicity study in healthy adult subjects. The objectives were to establish the safety and immunogenicity of D1ME100 and to compare the antibody and T cell responses seen in non-human primates with the responses in humans. Twenty-two flavivirus-naive subjects were assigned to one of two groups. One group (n=10) was to receive three doses (0, 1, and 5 months) of D1ME100 at 1 mg per dose and the remaining 12 were to receive three high-dose (5 mg) immunizations. All injections were via the intramuscular (IM) route using the needle-free Biojector 2000. The immunization phase of the study is completed and safety follow-up visits are currently being conducted. Nine subjects in the low-dose group and 12 in the high-dose group received two or more immunizations. The most commonly reported symptom was local tenderness, which resolved in one to two days following vaccination. The most serious adverse events reported as related to the vaccine were moderate fatigue and local tenderness. The most frequent and severe laboratory abnormality was elevated serum creatinine phosphokinase levels, however all were transient and clinically asymptomatic. The analysis of anti-DEN-1 antibody responses is partially completed. At study day 168, anti-DEN ELISA IgG was detected in 7/9 subjects receiving the low-dose. In the high-dose group, 10/12 subjects developed ELISA IgG antibody and 2/12 had detectable neutralizing antibody after only two immunizations. Analysis of antibody and cellular immune responses after high-dose immunization #3 are pending. These preliminary results demonstrate a favorable reactogenicity and safety profile of the first DEN DNA vaccine evaluated in humans.

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PROTECTIVE EFFECT OF PRIMARY HETEROLOGOUS DENGUE VIRUS INFECTION IN A MOUSE MODEL FOR SECONDARY INFECTION

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Dengue fever and dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS) are caused by the four dengue viruses (DENV1-4), with tens of millions of people infected every year across tropical and sub-tropical regions of the world. The most significant risk factor for the potentially fatal DHF/DSS has been shown through epidemiologic studies to be a secondary, heterologous infection with a distinct DENV serotype. The long-term presence of both serotype-specific antibodies and memory T cells has been demonstrated in human cases; however, it remains unclear which elements of the immune response are responsible for protection versus immunopathology during secondary DENV infections. Using a mouse model of DENV infection, we are investigating the mechanism of both serotype-specific and heterotypic protective immunity using sequential virus infections, passive transfer of DENV-immune serum, and adoptive transfer of DENV-immune cells. 129/Pas mice lacking IFN-α/β and -γ receptors (AG129) were infected sequentially with DENV1

followed by DENV2 or DENV2 followed by DENV4 at intervals of 4, 15, ~30, and ~52 weeks. At 3-4 days after infection with the second serotype, spleen, lymph node, and bone marrow were harvested for detection of infectious virus. Prior infection with one DENV serotype was protective against heterologous challenge up to one year post-primary infection. Characterization of the antibody response to multiple DENV serotypes up to 6 months post-infection by ELISA and PRNT showed that heterotypic neutralizing antibodies were present. Passive transfer of DENV2- and DENV4-immune serum was shown to be protective against DENV2 challenge in a dose-dependent manner. Experiments employing adoptive transfer of immune cells are underway to determine the contribution of T cells to protection. Understanding the mechanism of homologous as well as heterologous protection in DENV infections will be essential in designing and evaluating an effective tetravalent vaccine.

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MECHANISMS OF THROMBOCYTOPENIA IN DENGUE VIRUS-INFECTED MICE

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Thrombocytopenia is a defining feature of dengue virus (DENV) infection in humans, and platelet depletion may contribute to the hemorrhagic and plasma leakage phenotypes of severe dengue disease. Many hypotheses have been advanced to explain the origins of thrombocytopenia in DENV infections, such as reduced platelet output due to disturbances in the bone marrow or increased platelet consumption in peripheral tissues due to coagulopathy or platelet-reactive antibodies. However, the limitations of human studies have prevented detailed analyses of the mechanisms of thrombocytopenia in dengue. Recently, DENV infection of interferon-α/β and -γ receptor knock-out mice (AG129 mice) has been shown to mimic many important features of human infection, including replication in monocyte/macrophages and dendritic cells in lymphatic tissues and a vascular leakage/early death phenotype at high dose. We here demonstrate that circulating platelets are progressively depleted following DENV infection of AG129 mice, coincident with virus replication in bone marrow. To examine the role of bone marrow infection, immunohistochemical identification of infected cell types and histopathologic examination of megakaryocytes in infected bone marrow are underway. Serum fibrinogen and activated partial thromboplastin time (aPTT) assays are being used to detect systemic coagulopathy, and the role of anti-DENV antibody in platelet depletion will be assessed by ELISA and other assays. These studies will shed light on the origins of thrombocytopenia, one of the hallmark features of dengue disease.

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CLINICAL CHARACTERIZATION AND ECONOMIC IMPACT OF THE DENGUE EPIDEMIC IN PANAMA IN THE YEAR 2005

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In 2005, Panama experienced the largest dengue epidemic since the reintroduction of dengue in 1993. Based on a representative sample of 130 confirmed dengue cases treated in ambulatory settings, this study performed the clinical characterization of dengue and estimated the total cost of the epidemic in Panama Province. After giving consent, participants were interviewed using a standardized questionnaire was to obtain data on demographic, clinical and socioeconomic aspects of the disease. Additionally, unit costs for medical care and total cost for vector control borne by the Ministry of Health (MOH) was obtained through interviews with health officials. Measurement of costs related to an illness episode included: a) direct costs (medical and non-medical) and b) indirect cost resultants of lost days of work, school, either others by the patient or other household members. Costs related to the vector (*Aedes aegypti*) control program included: a) personnel b) supplies, c) fuel and d) equipment. Costs are reported in 2005 US dollars. In this study, 82% of patients were aged 18 plus and 62% were women. Clinical features of the illness included: fever (91%), muscle pain (87%), rash (85%), and retro-orbital pain (76%). Duration of fever averaged 6.1 (SD 5.3) days and duration of illness averaged 21.2 (SD=13.5) days. Patients reported feeling either "bad" or "very bad" for 9.9 (SD=6.5) days with a loss in quality of life of 67% (SD=21.2) during the worst days of illness. The estimated cost of an illness episode averaged US$ 336 distributed among medical costs (19%), non-medical costs (6%) and indirect costs (75%). In 2005, 3937 dengue cases were reported but the MOH estimated a notification rate of 17%. This implies 23,622 cases occurred with a total cost of dengue illness of US$7.9 million. Additionally, the government spending on vector control activities in the study area was of US$ 1.4 million. In conclusion, the study found a remarkable disease and economic dengue burden during the epidemic year with a probable total cost of US$9.3 million (85% for illness and 15% for vector control).

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INEFFICACY OF THE TREATMENT WITH A HIGH DOSE OF INTRAVENOUS IMMUNOGLOBULIN ON SEVERE THROMBOCYTOPENIA IN PATIENTS WITH SECONDARY DENGUE VIRUS INFECTION

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Our recent data indicate that the formation of platelet-associated IgG (PAIgG) results in thrombocytopenia in patients with secondary dengue virus infection, as reported previously. This disease can be recognized as a dengue virus-induced idiopathic thrombocytopenic purpura (ITP). Since intravenous immunoglobulin (IVIG) is currently a widely accepted as a treatment option for ITP, the Fc γ receptor blockade by a high dose of IVIG, therefore, may inhibit the development of severe thrombocytopenia due to secondary dengue virus infection. A randomized control study was conducted to determine whether a high dose of IVIG is effective in inhibiting development of thrombocytopenia in patients with secondary dengue virus infection at San Larazo Hospital, Manila between October and December 2005. The inclusion criteria for these patients were 1) acute phase of dengue illness, 2) a low platelet count between 20,000 and 80,000/ul without prominent bleeding manifestation. 31 patients with secondary infection were randomly assigned to two treatment groups; 1) IVIG group (0.4 g/kg per day for three days) and 2) No IVIG group. While IVIG group involves 8 DF cases and 7 DHF cases, non IVIG group involves 9 DF cases and 7 DHF cases. The mean of age, the period from the onset of illness to the time of enrollment, platelet count on admission in IVIG and non-IVIG group were; 16.3 and 14.2 y.o., 3.9 and 4.1 days, 54.9 and 48.0 x 103 /μl, respectively. IVIG (Gammamune, Bayer Health Care) at a dose of 0.4 g/kg per day was given to a patient of IVIG group during 2 nd to 4 th day of admission. Since the nadirs of platelet counts were found around the admission day in both groups, we could evaluate the recovery phase of platelet. Despite the treatment with IVIG, no significant difference was found in the kinetics of platelet counts of patients in between IVIG and non-IVIG group during and after the treatment. No significant difference was similarly found in the kinetics of PAIgG in between IVIG and non-IVIG group. Results shown in this study suggest the blockade of Fc γ receptor by IVIG has no effect on the recovery of thrombocytopenia during the acute phase of secondary infection. Platelet clearance by macrophage through Fc γ receptor may not be a primary mechanism, but other immune mechanisms, such as platelet clearance by macrophage through complement receptor 3 or complement-mediated platelet lysis, may be operative in this disease.

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IL-5 LEVELS AND PAIN INTENSITY CORRELATED TO HIGH DENGUE 3 VIRAL LOADS

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Dengue is the most important disease caused by an arbovirus worldwide. After the introduction of serotype 3 in Brazil, the incidence of the disease, especially of its hemorrhagic forms, has substantiality increased, representing a serious public health problem in the country. Despite the large amount of studies, the mechanisms involved in the pathogenesis of dengue virus infections remain unclear. The present study has been conduced based on the following objectives: evaluate the correlation between viremia and the production of specific cytokines and the correlation between these variables with the severity of clinical and laboratorial findings. From February 2003 to May 2003, 80 patients with suspected dengue were submitted to physical examination and laboratorial evaluation. The first clinical evaluation of these patients was conducted between 2 to 19 days after symptoms onset (average: 7,1 days). From the total of patients included, 52 (65%) were confirmed to have dengue infections based either on RT-PCR detection of viral RNA or the presence of specific IgM antibodies detected by MAC-ELISA. Age and sex distribution were very similar between the two groups (dengue patients and other febrile disease patients. The polymerase chain reaction was positive in 20 of the 47 serologically confirmed samples; only serotype 3 was identified. Among the 40 IgM positive samples tested by real time RT-PCR, 17 were positive, with detection and quantification of dengue viruses serotype 3. Only three patients met the World Health Organization criteria for dengue hemorrhagic fever, and all of them had favorable clinical outcome. Serum levels of IL-5, IL-12 and TNF-α did not show correlation with the severity of clinical and laboratorial findings. There was not a significant difference between TNF-α serum levels in patients with or without confirmed laboratorial diagnosis. IL-5 serum levels were detected in a greater proportion in dengue-positive samples. Correlation was found between dengue viremia and only two variables: pain intensity, measured by the visual analogue scale, and IL-5 serum levels. The present data suggests a correlation between the production of Th-2 cytokines and higher viral loads. Further studies evaluating the correlation between other Th-2 mediators and viremia kinetics may be useful for a greater understanding of the immunopathological mechanisms of the disease.

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ASSESSING THE IMPACT OF VIRAL ASSAYS METHODS IN THE ESTIMATION OF INFECTION RATES FROM FIELD CAUGHT MOSQUITOES

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A common assumption in arboviral surveillance is that the risk of human infection is correlated linearly with estimated infection rates in mosquitoes taken from the field. This assumption requires a linear, positive relationship between true and estimated infection rates, but there is little information available to support these assumptions. Using sampling simulations we observed that variations in pool size and the number of infected mosquitoes in the pool can result in sigmoidal or logistic rather than linear associations. However, not only the number of infected mosquitoes but also the virus titer and the type of assay used can have an impact on the estimation of infection rates. Virus titers in the mosquito body are highly variable, and the point in the extrinsic incubation period at which the mosquito is collected will be an important factor determining this titer. Available assays are based on different principles like antigen presence, RNA segment detection, or presence of live virus capable of infecting cells, and these tests probably have different thresholds for virus detection. In this study we assume that if infected mosquitoes are present on a given pool, the titer of virus would follow a probabilistic normal distribution. We explore the consequences in estimation if the assays had either a threshold above which virus is detected in pools, or if the probability of detecting virus was a function of the titer. We use a modeling approach to study how different scenarios of mosquito sample sizes, true proportion of infected mosquitoes, test sensitivity, and pooling will affect estimates of infection calculated by either of two methods: the minimum infection rate (MIR) and the maximum likelihood estimator (MLE). A good understanding of how variations in all these factors affect our interpretation of surveillance data is critical in assessing the risk of arbovirus transmission to humans.

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SAFETY AND IMMUNOGENICITY OF CONCOMITANT VACCINATION WITH IC51 AND HEPATITIS A VACCINE IN HEALTHY SUBJECTS. A SINGLE-BLIND RANDOMIZED CONTROLLED PHASE 3 STUDY

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Vaccination against Japanese encephalitis (JE) is the most important measure to prevent infection among residents and visitors to endemic areas. IC51, a second-generation $Al(OH)_3$-adjuvanted vaccine based on the purified, inactivated JEV strain SA_{14}-14-2, is in late stage development by Intercell AG. The aim of this Phase 3 trial was to investigate potential influence on immunogenicity and safety of a concomitant vaccination of IC51 with another vaccine frequently given to travellers to endemic areas. The primary objective of this trial was to demonstrate non-inferiority of IC51+HAVRIX®1440 as compared to IC51+placebo in terms of GMT (geometric mean titer) at Day 56, and IC51+HAVRIX®1440 as compared to HAVRIX®1440+placebo in terms of GMT at Day 28. The study was a multi-center (3 sites, 2 countries), single-blind, randomized controlled Phase 3 trial. 192 healthy subjects received either two injections of IC51 (6 mcg, Days 0 and 28) and one injection of placebo (0.5 mL, Day 0), or two injections of placebo (0.5 mL, Days 0 and 28) and one injection of HAVRIX®1440 (1.0 mL, Day 0) or two injections of IC51 (6 mcg, Days 0 and 28) and one injection of HAVRIX®1440 (1.0 mL, Day 0). Immune response by determination of PRNT50 (plaque reduction neutralization test) titers and HAV (Hepatitis A Virus) titers was assessed 4 weeks after the last vaccination. GMT for anti-JEV neutralizing antibodies at Day 56 were 202.7 for subjects treated with IC51+HAVRIX®1440, and 192.2 for subjects receiving IC51+placebo. At Day 28, the GMT for HAV were 24.0 for IC51+HAVRIX®1440 and 21.7 for HAVRIX®1440+placebo.For both comparisons, non-inferiority was demonstrated, as the lower bound of the 95% CI for the GMT ratio was >0.5 in both cases (0.7541 and 0.8115, respectively). Co-vaccination of IC51 with HAVRIX®1440 showed a favorable safety and tolerability profile. In conclusion, this Phase 3 study demonstrated that IC51 can be safely administered concomitantly with a Hepatitis A vaccine, with no negative effects on safety or immunogenicity of either vaccine.

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MOLECULAR CHARACTERIZATION OF ENVELOPE GENE FOR HUMAN FATAL AND NON-FATAL YELLOW FEVER ISOLATES: DETECTION OF SPECIFIC MUTATION AT POSITIONS E147 AND E154

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Yellow fever virus (YFV) is the prototype species in the genus *Flavivirus* (family *Flaviviridae*). Flaviviruses are characterized by a RNA with 11 000 bp which when cleaved generate 10 genes (3 structural C-PrM/M-E and 7 non-structural NS1-NS2a-NS2b-NS3-NS4a-NS4b-NS5). The structural region is very important, among other functional activities, to virus attachment, replication, and induction of immune response. This study was undertaken to perform nucleotide sequencing of structural genomic region of YFV isolates obtained in Brazil. Four yellow fever isolates obtained in Brazil between 1980 and 2004 from human (two fatal and two non-fatal) were growth in VERO cells and studied at their structural genes by direct nucleotide sequencing. A genotype II isolate from Rondônia state showed two amino acids substitution in the Envelope protein [positions E-147 (T>A) and E-154 (T>A)], while the other three genotype I isolates did not show these substitutions, neither the Asibi nor the parental 17DD vaccine strain that showed amino acid A in both E-147 and E-154 positions. In conclusion, as E protein is linked to the YFV attachment and the host immune response, these mutations could play a role in the phenotype of disease and YFV evolution. Experiments using animal models should be done to test this hypothesis.

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CHARACTERIZATION OF ANTIGENIC CHIMERIC ST. LOUIS ENCEPHALITIS VIRUS/DENGUE VIRUS TYPE 4 RECOMBINANT VIRUSES IN MICE AND MONKEYS

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St. Louis encephalitis virus (SLEV) is a mosquito-borne flavivirus that is endemic in the Americas and causes sporadic outbreaks of disease in humans. We previously developed a WNV/DEN4Δ30 antigenic chimeric virus as a live attenuated virus vaccine candidate for West Nile virus (WNV) that contains the WNV M and E proteins on a dengue virus type 4 (DEN4) backbone with a 30 nucleotide deletion (Δ30) in the DEN4 3′ UTR. Using a similar strategy, we sought to generate an antigenic chimeric SLE/DEN4, which could potentially be combined with WNV/DEN4Δ30 in a bivalent vaccine to maximize protection against the mosquito-borne flaviviruses in the Americas. The M and E genes of SLEV Hubbard were introduced into the cDNA clones of rDEN4 or rDEN4Δ30 and viruses were recovered which replicated to greater then 6.5 $\log_{10}$PFU/ml in Vero cells and designated SLE/DEN4 and SLE/DEN4Δ30, respectively. Neuroinvasiveness of the chimeric viruses was compared to that of wild-type (wt) SLEV following i.p. injection of Swiss Webster mice or SCID mice. In Swiss mice, SLE/DEN4 and SLE/DEN4Δ30 lacked detectable neuroinvasiveness, and they were at least 10^5-fold less neuroinvasive than wt SLEV in SCID mice. The neurovirulence of SLE/DEN4 and SLE/DEN4Δ30 was found to be comparable to wt SLEV upon i.c. infection of Swiss suckling mice. In rhesus monkeys following a single s.c. dose of 10^5 PFU, wild-type SLEV replicated to greater than 2.0 $\log_{10}$PFU/ml serum and achieved mean neutralizing Ab titers of 1:66. SLE/DEN4 was only slightly attenuated in rhesus monkeys with a viremia of 1.3 $\log_{10}$PFU/ml and induced neutralizing Ab at levels comparable to wt SLEV. However, the presence of the Δ30 mutation significantly restricted replication in rhesus monkeys since each of 4 monkeys lacked signs of infection after inoculation with 10^5 PFU SLE/DEN4Δ30. These results indicate that chimerization of SLE with DEN4Δ30 decreased neuroinvasiveness in mice, did not affect neurovirulence in mice, and overattenuated the virus for monkeys. Studies to address these findings are in progress.

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ASSAY FOR AND REPLICATION OF KARSHI (MAMMALIAN TICK-BORNE FLAVIVIRUS GROUP) VIRUS IN MICE

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Karshi virus (genus *Flavivirus*, family *Flaviviridae*) is a member of the mammalian tick-borne flavivirus group, previously known as the "tick-borne encephalitis virus serocomplex." Karshi virus is not considered to be highly pathogenic for humans; however, severe disease, including encephalitis, has been reported in a few individuals, and large outbreaks of febrile illness associated with infection with Karshi virus have been observed in Uzbekistan. Recent studies have also shown that argasid ticks in the genus *Ornithodoros* are competent vectors of Karshi virus. However, little is known about the replication of Karshi virus in its rodent hosts. Therefore, we developed a novel, quantitative, real-time RT-PCR assay and measured the amount of viral RNA in selected tissues of infected Swiss Webster mice. Two-day-old mice were highly susceptible, with 100% fatality between days 9 and 12 after infection, while 9-day-old mice were less susceptible, with death occurring only rarely. In nearly all cases, mice inoculated when 2-days old contained similar numbers of viral genome equivalents from blood and liver samples from any given mouse, with titers declining after day 7. In contrast, the amount of viral RNA in the brain began to rise rapidly 4 days after exposure, peaked at about 6 days after viral exposure with a titer of $>10^{13}$ genome equivalents/g, and remained at that level until euthanasia or death. Viral profiles were similar in needle-inoculated or tick-exposed mice. These data support the assumption of neurotropism by this virus, and suggest that further investigation into the pathogenicity of Karshi virus in humans is warranted.

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DEMONSTRATION OF RNA RECOMBINATION IN JAPANESE ENCEPHALITIS VIRUS

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Due to instability of genonic RNA, accumulated mutations are known to be a force driving viral evolution in the genus *Flavivirus*, including the Japanese encephalitis (JE) virus. Based on sequencing data, RNA recombination was recently postulated to be another factor associated with genomic variations in these viruses. We herein provide experimental evidence to demonstrate the occurrence of RNA recombination in the JE virus using 2 local pure clones (T1P1-S1 and CJN-S1) respectively derived from local strains T1P1 and CJN. Based on results from the restriction fragment length polymorphism (RFLP) assay on the C/preM junction comprising a fragment of 868 nucleotides (nt 10~877), the recombinant progeny virus was formed primarily in BHK-21 cells that had been co-infected by the 2 clones used in this study. Nine of 20 recombinant forms of the JE virus had a crossover at the region of nt 123~323. Sequencing data derived from these recombinants revealed that no nucleotide deletion or insertion had occurred in this region favoring crossovers, indicating that precisely but not aberrantly homologous recombination was involved. Since rates of recombination decreased after 1 of 3 stem-loops in the corresponding region of the viral genome was destabilized, the secondary structure may be important in modulating RNA recombination.

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ENZYME-LINKED IMMUNOSORBENT ASSAY USING CROSS-REACTIVITY REDUCED VIRUS-LIKE PARTICLES TO DETECT ANTIBODIES AGAINST JAPANESE ENCEPHALITIS VIRUS

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The cross-reactive antibodies, induced by flavivirus infections, create problems for serodiagnosis and pathogenesis, especially in secondary infections caused by antigenically closely related flaviviruses. The highly conserved envelope (E) glycoprotein fusion peptide residues Gly104, Gly106, and Leu107, constitute immunodominant flavivirus cross-reactive antigenic determinants. Using recombinant plasmid expressing Japanese encephalitis (JE) virus-like particles (VLPs), we altered and expressed fusion peptide mutated JEV VLPs that produced dramatic reactivity reductions against a diverse panel of flavivirus cross-reactive murine monoclonal antibodies, in particular for G106K and L107D (KD) double-mutant antigen. Panels of human sera were assembled from patients having recent flavivirus or non-flavivirus infections to assess the specificity and sensitivity of the JEV wild-type (WT) and cross-reactivity reduced KD VLPs in IgM and IgG ELISA. Receiver operating characteristic (ROC) analysis indicated that there were significant differences in assay performance between WT and cross-reactivity reduced KD antigens. ELISAs with either antigen exhibited comparable sensitivities for the detection of IgM and IgG antibodies against JEV. Importantly, we observed higher specificity, positive predictive values (PPV) and likelihood ratios (LR) using the cross-reactivity reduced KD antigen than with the WT antigen. These results provide insight into the antigenic structure of the flaviviral E protein and support the continued development of novel, species-specific diagnostic antigens that should improve both flavivirus serodiagnosis and estimates of disease burden.

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EVALUATION OF VIRUS ISOLATION TECHNIQUES FOR JAPANESE ENCEPHALITIS

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Virus isolation is the gold standard for identification of etiology of virus infection, as it is highly specific. However, due to the low-level, transient viremia seen in patients presenting to hospitals with arthropod borne virus (arbovirus) infections such as Japanese encephalitis virus (JEV) virus isolation has low sensitivity and is not practical for diagnosis. Development of new techniques for arbovirus isolation is an active field of research. To enhance sensitivity of cell culture isolation for JEV, we have developed a centrifugation shell vial assay in both Vero and PK15 cells. This method was compared to traditional T-25 flask virus isolation in both cell types. Outcomes of the assay were measured as day of presentation of cytopathic effect (CPE) and detection of virus nucleic acid by real-time RT-PCR and plaque assay. CPE results show that the PK15 cells in shell vials are more sensitive to JEV infection, and can be used as a rapid test for infection, because CPE is present within 24 hours.

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SEROLOGICAL EVIDENCE OF POWASSAN VIRUS TRANSMISSION IN SMALL MAMMALS COLLECTED IN RUSSIA, ALASKA AND THE WESTERN UNITED STATES

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Powassan virus (POW), the sole North American representative of the tick-borne encephalitis (TBE) serogroup of the flaviviruses, appears to derive from a Eurasian progenitor that was introduced into North America via the Bering land bridge. In North America, POW has diversified into at least two genetic lineages that perpetuate in distinct transmission cycles involving ticks and small to medium sized mammals. In regions of the United States and Canada, the virus is an emerging cause of human morbidity and mortality. Although ample evidence has been presented for enzootic transmission and concomitant human disease in Southern Canada and the Northeastern United States, there is limited information on POW perpetuation in the Beringian region. We therefore sought to determine whether POW or a related flavivirus perpetuates in Russia (Siberia), Alaska and the Western United States by screening small mammals for serologic and molecular evidence of infection. Small mammals were collected in collaborative efforts with the Museum of Southwestern Biology (MSB). Serological evidence of exposure to POW was detected by screening blood, serum or liver translucidates using a recombinant POW envelope (E) protein in a strip immunoblot assay (SIA) format. 408 individuals, representing 21 species were tested. Two of 21 species were seropositive by SIA. The only species collected in Beringia that was seropositive was *Myodes rutilus*. Six percent of *M. rutilus* (14/243 from Alaska and 2/31 from Russia) were seropositive. 28% of *Peromyscus truei* collected in the Western US were seropositive. Thus far, viral RNA has not been detected in any of 34 samples tested by RT-PCR. Serological and molecular testing of additional specimens are ongoing. These results suggest that POW or a related flavivirus perpetuates in an enzootic transmission cycle involving *M. rutilus* along the central coast of Alaska, and in a cycle involving *P. truei* elsewhere in western North America.

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POTENTIAL FOR INTRODUCTION AND ESTABLISHMENT OF JAPANESE ENCEPHALITIS VIRUS INTO NORTH AMERICA

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Japanese Encephalitis (JE) is an emerging arboviral disease that threatens public health and agriculture. JE expanded west across Asia with the Green Revolution, and recently into Australia and West Pacific. JE has not been introduced into North America. Like West Nile virus, JE has a broad range of vectors and reservoirs, making enzootic establishment possible. This study analyzes existing data on commerce, travel, avian migrations, exotic mosquito introductions, and mosquito vector competence to determine the most likely routes and locations of introduction. It is recommended that surveillance be conducted in coordination with WNV surveillance in key ports and the Pacific Northwest. Avian and mosquito surveillance should be conducted along the Trans-Pacific avian flyway, possibly with existing avian influenza surveillance. Vector and reservoir competence studies are required to focus control efforts and prevent establishment. Greater cooperation between commerce, public health, and veterinary health agencies is needed to prevent introduction and establishment of emerging zoonoses such as JEV.

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DIFFERENT PATTERN OF LIVER LESIONS IN GOLDEN HAMSTERS FOR YELLOW FEVER FATAL AND NON-FATAL HUMAN ISOLATES

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Recently the golden hamsters (*Mesocricetus auratus*) have been established as a simple, efficient and cost-beneficial model to experimental studies of yellow fever. Objective: To perform experimental studies with yellow fever virus isolates from fatal and non-fatal cases, in order to compare the pathogenesis in the golden hamsters. Four yellow fever isolates obtained in Brazil between 1980 and 2004 from human (two fatal and two non-fatal) were growth in VERO cells and infected in golden hamsters using a dose of $10^{3.0}$ LD_{50}/ml. Infected and control animals were sacrificed at 24h intervals for 1 to 10 days, and 21 days post inoculation when experiment was ended. Viscera fragments were paraffin embedded and hematoxilin and eosin slides were semi-quantified for the presence of necrosis, apoptosis, steatosis, and intensity of tissue lesion, as well as qualitatively analyzed for the presence of specific yellow fever antigens by immunocytochemical assay. In the infected hamsters, liver lesions for a genotype II isolate from a fatal yellow fever case and other two isolates of both South American genotype I (one fatal and other severe non-fatal yellow fever case) showed to be more intense than for a non-fatal strain (genotype I) obtained from a mild yellow fever case, whose infected hamsters showed scarce and mild grade of liver lesion. In conclusion, our results showed that fatal and severe yellow fever isolates were more pathogenic for golden hamster than a non-fatal strain. Further experiments with other yellow fever virus strains from fatal and non-fatal human cases should be performed to confirm these findings.

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DETERMINATION OF IMMOBILIZATION AND LETHAL DOSES (MG/ML) OF ERYNGIAL (TRANS-2-DODECENAL), USING *STRONGYLOIDES STERCORALIS, HAEMONCHUS CONTORTUS, ANCYLOSTOMA CANINUM* AND *PARASTRONGYLOIDES TRICHOSURI* INFECTIVE LARVAE *IN VITRO*, AND A COMPARISON OF ITS ANTHELMINTIC ACTIVITY WITH IVERMECTIN

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The anthelmintic activity of eryngial (trans-2-dodecenal), a plant compound from *Eryngium foetidum*, was investigated *in vitro* using *Strongyloides stercoralis*, *Parastrongyloides trichosuri*, *Haemonchus contortus* and *Ancylostoma caninum* infective larvae. The 24-hr 50% immobilization doses (ID_{50}) of eryngial (mg/ml) using *S. stercoralis*, *P. trichosuri*, *H. contortus* and *A. caninum* infective larvae were 0.058 (0.054 - 0.061), 0.013 (0.007 - 0.030), 0.132 (0.078 - 0.186) and 0.545 (0.440 - 0.641), while the 24-hr 90% immobilization doses (ID_{90}) were 0.081 (0.076 - 0.088), 0.092 (0.051 - 0.201), 0.782 (0.641 - 0.998) and 0.867 (0.717 - 1.445), respectively. Using *S. stercoralis* L3 larvae, the 48 hr ID_{50} and ID_{90} values for eryngial were 0.054 (0.047 - 0.061) and 0.077 (0.066 - 0.101), while for ivermectin, the values were 0.136 (0.114 - 0.157) and 0.297 (0.251 - 0.377), respectively. Using *H. contortus* L3 larvae, the 48 hr ID_{50} and ID_{90} values for eryngial were 0.313 (0.143 - 0.453) and 0.761 (0.530 - 1.488), while for ivermectin, the values were 0.011 (0.001 - 0.022) and 0.059 (0.043 - 0.092), respectively. The 24-hr 50% lethal doses (LD_{50}) for eryngial using *S. stercoralis*, *P. trichosuri*, *A. caninum* and *H. contortus* were 0.085 (0.073 - 0.102), 0.105 (0.084 - 0.133), 0.608 (0.429 - 0.765) and 1.793 (1.516 - 2.558), while the 24-hr 90% lethal doses (LD_{90}) were 0.143 (0.115 - 0.245), 0.297 (0.214 - 0.515), 1.178 (0.913 - 2.100) and 2.765 (2.010 - 5.915), respectively. Using *S. stercoralis* L3 larvae, the 48 hr LD_{50} and LD_{90} values for eryngial were 0.069 (0.058 - 0.085) and 0.105 (0.085 - 0.174), while for ivermectin, the values were 0.306 (0.203 - 0.442) and 1.038 (0.665 - 2.498), respectively. Using *H. contortus* L3 larvae, the 48 hr LD_{50} and LD_{90} values for eryngial were 1.361 (1.284 - 1.449) and 2.360 (2.109 - 2.768), while for ivermectin, the values were 4.990 (4.572 - 5.954) and 6.949 (5.858 - 10.10), respectively. The results demonstrate anthelmintic activity of eryngial, which compares favorably with ivermectin *in vitro*.

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DEVELOPMENT OF DNA ASSAYS, IN SOIL-TRANSMITTED NEMATODE PARASITES OF HUMANS, FOR THE DETECTION OF SINGLE NUCLEOTIDE POLYMORPHISMS (SNPS) ASSOCIATED WITH BENZIMIDAZOLE RESISTANCE

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The Focussing Resources on Effective School Health (FRESH) Partnership has been implemented to remove soil-transmitted nematodes from school-aged children by using single annual doses of either albendazole or mebendazole. There is concern that increased use of anthelmintics in children could select for resistant populations of these human parasites. In human filarial nematodes, a single nucleotide polymorphism (SNP) which causes an amino acid substitution from phenylalanine to tyrosine in parasite β-tubulin at position 200, associated with benzimidazole resistance in other nematodes, has been detected. We have developed Pyrosequencer assays for determination of the codon 200 in β-tubulin in *Trichuris trichiura* and *Ascaris lumbricoides*, to screen for this SNP. These assays are being applied to eggs, larvae and adult worms from patients in order to determine whether repeated treatment with albendazole or mebendazole changes the frequency of β-tubulin alleles and selects for this resistance-associated SNP in these human soil-transmitted nematodes.

(ACMCIP Abstract)

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NATIONAL SEROPREVALENCE AND RISK FACTORS FOR ZOONOTIC *TOXOCARA* SPP. INFECTION

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Larval stages of *Toxocara canis* and *Toxocara cati*, common roundworms of dogs and cats, respectively, frequently infect humans worldwide. However, many people infected with *Toxocara* spp. remain asymptomatic throughout the duration of the infection. To estimate the prevalence of *Toxocara* spp. infection in a representative sample of the United States population, sera from participants ≥6 years of age in the Third National Health and Nutrition Examination Survey (1988-94) were tested for antibodies to *Toxocara*. Among persons tested (n=20,395), age adjusted seroprevalence was 13.9% (95% confidence limits [CL] 12.5, 15.3). Seroprevalence was higher in males (15.6%; 95% CL 13.8, 17.5) than females (12.4%; 95% CL 10.9, 13.8), and higher in non-Hispanic blacks (21.2%; 95% CL: 19.7, 22.8) than non-Hispanic whites (12.0%; 95% CL 10.2, 13.8) or Mexican Americans (10.7%; 95% CL 9.5, 11.9). Seroprevalence was 22.2% (95% CL 18.1, 26.2) among the foreign born population compared to 12.7% (95% CL 11.2, 14.2) for those born in the U.S., higher among persons living below poverty (22.9%; 95%

CL 19.7, 26.2) than those living at or above the poverty level (12.3%; 95% CL 10.9, 13.6), and higher among persons with elevated blood lead levels (26.9%; 95% CL 21.0, 32.8) compared to those with normal blood lead concentrations (13.5%; 95% CL 12.0, 15.0). Seroprevalence was 21.8% (95% CL 19.1, 24.5) for persons with only some high school education, decreased to 14.1% (95% CL 12.3, 15.9) with a high school education, and was lowest (9%; 95% CL 7.8, 10.3) for those with some college education (p-values <0.001 for all comparisons listed above). In multivariate analyses, dog ownership was associated with *Toxocara* seropositivity (OR 1.22; 95% CL 1.05, 1.40). Prevention efforts such as hand washing after soil contact, prevention of soil contamination in public areas by dog and cat feces, and anti-helminthic treatment of puppies and kittens may minimize exposure to *Toxocara* spp.

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DEVELOPMENT OF A RAPID AND SPECIFIC IMMUNODIAGNOSTIC ASSAY FOR *STRONGYLOIDES* INFECTION USING A LUCIFERASE IMMUNOPRECIPITATION SYSTEM

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Serological approaches to the diagnosis of *Strongyloides stercoralis* (Ss) infection have been hampered by poor specificity, their reliance on crude parasite extracts, and the time needed to perform the assays. To develop a more rapid, specific and standardized assay, we have developed a luciferase immunoprecipitation systems (LIPS) assay based on antibody to a strongyloides-specific (NIE) recombinant antigen and compared it to a standard ELISA using NIE. By ELISA, 96.7% of 31 patients with parasitologically proven Ss infection had antibodies to NIE antigen compared to 2.8 % of 35 sera from normal, uninfected controls giving a calculated sensitivity and specificity for the NIE ELISA of 97 and 98%, respectively. For the same group of patients, the LIPS assay demonstrated a sensitivity and specificity of 100%. The difference between the highest normal value and the lowest strongyloides titer was 10759 Luminescense Units (LU). There was a good correlation between the values obtained by LIPS and by ELISA (R^2= 0.810, p value < 0.0001). Sera from 39 expatriate patients with filarial infections (*Onchocerca volvulus and Loa loa*) and negative stool examination for Ss were selected to examine crossreactivity of both assays. By NIE ELISA, 87.2% of filarial infected patients were 'positive' using our cutoff of 150 U ml^{-1}. By contrast, LIPS effectively distinguished between filarial infected and Ss infected patient sera, with the highest LIPS value for a filarial infected patient (5804 LU) falling below the lowest value for Ss-infected patients (7292 LU). In conclusion, LIPS using NIE recombinant antigen can accurately and rapidly identify sera from patients with Ss infection and can easily distinguish between patients infected with strongyloides and other infections that typically cross react in ELISA-based assays.

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PCR ASSAY FOR THE DETECTION OF *ANGIOSTRONGYLUS COSTARICENSIS* DNA

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Angiostrongylus costaricensis is a parasitic nematode of rodents that occasionally infects human beings causing a disease called abdominal angiostrongyliasis. In complicated cases, abdominal angiostrongyliasis can result in death due to occlusion or perforation of the intestine. Diagnosis of this illness is made upon the finding of the parasite in tissue samples, but most of the time is based solely on clinical symptoms that can be confused with other conditions. Because of the absence of a specific diagnostic tool, no data is available on the incidence of this disease or the population that is most affected by it. However, for many years it has been suggested that abdominal angiostrongyliasis is primarily a disease that affects children as a result of the first described cases in Costa Rica. The goal of this project is to develop a PCR assay that can be used for detection of *A. costaricensis* DNA based on the amplification of a highly repetitive sequence within the parasite's genome. We have identified a repeated sequence of 170 bp in length. Based on this sequence, we designed primers that can be used to amplify small quantities of *A. costaricensis* DNA using PCR. This PCR assay should prove very useful in the early diagnosis of abdominal angiostrongyliasis in humans. A more specific test for the diagnosis of abdominal angiostrongyliasis will help elucidate the dynamics of the disease in terms of geographic distribution, prevalence target population and risk factors. Hopefully a better understanding of this disease will translate into the development of effective treatments, preventive measures and appropriate patient care.

(ACMCIP Abstract)

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MODULATION OF THE INNATE AND ACQUIRED IMMUNE RESPONSE IN THE MICE REINFECTED WITH *STRONGYLOIDES VENEZUELENSIS*

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It is clear that helminth infections induce immune responses which are characterized by production of eosinophilia, Th_2-associated cytokine, and antibodies. The aim of this study was to investigate the participation of innate and acquired immune response in the Balb/c mice infected and reinfected with *Strongyloides venezuelensis*. On the 2nd and 7th day post-infection, the mice were killed and eosinophil (EO), mononuclear cells (MC), and neutrophil (NE) numbers were count in the blood, peritoneal cavity fluid (PCF), and broncoalveolar fluid (BALF). As well as, the number of larvae, worm, specific IgE, IgG1, IgG2a antibodies, IL-3, IL-4, IL-5, IL-10, IL-12, IL-13, and IFN-γ cytokine levels were measured. Memory cells, CD4+ T, and CD8+ lymphocytes were identified. Uninfected mice were used as control. The EO and MC numbers increased in the infected mice in the blood, PCF and BALF, when compared with uninfected mice, but NE was increased only in the blood. Memory cells and CD4+ T lymphocytes were significantly increased in the PCF and BALF after second infection. IgE and IgG1 levels increased significantly after reinfection. Nevertheless, IgG2α only increased significantly on the 2 day after 2nd reinfection. However, IL-3, IL-4, IL-5, IL-10, and IL-13 level were high on the 2nd day after reinfection, IL-12 was high on the 7th day of the reinfection, and IFN-γ was increased on the 2nd day post-infection. The number of larvae and recovered worm decreased after reinfection, and those were completely eliminated after a 2nd one. Our data show high immune response of the Th_2 pattern in the mice reinfected with *S. venezuelensis* and it is protective after second reinfection.

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HOUSING CONDITIONS AND SURVIVAL OF PEOPLE WITH HIV INFECTION IN THE DOMINICAN REPUBLIC

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Household conditions can affect health. Building materials, household hygiene, and factors such as cooking fuel and kitchen ventilation can be

associated with disease. This investigation examined whether there was an association between household conditions and all-cause mortality for HIV-infected people. Completion of a Dominican public health household survey that rated 13 separate characteristics (including construction materials, water security, and hygiene questions) was one component of home evaluations for families inscribed into an HIV/AIDS services project. 184 HIV-infected people were enrolled over 18 months (Apr 2005-Sep 2006); 134 of these HIV-infected people (73%) had household surveys completed (many people declined a home visit because of fear that it might compromise the confidentiality of their HIV status). Follow-up was available to a maximum of 23 months and one person was lost to follow-up. There was no association between any of the housing characteristics and all-cause mortality for the HIV-infected people (30 deaths). However, this project occurred during roll-out of HIV therapy in the Dominican Republic. During the first five months of this project, there was no reliable local source of antiretroviral therapy. For the subset of HIV-infected people inscribed during the first five months of the project (n = 51), there was a relationship between deaths and having animals ("Dog, cat, pig, others") in the house (p = 0.05, Fisher exact test). Comparing people inscribed in the first five months (n = 51) to those from the subsequent 13 months (n = 133), there was a difference in the probability of survival favoring those entering after antiretroviral therapy was available (p = 0.03, logrank test for censured data). With the exception of animals in the homes of the sub-group enrolled before antiretroviral therapy was available, household characteristics had no association with mortality in this small group of HIV-infected people. Availability of antiretroviral therapy at time of inscription was associated with an increased probability of survival. As antiretroviral therapy becomes more available in mid- and low-income countries, emphasis on providing antiretroviral therapy and home-based interventions in support of that therapy (such as adherence and nutrition services) may produce more impact than interventions aimed at improving the household environment.

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THE SIX SYSTEMIC COMPONENTS FOR DEVELOPMENT AND IMPLEMENTATION OF SUCCESSFUL INTERNATIONAL PUBLIC HEALTH REGIMES

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Despite the plethora of functioning international and multi-national public health programs, little systematic research has been done which explores the necessary and sufficient components of successful international public health regimes. This research explores public health programming holistically in an attempt to determine which of the components are common among the most successful public heath regimes and which are lacking among those which have failed. The research identifies six necessary components for successful international public health regimes which are grouping into two categories: the considerations, and the operative levels. The considerations include three units. Science - does the proposed regime have its base in the results of legitimate scientific research? Programming - have the specific programs been designed to minimize costs and maximize the prospect of success? And policy - have political and administrative routines been established which support programs which are based on legitimate science? The second category, operative levels discusses the three levels at which continuity of purpose must be maintained. These include national governments, Non-Governmental Organizations (NGOs)/ Inter-governmental Organizations (IGOs), which are discussed concurrently, and field level workers. Examples are drawn from HIV/AIDS regimes, Polio campaigns, and anti-malaria programs.

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IDENTIFICATION AND CHARACTERIZATION OF TWO 14-3-3 PROTEINS IN THE HUMAN PARASITE *TRYPANOSOMA CRUZI*

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The protozoan parasite *Trypanosoma cruzi* is the causative agent of Chagas Disease- a serious illness that leads to potentially fatal heart or digestive pathology in up to one-third of cases. The World Health Organization estimates that 16-18 million people in Latin America are currently infected and another 100 million people are at risk of infection. Although diagnostic tests have been developed, there is currently no effective drug to treat chronic infections. This is due in part to the fact that little is understood about the complex and unique molecular biology of the parasite and the pathways and events associated with host cell invasion. Because the *T. cruzi* genome was recently sequenced and reporter constructs are now available, it is possible to use molecular biology techniques to study this organism. *Trypanosoma cruzi* has a complex life cycle involving four distinct life stages. The parasite must differentiate among these stages in response to external environmental cues depending on whether it is infecting a mammalian host or an insect vector and whether it is intracellular or extracellular. One family of proteins that is known to function in a wide variety of cellular processes in eukaryotes is the 14-3-3 family. These proteins are signaling modulatory molecules that may be involved in crosstalk among diverse signaling cascades. We feel that studying the function of 14-3-3 proteins in *T. cruzi* will lead to a better understanding of how the parasite adapts to its host environments. We have identified two 14-3-3 genes in *T. cruzi* (Genbank Accession Numbers AY672992, AY672993) and labeled their corresponding proteins with a fluorescent tag to study localization of each isoform *in vivo*. At least one of the two isoforms does localize to a specific compartment within the cell. More detailed studies should elucidate the exact location of the labeled protein and, by inference, hint at what roles the protein plays in the cell. Pull down assays to determine the potential binding partners of each 14-3-3 isoform will further elucidate in which pathways each isoform plays a role. Because there is a strong homology among the 14-3-3 proteins of *T. cruzi* and other medically important parasites, a better understanding of the roles 14-3-3 plays in *T.cruzi* could potentially lead to the development of broad spectrum anti-parasitic therapies.

(ACMCIP Abstract)

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ANTIGEN DISCOVERY FOR CONTROL OF VISCERAL LEISHMANIASIS

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Visceral leishmaniasis (VL) is the most severe form of leishmaniasis and is often fatal unless properly treated. Thus, development of accurate diagnostic tests and effective vaccine may play a very important role in control of the disease. We have identified a number of antigens with serological significance from parasites of the *Leishmania donovani* complex by a conventional serological screening and a novel bioinformatic screening based on searching for genes containing tandem repeat (TR) domains. Some of these antigens were further evaluated the potential for diagnostic use and were demonstrated the compensative potency for the current leading diagnostic antigen rK39. Next, using a mouse model we evaluated potency as a vaccine antigen of sterol 24-c-methyltransferase (SMT), which is an enzyme involved in biosynthesis of ergosterol in protozoa, fungi and plants. C57BL/6 mice immunized with SMT formulated in an adjuvant MPL-SE showed Ag-specific Th1 immune responses characterized by robust production of IFN-γ upon specific Ag re-exposure *in vitro*. Upon challenge with *L. infantum*, mice immunized

with SMT plus MPL®-SE showed significant lower parasite burdens in both spleens and livers compared with non-immunized mice or mice injected with adjuvant alone. The results indicate that SMT can be a safe and effective vaccine candidate against VL.

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ROLE OF MAP KINASE ERK IN ALTERING DENDRITIC CELL MATURATION AND CELL-MEDIATED IMMUNE RESPONSE TO *LEISHMANIA AMAZONENSIS* INFECTION

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Leishmaniasis is a group of diseases caused by obligate intracellular protozoan parasites of the genus *Leishmania*. Protection from disease requires the early establishment of a T helper 1 (T_H1) response characterized by production of interleukin (IL)-12 and the activation of interferon (IFN)-γ-producing $CD4^+$ T cells. *L. amazonensis* infection induces an ineffective, non-polarized immune response that leads to chronic disease. Dendritic cells (DCs) play a pivotal role in the induction of immune responses. Pathogen-mediated regulation of DC maturation can therefore be detrimental to the host. Mitogen-activated protein kinase (MAPK) pathways play essential roles in both innate and adaptive immunity as both initiators and regulators. Phosphorylation of MAP kinase ERK1/2 is associated with down-regulation of immune responses as well as a negative regulator of DC maturation. In our model of cutaneous leishmaniasis, study of bone marrow-derived DCs (BMDCs) has indicated that *L. amazonensis* infection modulates the maturation of these cells by suppressing both IL-12p40 production and CD40 cell surface expression via increased ERK1/2 phosphorylation. We have shown that *in vitro* inhibition of ERK1/2 phosphorylation can restore DC maturation. We sought to investigate the role of ERK1/2 during *in vivo* infection using an orally available MEK inhibitor, CI-1040. Treatment of *L. amazonensis*-infected mice with CI-1040 leads to a decrease in both lesion progression and parasite load in the infected footpad. Moreover, ERK1/2 inhibition resulted in enhanced IFN-γ production in recall responses of total draining lymph nodes of *L. amazonensis*-infected mice, suggesting the presence of a productive adaptive immune response. These findings reveal the important role of ERK during *L. amazonensis* infection which provides an excellent target for the development of therapeutic treatments using safe, orally available, ERK inhibitors.

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THE ROLE OF B CELLS IN THE CELL-MEDIATED IMMUNE RESPONSE TO *LEISHMANIA AMAZONENSIS*

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C3HeB/FeJ mice infected with *Leishmania major* develop a $CD4^+$ Th1 response and resolve infection, while infection with *Leishmania amazonensis* stimulates a poor T cell response, resulting in chronic disease. When C3HeB/FeJ mice are co-infected with both species of *Leishmania* we observe a healing phenotype similar to infection with *L. major* alone. In contrast, co-infected C57BL/6 mice have a non-healing phenotype similar to infection with *L. amazonensis*. We have developed an *in vitro* assay in which macrophages from C3HeB/FeJ mice infected with *L. amazonensis* can be activated to kill internalized parasites when co-cultured with lymphocytes isolated from the draining lymph node of a *L. major*-infected C3HeB/FeJ mouse. Specifically, we have found that $CD4^+$ T cells and B cells from the draining lymph node of *L. major*-infected C3HeB/FeJ mice are sufficient and necessary for macrophage activation and parasite lethality. When C57BL/6 mouse-derived total lymph node cells or purified $CD4^+$ T cells and B cells from a C57BL/6 mouse infected with *L. major* are co-cultured *in vitro* with *L. amazonensis* infected macrophages parasite killing is not observed. A series of cell transfer studies have demonstrated that B cells from the C57BL/6 mice do not promote killing of intracellular *L. amazonensis*. These findings from our *in vitro* parasite killing assay indicate that B cells play a prominent role in the cell-mediated immune response against *L. amazonensis* and that B cells from C57BL/6 mice are killing deficient compared to cells from C3HeB/FeJ mice.

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THE MAJOR SURFACE PROTEASE OF THE AMASTIGOTE STAGE OF *LEISHMANIA CHAGASI*

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The major surface protease (MSP) is an abundant surface metalloprotease of *Leishmania* spp. encoded by three distinct gene classes in *Leishmania chagasi* (*MSPS, MSPL, MSPC*). Although MSP was discovered and initially characterized in extracellular promastigotes, MSP is also expressed by obligate intracellular amastigotes. We compared MSP expression in the *L. chagasi* LcJ line that cycles between axenic amastigotes and promastigotes. Promastigotes express *MSPS, MSPL*, and two forms of *MSPC* RNA, whereas amastigotes only express *MSPL* and one *MSPC* transcript. Using two-dimensional immunoblots, amastigotes were found to express more than 10 MSP isoforms, similar to promastigotes. However, promastigote MSPs focus between *pI* 5.2 and 6.1, whereas amastigote MSP isoforms migrate at a more acidic *pI*. Promastigote MSP isoforms are N-glycosylated, whereas most amastigote MSPs are not. Immuno-electron microscopy showed 2/3 of total promastigote MSP is localized on the surface and 1/3 is intracellular. In contrast, most amastigote MSP is intracellular and localized at the flagellar pocket, the major site of endocytosis/exocytosis in leishmania. Alkaline carbonate treatment and high-salt washing indicated that the majority of amastigote MSP is cytosolic, whereas promastigote MSP is mostly membrane-associated. Furthermore, promastigote MSP is shed extracellularly whereas amastigote MSP is not despite the fact that MSP acts as a most active metalloprotease in both forms of the parasites. Moreover, immunoelectron microscopy indicated that MSP concentrates at this site of contact between parasite and parasitophorous vacuole (PV). We conclude that intracellular *L. chagasi* amastigotes express multiple MSP isoforms similar to promastigotes, but amastigote MSPs differ from promastigote MSPs biochemically, localize differently in the parasite cell. These observations suggest the abundant MSP protease plays different roles in the extracellular and intracellular environments.

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POST TRANSLATIONAL REGULATION OF MYOBLAST CYCLIN D1 BY *TRYPANOSOMA CRUZI*

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Infection with the parasite *Trypanosoma cruzi* causes Chagas' disease. Previously, we established that infection with this parasite results in an increased expression of the cell cycle regulatory protein, cyclin D1. Immunoblot analysis revealed that that there was an increase in cyclin D1 expression in *T. cruzi* (Tulahuen strain)-infected myoblasts. To examine a possible mechanism for the increased cyclin D1 expression we infected and transfected L_6E_9 myoblasts with cyclin D1 wild type and mutated cyclin D1 promoter luciferase reporter constructs. The constructs were mutated at the AP-1 and ATF binding sites. There was no evidence of an increase in promoter activity following infection. Additionally, quantitative PCR failed to demonstrate any change in cyclin D1 mRNA during infection. The proteosomal pathway was examined both in infected and uninfected myoblasts. We found that cyclin D1 was degraded by proteosome-dependent mechanisms in both cases. Furthermore, the cyclin D1 protein as well as the transcription factor E2F was significantly stabilized after infection. Collectively, these data indicate that the increased cyclin D1 protein expression that results from *T. cruzi* infection is regulated at the post translational level.

(ACMCIP Abstract)

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CHARACTERIZATION OF *TRYPANOSOMA BRUCEI* CA^{2+} CHANNNEL: A POTENTIAL DRUG AND VACCINE TARGET IN TRYPANOSOMES

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Trypanosoma brucei spp. causes Human African Trypanosomiasis (HAT, sleeping sickness) disease. 2 - 300 million people are exposed to this devastating disease along with an annual reported estimate of 50,000 new cases and equal number of deaths in sub-saharan Africa. HAT invades the CNS and can result in coma and death. Drugs used to treat HAT are toxic, and parasite resistance is common. Past attempts to develop vaccines against HAT were unsuccessful because of host immune system evasion by antigenic variation and an impervious membrane. Thus, there is an immense need for the development of an effective vaccine that targets the flagellar pocket membrane proteins and is capable of protecting against infection. We are targeting the Ca^{2+} channel as a novel strategy to interrupt calcium channel function in *T. brucei*. We have characterized, and synthesized a recombinant *T. brucei* Ca^{2+} channel peptide, TBCC1, corresponding to the most immunogenic region of the putative channel peptide sequence. Mice were immunized with TBCC1 to produce specific anti-TBCC1 antibody. Using anti-TBCC1 antibody, we assessed expression and localization of the Ca^{2+} channel in the membrane and flagellar pocket of procyclic and blood form parasites. These results confirmed the immunogenic properties of the TBCC1 Ab and formed the basis of our hypothesis that vaccinating with TBCC1 conjugated to KLH (TBCC1-KLH) will target Ca^{2+} channels in the flagellar pocket thereby disrupting Ca^{2+} channel function, $[Ca^{2+}]$ homeostasis and parasite survival. In testing our hypothesis, we vaccinated mice with or without TBCC1-KLH and later collected serum and splenic T lymphocytes to assess the level of antibody and cytokine production. Immunized mice were challenged with *T. brucei* to assess parasitemia and survival. This rapid identification, characterization and validation of immunogenic protein targets in trypanosomes may be a novel shotgun approach to developing vaccines against HAT.

(ACMCIP Abstract)

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THE DETERMINATION OF SPECIES AND GENOTYPES OF *LEISHMANIA* SPP. USING PCR-RFLP ASSAYS IN CLINICAL SAMPLES OF PATIENTS AND RESERVOIRS IN TURKEY

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Leishmaniasis is an infection that caused by the parasites belonging to the genus *Leishmania.* According to WHO, 350 million people confront the risk of having leishmaniasis every year. The disease can be fatal if it is complicated with AIDS. The clinical forms of the disease can be summarized in three topics: Visceral (VL), cutaneous (CL) and mucocutaneous (MCL) leishmaniasis. The first two of these forms are seen in Turkey and the reservoirs are the dog and the man respectfully. In our study, 37 *Leishmania* isolates that have been cultivated in the NNN medium since 1996 were analyzed by using molecular tools comparing the restriction patterns of the DNA bands belonging to the ribosomal ITS region amplified by PCR method. By using one of the restriction nucleases, Taq I, different restriction patterns were obtained between the isolates from VL and CL, and also CL isolates from Sanliurfa and Ege Region. There were different patterns in three of dog isolates while rest of the VL isolates that originated from the man and the dog were similar. In addition to this, RFLP patterns of 14 Sanliurfa isolates out of 21 CL isolates were almost totally homogenous except one. The causative agents were confirmed as *Leishmania infantum* for VL, and *Leishmania tropica* for CL in these regions in Turkey. But the RFLP patterns of the isolates obtained from the patients with CL in Sanliurfa were not the same as the isolates from Ege Region except one in comparison by the genotype. The analyses of the molecular results together with izoenzyme typing will be supportive for the epidemiological aspect.

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THE ROLE OF ACRIFLAVIN IN THE PROLIFERATION OR INHIBITION OF *TRYPANOSOMA MUSCULI* BY INDUCING APOPTOSIS WITH SPECIFIC BINDING AFFINITY TO KDNA OF THE PARASITE *IN VITRO* AND *IN VIVO*

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Acriflavin (Euflavin) is an intercalating agent and inhibitor of mitochondriogenesis. It also intercalates with the major and minor helices of DNA in bacteria and prokaryotes. When *Trypanosoma musculi* were treated with acriflavin *in vitro*, it caused the destruction of the kinetoplast and respiratory defects. The binding affinity of acriflavin, its role in inducing apoptosis, biological effects on proliferation of the parasite, and its distribution were also studied both *in vitro* and *in vivo*. The parasitemia levels, at different time of infection demonstrated anti-trypanosomal and prophylactic activities of acriflavin *in vivo*. The specific binding of acriflavin to kDNA was demonstrated using fluorescence microscopy. The staining of fluorescent dye 4', 6-diamidino-2-phenylindole (DAPI) showed the effect of acriflavin in inducing fragmentation of kDNA. The fragmentation of the parasites' kDNA was further established using gel electrophoresis assay. The histological effect of the acriflavin in the kDNA of the parasite was demonstrated using transmission electron microscopy. The function of acriflavin on the parasites' proliferation was studied *in vitro* using 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay. Western blot analysis showed the release of cytochrome C from the

kDNA to the cytoplasm and subsequent activation (cleavages) of caspase 3 and 9 proteins. The western blot analysis showed acriflavin treatment did not affect the immune response of the host. The membrane potential difference of the kDNA between the control and acriflavin treated parasites indicates the oxidizing power of acriflavin and its interference on the respiratory chain of the parasite and hence the parasite's ATP production activity. The results of this study suggested that acriflavin treatment caused swelling of the kinetoplast and condensation of the kDNA, which lead to ultimate death of the trypanosome.

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THE ROLE OF LIVER-X RECEPTOR (LXR) IN *LEISHMANIA CHAGASI* INFECTION IN MICE

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The liver X receptors (LXRs) are a family of nuclear receptors that have defined roles in lipid metabolism and have been implicated in inflammation and immune regulation. LXRs have previously been shown to have anti-inflammatory effects in macrophages via NF-κB signaling, as well as to affect antimicrobial responses. Previous work from our laboratory has shown that mice lacking LXRs are susceptible to infection by *Listeria monocytogenes*, an intracellular bacterium. This finding raised the possibility that LXR-mediated pathways might also be implicated in macrophage responses to other intracellular pathogens. To examine this question, we challenged mice lacking LXRs with the intracellular parasite *Leishmania chagasi*. Surprisingly, we found that LXRα knock-out and LXR-α/β double-knock-out (DKO) mice were markedly resistant to infection compared to wild-type mice. Specifically, parasite loads in livers and spleens of LXR-DKO mice were approximately eight-fold lower than in WT mice. Results from *in vitro* assays using bone marrow-derived macrophages (BMDMs) infected with *L. chagasi* revealed increased nitric oxide production as well as increased expression of inflammatory genes in LXR-DKO macrophages. Additionally, in wild-type BMDMs, LXR ligands abrogated nitric oxide production in response to *L. chagasi* infection. This observation suggests that LXR-DKO macrophages are able to mount a more vigorous antimicrobial response to *Leishmania* infection. These results indicate that LXR signaling pathways modulate host immune responses in a complex and pathogen-dependent manner. The LXR pathway may represent a potential therapeutic target for modulating immunity against *Leishmania* or other intracellular parasites.

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EFFICACY OF A DNA VACCINE AGAINST *LEISHMANIA MEXICANA* IN GOLDEN HAMSTERS

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The Leishmaniases are a group of diseases caused by protozoan parasites of the *Leishmania* genus. We previously found that a DNA vaccine encoding *L. donovani* antigen NH36 could induce protection against both *L. chagasi* (visceral leishmaniasis) and *L. mexicana* (cutaneous leishmaniasis). We further optimized this vaccine by combining it with a plasmid encoding *L. mexicana* GP63 and this vaccine provided good protection in BALB/c mice. To further evaluate this DNA vaccine, we investigated here its efficacy to induce protection in golden hamster, an alternative animal model for leishmaniasis. Female golden hasmters were immunized with 2 doses of 100 µg of DNA vaccines encoding NH36 and GP63, with aluminium phosphate as adjuvant. Contro, groups received saline solution or the empty plasmid. The animals were infected with 500 *L. mexicana* parasites in the hindpaw 2-3 weeks after immunization. Lesion measurement during 17 weeks indicated that the DNA vaccine was able to reduced lesion size in vaccinated hamsters, compared to controls. Further evaluation of the immune response in underway, but these results suggest that this DNA vaccine is able to partially protect hamsters against *L. mexicana* infection.

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EVALUATION OF THE EFFICACY OF A COMBINATION OF DNA VACCINES ENCODING TSA-1 AND TC24 ANTIGENS IN MICE INFECTED WITH *TRYPANOSOMA CRUZI*

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Chagas disease, caused by the protozoan parasite *Trypanosoma cruzi* is one of the main public health problems in Latin America. Due to the lack of effective drug treatment, we have previously demonstrated that therapeutic DNA vaccines encoding *T. cruzi* antigens TSA-1 or Tc24 could partially control an ongoing infection with *T. cruzi* in ICR and BALB/c mice. In this study, we investigated if treatment with a combination of both plasmids could increase therapeutic efficacy compared to each plasmid alone in mice. ICR and C57BL/6 mice were infected with 500 or 20,000 blood parasites, respectively, and treated at day 5 and 12 post infection with two doses of DNA vaccines encoding TSA-1 and Tc24 antigens either alone or in combination. Treatment with the combination of 2 plasmids was able to reduce parasitemia in both ICR and C57Bl/6 mice and reduced cardiac tissue inflammation. Further studies are underway to evaluate parasite burden in the heart. These results suggest that treatment with a combination of DNA vaccines provides a better therapeutic effect in mice infected with *T. cruzi* and this treatment thus presents an attracive alternavive for further evaluation.

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T-CELL EPITOPE MAPPING OF MAJOR MSP-1_{33} ALLELES IN HUMAN CORD BLOOD FROM KENYAN NEWBORNS

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Merozoite Surface Protein 1 (MSP-1) is the most abundant surface protein on the *P. falciparum* merozoite. The 33kDa region of MSP1 is highly polymorphic with two distinct allelic families. Systematic mapping of T cells epitopes in the 33kDa region and identification of allele-specific differences has not been described in cord blood. Eighteen-mer peptides overlapping by 9 amino acids were synthesized spanning the 33kDa fragment for both allelic families (MAD20 and Wellcome [K1]) for a total of 58 peptides. Lymphocyte proliferation, IFN-g, IL-10 and IL-13 responses were evaluated in cord blood lymphocytes (CBL) from Kenyan newborns (N=48) to all peptides and to recombinant $MSP1_{42}$ (N= 21). Overall 52% demonstrated a lymphocyte proliferation responses and 35% a cytokine responses to ≥2 peptides whereas recombinant $MSP1_{42}$ generated proliferative and cytokine response to 7% and 14% respectively. Two dominant T cell epitopes for the MAD20 and K1 allele were identified as distinct and non-overlapping; often the same individual recognized both. Most peptides failed to stimulate any response in CBL from North America. CBL developed a specific and restricted lymphocyte response to $MSP1_{33}$ similar to that acquired in adults. This indicates maturation of the Tc-receptor repertoire and an intact ability of neonatal APC to process

antigen *in vivo*. The reduced response of CBL to recombinant $MSP1_{42}$ relative to peptides suggests that neonatal peripheral blood APC may have an impaired ability to process and/or present antigen. Finally the presence of distinct immunodominant epitopes for different $MSP1_{33}$ alleles has important implications for design of a malaria blood stage vaccine.

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RNAI OF EXTRACELLULAR MATRIX GENES THAT ARE REGULATED BY *TRYPANOSOMA CRUZI* BLOCKS *T. CRUZI INFECTION*

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It is thought that *Trypanosoma cruzi*, the protozoan that causes Chagas heart disease, modulates the extracellular matrix network to facilitate infection of human cells. However, direct evidence to document this phenomenon is lacking. Here we show that *T. cruzi* gp83 ligand, a cell surface trans-sialidase like molecule that the parasite uses to attach to host cells, increases the level of laminin γ-1 transcript and its expression in mammalian cells, leading to an increase in cellular infection. *T. cruzi* induces a peak of thromobospondin-1 gene transcripts in cells at 60 min, followed by a laminin γ-1 transcript peak at 120 min. Stable RNA interference (RNAi) of host cell laminin γ-1 and thrombospondin-1 gene transcripts in cells knocks down the levels of laminin γ-1 transcripts and thrombospondin-1 transcripts and laminin γ-1 and thrombospondin expression in mammalian cells causing a dramatic reduction of cellular infection by *T. cruzi*. Thus, host thrombospondin-1 and laminin γ-1, which are regulated by the parasite, play crucial roles in the early process of infection. This is the first report showing that knocking down the expression of human genes by RNAi inhibits the infection of an intracellular parasite.

(ACMCIP Abstract)

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THE ROLE OF OXIDATIVE STRESS AND MALARIA INFECTION ON ANAEMIA IN PREGNANCY

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Anaemia is common among pregnant women, especially in developing countries. There is not enough information about factors responsible for this condition in Akoko area of Ondo state in Nigeria. Therefore, this study assessed the role of oxidative status and malaria infection on anaemia in pregnancy. A total of 210 and 100 pregnant and non-pregnant women respectively were recruited for this study from Ikare specialist hospital and Iwaro general hospital in Akoko area. Pregnant women were grouped into primigravidae and multigravidae. *Plasmodium falciparum* was determined by microscopy. Haemoglobin (Hb) level was quantified by colorimetric method using Drabkin's solution. Oxidative status was determined by measuring malondialdehyde (MDA), ascorbic acid, and superoxide dismutase (SOD) spectrophotometrically. Mean parasitaemia was significantly higher ($p<0.05$) among pregnant women (2300±101) than non-pregnant women (910± 87). Hb level was significantly reduced ($p<0.05$) among malaria positive pregnant and non-pregnant women than malaria negative (Hb levels of 8.3 - 10.0g/dl). The oxidative status indicated that MDA was significantly increased ($p<0.05$) among pregnant (2.5±0.7nmol/ml) than non-pregnant women (1.8±0.1nmol/ml), while ascorbic acid and SOD levels were significantly reduced ($p<0.05$) among pregnant than non-pregnant women. MDA was significantly increased among malaria positive than malaria negative, while SOD and ascorbic acid was were not. There was increase in MDA and decrease in SOD and ascorbic acid in malaria positive primigravidae as compared with malaria positive multigravidae. The increased in MDA among malaria positive was correlated to decrease in Hb levels. This study shows that oxidative stress due to malaria infection in pregnancy could be one of the contributing factors responsible for anaemia in pregnancy.

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INDUCTION OF *PLASMODIUM* SPOROZOITE MOTILITY BY ALBUMIN IS ASSOCIATED WITH MOBILIZATION OF INTRACELLULAR CALCIUM

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One of the most striking features of the invasive stages of apicomplexan parasites, including *Plasmodium* sporozoites, is that they exhibit gliding motility. This has been extensively studied with *Plasmodium* sporozoites because of the ease of initiating motility *in vitro* when albumin is added to the medium. We performed studies with sporozoites of *Plasmodium berghei* and *P. yoelii* and now report, that $Ca^{2+}$and cAMP act as intracellular second messengers leading to microneme release of proteins from the anterior end of the parasite, as occurs with other apicomplexans. The transport of these microneme proteins posteriorly along the sporozoite is associated with the gliding forward of the parasite. We found that a specific chelator of intracellular calcium ions (BAPTA-AM) inhibited sporozoite motility in the presence of albumin; this was reversed by the inflow of calcium ions produced by the calcium ionophore A23187. When sporozoites were placed in calcium-free medium with albumin present, they gradually lost their motility over a 90 min period but replenishment of medium with one containing calcium ions restored motility, suggesting that intracellular stores of calcium had been depleted over time but that this depletion was reversible. The role of cAMP as an additional second messenger leading towards induction of sporozoite motility was established by showing that a) inhibition of intracellular formation of cAMP by inhibiting adenylyl cyclase activity, and b) inhibition of cAMP-dependent protein kinase A each reduced motility. Finally, we were able for the first time to induce sporozoite motility in the absence of albumin by any of the following: a) enhancing the synthesis of cAMP with an activator of adenylyl cyclase, b) inhibiting the degradation of cAMP with an inhibitor of phosphodiesterase, c) addition of dibutyryl cAMP (a cell-permeant cAMP agonist). The mechanism by which extracellular albumin acts at the sporozoite surface to induce activity of the intracellular second messengers remains to be established.

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MAGNETIC SEPARATION: A VERY EFFECTICE METHOD FOR THE SYNCHRONIZATION OF *PLASMODIUM FALCIPARUM* IN CULTURE

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The most unique biological characteristic of *Plasmodium falciparum* during schizogony is the synchronicity. Synchronous release of merozoites from infected erythrocytes makes malaria parasites minimize the exposure time to an extracellular environment. It results in a successful escape of the parasite from humoral immune system of the host. Since the first introduction of *in vitro* cultivation of *P. falciparum* in 1976, various kinds of methods for synchronization have been tried. However, in spite of partial successful fulfillment, the efficiency of synchronization reached at most 80%, which was not enough to mimic natural condition. The magnetic separation is a method to select or to deplete the cells by their magnetic properties using high-gradient field magnet. In particular, erythrocytes infected by *Plasmodium* of later stages of schizony, i.e. old trophozoites or schizonts, can be separated effectively by the magnetic separation. In this study, we tried to synchronize *P. falciparum* in culture using the magnetic separation. The efficiency of synchronization by the magnetic separation was not less than 99%, which was fully sufficient to reproduce natural synchronicity of malaria parasites. The magnetic separation combined with the existing synchronization methods was very simple and useful to synchronize malaria parasites in culture.

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PLASMODIUM FALCIPARUM LIVER STAGE ANTIGEN-1 IS CROSSLINKED BY TISSUE TRANSGLUTAMINASE

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The protozoan parasite *Plasmodium falciparum* is the causative agent of cerebral malaria, the most lethal variant of human malarial disease. Sporozoites, injected by feeding mosquitoes, rapidly enter the liver where they undergo pre-erythrocytic developmental schizogony before forming tens of thousands of merozoites that are released into the blood stream. Shortly after hepatocyte invasion the parasite starts to produce Liver Stage Antigen-1 (LSA-1) which accumulates within the parasitophorous vacuole. While the role of LSA-1 has yet to be elucidated, we have identified a tissue transglutaminase-2 (TG2) substrate motif within the central repeat region of LSA-1. In general, the function of TG2 is to post-translationally modify proteins by the formation of isopeptide ε-(γ-glutamyl)lysine cross-bridges between glutamine and lysine residues. We have shown that a recombinant LSA-1 protein is cross-linked *in vitro* by both purified guinea pig and human TG2, as well as cell extracts of a human cell line that over expresses human TG2. Characteristic of TG2-mediated polymerizations, the reactions are calcium dependent and result in a flocculent mass of protein that is highly resistant to degradation. In addition, we have studied native LSA-1 expression in transgenic, chimeric mice containing functional human livers. Furthermore, we show by immunofluorescence that antibodies specific to TG2 catalysed ε-(γ-glutamyl)lysine cross-bridges colocalize with LSA-1 in infected human hepatocytes.

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REDUCED RISK OF *PLASMODIUM VIVAX* AND *P. MALARIAE* INFECTIONS ASSOCIATED WITH BAND 3 DELETION IN PAPUA NEW GUINEAN CHILDREN

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Since Haldane's proposal of the 'Malaria Hypothesis', human *Plasmodium* species parasites have been viewed as a major force shaping evolution of the human genome in endemic areas. With the exception of the Duffy blood group, which prevents infection with *P. vivax*, research has focused on host genetic adaptations to *P. falciparum*, the deadliest of the human malarias. In a study of Papua New Guinean school children, we have now demonstrated that the band 3 deletion (SLC4A1 Δ21) that causes South-East Asian Ovalocytosis (SAO) is associated in risk of acquiring blood stage infections with *P. vivax* and *P. malariae*. During 6 months of active fortnightly follow-up at least one post-PCR LDR-FMA positive *P. vivax* infection was observed in 70.3% (19/27) of SAO but 83.8% (98/179) of non-SAO children ($p = 0.001$). Similarly, LM positive *P. vivax* infections were observed in only 37% of SAO (10/27) but 54.7% (98/179) of non-SAO children ($p = 0.013$). Only 3 of 27 SAO children (14.8%) were re-infected with an LDR-FMA positive PM infections (all of these infections were below LM detection threshold) compared to 38.0% (57/179) and 9.5% (17/179) non-SAO children with LDR-FMA ($p = 0.02$) and LM ($p = 0.09$), respectively. The SAO genotype was thus associated with a 52% reduction in LDR-FMA and 60% reduction in risk of LM-detectable *P. vivax* infections and a 71% reduction for LDR-FMA diagnosed *P. malariae* infections but had no effect on the risk of acquiring *P. falciparum* infections. This observation suggests that invasion of the human red blood cell by *P. vivax* and P. malariae is compromised by the SAO mutation and may suggest a contribution of non-*falciparum* malarias in shaping the unique host genetic adaptations to malaria in Asian and Pacific populations.

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INTEGRATING QUANTITATIVE TRAIT LOCI (QTL) WITH WHOLE-GENOME DATA TO IDENTIFY CANDIDATE GENES CONTROLLING GROWTH TRAITS IN *PLASMODIUM FALCIPARUM*

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The molecular mechanisms controlling differential malaria parasite growth rates are not well known. Disease severity is influenced by high proliferation of *Plasmodium falciparum* within erythrocytes. Recent work in our lab has highlighted discrete growth-related traits that contribute to a faster growth rate of the Dd2 clone over HB3. Quantitative trait loci (QTL) mapping in the progeny generated from the HB3 × Dd2 cross has identified genomic regions contributing to growth phenotypes and has provided portals into the genome, facilitating the discovery of key genes and polymorphisms. To sift these loci for molecular determinants regulating the variation observed in growth, the development of unbiased methods that draw upon the accumulating wealth of genome-wide data and that are not solely dependent on gene annotations is essential. Here, we describe a series of filters to refine a list of candidate genes that influence the duration of the erythrocytic cycle and merozoite production in the cross. Genes from within the QTL are prioritized based on: 1) gene functions, if known, 2) coding sequence polymorphisms (*i.e.* SNPs) between HB3 and Dd2, 3) density of SNPs, and 4) variation between HB3 and Dd2 gene expression profiles. Correlations of more than 7000 gene expression traits from the HB3×Dd2 progeny with our growth traits can identify suites of co-varying transcripts that can be screened for functional

enrichments, highlighting candidate biological processes contributing to growth differences. Finally, putative functions can be assigned to 'hypothetical' genes based on interactions in network databases. The intersection between these lists of candidate genes defines reliable, manageable suites of genes likely impacting the growth of the parasite.

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MULTI-FACETED IMPACT OF MSP-1P42 SPECIFIC ANTIBODIES ON BLOOD STAGES OF *PLASMODIUM FALCIPARUM*

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Antibodies are the main effector molecules in the defense against blood stages of the malaria parasite *Plasmodium falciparum*. Understanding the mechanisms by which vaccine-induced anti-blood stage antibodies work to protect against malaria is essential for successful vaccine design and testing. Previously, we showed *in vitro* antibody modality depends on both antigen specificity and target strain. Although AMA-1-specific Ab inhibit erythrocyte invasion by both FVO and 3D7 *P. falciparum* merozoites, MSP-1p42-specific Ab inhibit FVO by interfering with invasion and 3D7 by interfering with intracellular development. The present study shows that in arresting invasion of FVO strain *P. falciparum*, MSP-1p42-specific antibodies either prevent schizont rupture or agglutinate released merozoites. Alternatively, anti-MSP-1p42 antibodies do not prevent the rupture of 3D7 schizonts; instead, they agglutinate merozoites and arrest the development of young parasites at the early trophozoite stage. We will also report the contributions of fine-specificity to these mechanisms as defined by results from testing monoclonal antibodies whose specificities have been mapped to the various domains of MSP-1p42.

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IDENTIFICATION OF A NOVEL FAMILY OF VARIANT SURFACE ANTIGENS IN *PLASMODIUM FALCIPARUM*

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Plasmodium falciparum variantly expressed surface antigens (VSA) have been proposed as an escape mechanism from the host immune response giving rise to persistent infections in humans. With 60% of the genome still uncharacterized, we sought a means to identify novel VSA that might play an important role in pathogenesis. We hypothesized that nucleotide diversity, variant expression, and presence of the Pexel motif could be used to filter the genome into a testable set of candidate VSA. To identify single nucleotide polymorphisms (SNPs) and evaluate genetic diversity, we have sequenced the HB3 and Dd2 genomes to 8x coverage, and an additional nine parasite samples to 1.25x coverage and compared them to the 3D7 genome sequence. We identified over 93,000 high confidence SNPs and calculated the pairwise nucleotide diversity (π) for every gene. Most genes demonstrate low nucleotide diversity, with 85% of the genome having a π value less than 2.0×10^{-3}. To identify novel antigens, we focused on the top 5% of highly diverse genes and further evaluated their expression profiles in a set of five patient transcriptomes and the 3D7 transcriptome. To discriminate genes that are exported to the surface of the infected red blood cell, we also factored the presence of the Pexel motif into our analysis. Most of the candidates that fulfilled our filter criteria are known antigens, but some were uncharacterized genes that warranted further study. One small paralogous gene family demonstrated significantly higher nucleotide diversity than other Pexel containing genes, and we predict they represent a novel family of VSA. Preliminary analysis suggests these genes are variantly expressed across parasite lines and are generally up-regulated *in vivo*. We are currently determining the cellular localization of these proteins and testing their antigenicity. Our study demonstrates that nucleotide diversity, along with other bioinformatic parameters, represents a powerful tool for identifying novel genes involved in pathogenesis and predicting new targets for vaccine development.

(ACMCIP Abstract)

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VARIANT MEROZOITE PROTEIN EXPRESSION IS ASSOCIATED WITH ERYTHROCYTE INVASION PHENOTYPES IN *PLASMODIUM FALCIPARUM* ISOLATES FROM TANZANIA

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Invasion of erythrocytes by *Plasmodium falciparum* merozoites involves multiple ligand-receptor interactions, known as invasion pathways, defined by the erythrocyte receptor sensitivity to neuraminidase, chymotrypsin and trypsin. Recognition of alternate receptors on the erythrocyte surface by *P. falciparum* merozoites is thought to be mediated by members of the Reticulocyte-Binding homolog (PfRh) and Erythrocyte Binding Antigen (PfEBA) ligand families. Laboratory strains of *P. falciparum* vary in their dependence on alternate receptors for invasion and also express different members of the PfRh family. This variant expression could explain the use of alternative invasion pathways by different parasite lines. Our current understanding of alternative erythrocyte invasion patterns relies on either laboratory adapted strains that have been passaged for many generations or on wild isolates that have been recently adapted to *in vitro* culture. This study was initiated to determine the use of alternative ligand-receptor interactions in a natural population of Tanzanian parasites, to further our understanding of the dynamics of invasion in the natural context. We also sought to assess the extent of differential expression of the PfRh and PfEBA ligands, and the role this may play in defining invasion pathway utilization. Our results indicate that Tanzanian parasites display variant expression of Rh and EBA proteins, even in multiclonal infections. We find significant coordinate expression of PfRh2a and PfRh2b, PfRh2b and EBA-181, and EBA-175 and EBA-140 whereas there is a negative correlation between expression of Rh2a/Rh2b and Rh1. Further, we find an association between expression and invasion phenotype: Rh1 expression is associated with invasion by a trypsin/chymotrypsin-resistant pathway whereas expression of Rh2a/2b resulted in invasion by a trypsin/chymotrypsin-sensitive pathway. Such variant expression of invasion ligands and the corresponding association with invasion pathway usage could reflect frequency-dependent selection by host receptor polymorphisms or immune evasion. We are currently conducting experiments to assess the immune response against these variantly expressed ligands and are conducting antibody inhibition studies with purified IgG from patient serum to determine if the immune response directed against these ligands functionally inhibits parasite invasion.

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STATINS ENHANCE HOST INFLAMMATORY RESPONSES TO *PLASMODIUM FALCIPARUM* GPI *IN VITRO* AND DYSREGULATE INNATE RESPONSE TO BLOOD STAGE INFECTION *IN VIVO*

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Cerebral malaria (CM) results, at least partly, from a dysregulated host inflammatory response to infection. We hypothesized that deleterious components of the innate immune response can be elucidated and modified to improve outcome. The objective of this study was to examine the ability of classes of FDA-approved compounds to modify excessive inflammatory responses and improve outcome. Based on a number of reports indicating potent activity against host-mediated inflammatory disorders such as sepsis and autoimmune disease, we examined the activity of statins (HMG-CoA Reductase inhibitors) on malaria-associated inflammation *in vitro* and *in vivo*. We investigated the effect of co-treatment of statins on *pf*GPI-induced MAPK activation and cytokine secretion from primary murine and human monocyte-derived macrophages. We demonstrated that ERK1/2, JNK, p38, c-Jun, and ATF-2 became phosphorylated in *pf*GPI-stimulated macrophages. Contrary to expectations, we observed a marked increase in *pf*GPI-induced TNFa when macrophages were treated with simvastatin and atorvastatin. Since *Pf*GPI is known to act through TLR2, we examined other TLR ligands. Using wild type, *Tlr2*$^{-/-}$ and *Tlr4*$^{-/-}$ macrophages and a panel of TLR ligands we found a similar statin-mediated enhancement of cytokine induction across a range of TLR-ligand interactions. We extended these studies to investigate the role of statins in a *Plasmodium berghei* ANKA (PbA) experimental model of cerebral malaria *in vivo*. Compared to control mice, statin-treated C57BL/6 mice were not protected from PbA-induced CM, displayed evidence of dysregulated cytokine response to infection and rapidly succumbed to infection. Statins are among the most widely-prescribed drugs available and have been touted as the solution to a multiplicity of diseases, in particular inflammatory disorders. Our results provide potential cautionary notes about the use of these drugs in malaria-endemic areas; however further studies are required to fully elucidate the molecular mechanisms responsible for the interaction between statins and host response to malaria.

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PLASMODIUM FALCIPARUM HISTONE DEACETYLASES: ENZYMES INVOLVED IN GENE REGULATION AS NEW ANTIMALARIAL DRUG TARGETS

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Parasite resistance to current antimalarials is driving the search for new agents that act on novel parasite targets. One drug discovery strategy is to identify molecular targets in *Plasmodium* that are already being pursued for other diseases. Using this 'piggy-back' approach we are investigating a class of poorly studied *P. falciparum* enzymes, histone deacetylases (HDACs), which have been the target of anti-tumour drug development for decades. While some natural product HDAC inhibitors (eg apicidin and TSA) kill *Plasmodium* species *in vitro*, they also kill human cells at comparable concentrations, compromising their potential as drug candidates. We have since shown that a range of HDAC inhibitors are active against *P. falciparum* at μM concentrations and *in vivo* in a rodent malaria model. These HDAC inhibitors are more bioavailable than TSA and apicidin and support the idea that *Pf*HDACs may be useful antimalarial targets. We have now engineered new compounds that are an order of magnitude more cytotoxic *in vitro* to *P. falciparum* (IC_{50}, 3-334 nM) and display significantly less anti-proliferative activity against healthy host cells. Treatment of *P. falciparum* infected erythrocytes with these compounds causes stage-specific growth inhibition, results in hyperacetylation of parasite histones, and alters the RNA expression profile of several *P. falciparum* genes. These compounds are potential leads for the development of a new class of antimalarials with a different mechanism of action against the parasite than existing agents and may be useful tools in the study of *P. falciparum* gene regulation.

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POTENT ANTIMALARIAL ACTIVITY OF THE A/T-SPECIFIC ALKYLATING AGENT AS-I-145 AGAINST *PLASMODIUM IN VITRO* AND *IN VIVO*

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One strategy to develop new anti-malarial drugs is to exploit the unusual proportion of adenine and thymine nucleotides within the *Plasmodium* genome. A/T-specific binding agents, such as adozelesin, display antimalarial activity *in vitro* and *in vivo*, but their toxicity is too severe for clinical development. We evaluated the antimalarial activity of the compound AS-I-145 that covalently binds to adenine-N3 within A/T-specific motifs and is not overtly toxic to C57/BL6 mice at doses as high as 15 mg/kg. This compound was highly active against *P. falciparum in vitro* with an IC_{50} of 2 nM for both drug-sensitive and drug-resistant strains. A single intraperitoneal injection of AS-I-145 (15 mg/kg) rapidly suppressed parasite levels in mice infected with the avirulent *P. chabaudi adami* DK strain and the parasitemia remained subpatent for at least 14 days. A single injection of AS-I-145 also reduced the parasitemia in mice infected with the virulent *P. c. adami* DS strain and protected mice from a second homologous challenge. AS-I-145 exhibited 100% activity against *P. berghei* ANKA in the four-day suppression test and promoted the survival of 3/8 mice for over 45 days. We propose that the mechanism of parasite death is associated with modification of *Plasmodium* genomic DNA. Using real-time PCR, we detected DNA damage in circulating parasites isolated from mice 24 h after a single treatment with AS-I-145. Given its low toxicity and potent activity against blood-stage parasites *in vitro* and *in vivo*, AS-I-145 could represent a powerful new class of antimalarial drugs.

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PHARMACOKINETIC COMPARISON OF ARTESUNATE (AS) FOLLOWING MULTIPLE INTRAVENOUS INJECTIONS IN THE *PLASMODIUM BERGHEI* INFECTED AND UNINFECTED RATS

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In our previous research, injectable artesunate (AS) exhibited less toxic effects in *P. berghei* infected rats than that in uninfected rats. The data showed that the minimal dose of AS required to clear parasitemia in 39-67% of animals was 37 mg/kg and no significant toxicity was observed. In order to investigate the pharmacokinetic comparison, intravenous AS of 37 mg/kg was given to *P. berghei* malaria-infected and uninfected rats by using 3 daily multiple injections. Drug concentration of AS was one-third to a half less on day 3 than that on day 1 in both infected and uninfected rats, suggesting an auto-induction of hepatic drug-metabolizing enzymes for AS, which was similar to other artemisinin drugs. Although artesunate is the pro-drug of DHA, the DHA/AS ratio of 5.26 in the infected rats was almost 8-fold higher than that (DHA/AS ratio = 0.67) in the uninfected animals, resulting in the total $AUC_{D1\text{-}3}$ (13051 ng·h/ml) of DHA in the infected rats to be about 3.3 folds higher than that (3935 ng·h/ml) in the uninfected animals during the three days treatments. The total concentration of AS plus DHA in infected rats ($AUC_{AS + DHA\ D1\text{-}3}$ = 15768 ng·h/ml) was also 1.5 folds higher than that in uninfected rats (9886 ng·h/ml). A question is then raised on why the higher drug exposure level of DHA, which was 2-4 folds more toxic than AS level in rats, does not cause greater toxicity in malaria-infected rats. On the contrary, the result surprisingly shows less toxic effects presented in the infected rats. Although DHA is known to be more highly bound (100-300 folds) to

malaria-infected red blood cells than uninfected cells, and this can reduce DHA concentration in plasma, a more detail reason to reduce toxicity in infected rats is unclear.

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PHARMACOKINETIC PROFILES OF INJECTABLE ARTESUNATE (AS) IN THE PREGNANT AND NON-PREGNANT RATS IN RELATION TO ITS EMBRYOTOXICITY

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It has been demonstrated that injectable artesunate (AS) can cause fetal death and teratogenic effects in animals when given at a level (0.6-1.0 mg/kg) below human therapeutic dose (2-4 mg/kg). Similar toxicity has also been found for oral artemisinins in various animal species at dosage levels higher than 2-4 mg/kg. However, these clinical observations were not found in humans. In order to investigate the severe embryotoxicity, the pharmacokinetics of AS following single and multiple intramuscular injection was conducted in pregnant and non-pregnant rats. Results demonstrated that the pharmacokinetic profile of AS was similar by single dose but was significantly different after multiple injections between the two groups of animals. Following multiple injections the drug concentration accumulated in the pregnant rats and declined in the non-pregnant rats. In addition, important higher conversion rate of AS to dihydroartemisinin (DHA) was detected in the pregnant rats after either single or multiple doses. The ratios of AUC_{DHA}/AUC_{AS} were 0.99-1.02 for the pregnant rats and 0.42-0.48 for the non-pregnant animals, indicating that the total AUC_{D1-3} (15049 ng·h/ml) of DHA during the three days treatment in the pregnant rats was about 3.7 folds higher than that (4015 ng·h/ml) in the non-pregnant rats. Previous studies also showed that DHA was 2-4 fold more toxic than AS in animal species and more sensitive on the embryotoxicity of rats. This evidence confirms that the AS and DHA concentrations (28679 ng·h/kg) in plasma of the pregnant rats was significantly higher than that (12908 ng·h/ml) of the non-pregnant animals, which may be a concern in the severe embryotoxicity of AS after intramuscular administration even with low dosage regimen in the pregnant animals.

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EFFICACY, TOXICITY AND THERAPEUTIC INDICES OF ARTESUNATE (AS) AND DIHYDROARTEMISININ (DHA) IN *PLASMODIUM BERGHEI* INFECTED AND UNINFECTED RATS

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Artesunate (AS) is being developed as a potential agent for the treatment of severe and complicated malaria. Dihydroartemisinin (DHA) has been found to be an active metabolite of AS in human and animal species. In order to assess the efficacy potency and therapeutic indices of AS and DHA, a study following daily intravenous injection for three days was conducted with P. berghei infected rats. The minimum clearance doses AS and DHA for parasitemia were 60.0 and 22.2 mg/kg, respectively, and the maximal tolerant doses of AS and DHA were 240.0 and 33.3 mg/kg. Thus, the therapeutic index was calculated 4.0 for AS and 1.5 for DHA. In the acute toxicity study, the LD_{50} studies were conducted in both *P. beighei* infected and non-infected rats. The LD_{50} studies in infected animals after consisted of 3 multiple doses given daily over three consecutive days, and the LD_{50} was determined to be 90.6 and 487.5mg/kg for DHA and AS, respectively, with most of the deaths occurring on the first day of dosing. The LD_{50} study in non-infected rats consisted of a single intravenous dose, and interestingly, the LD_{50} values obtained in non-infected rats following a single dose treatment were 69.1 and 351.2 mg/kg for DHA and AS, respectively, which doses were lower than that obtained in infected animals when receiving three multiple doses. Based on the comparison data, the potency of DHA seems to be 2-3 times higher than that of AS in antimalarial effect, but AS is 2-4 times safer than DHA following daily intravenous injection for three days in *P. berghei* infected rats or single injection in uninfected subjects. In addition, the malaria infected rats are more tolerant with the two drugs than uninfected rats.

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EFFICACY EVALUATIONS OF 13 METABOLITES OF ARTESUNATE IN CULTURE WITH VARIOUS CLONES AND ISOLATES OF *PLASMODIUM FALCIPARUM*

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Metabolism studies have traditionally used animal model systems to predict metabolic fates in humans. Recently, tissue distribution and metabolic pathway studies of radiolabeled artesunate (AS) has been conducted in rats and most of the major metabolites of AS were revealed in the plasma and urine. In order to assess the relative antimalarial potency of these metabolites, AS and its 13 unknown metabolites plus a known active metabolite of AS, dihydroartemisinin (DHA), were analyzed on efficacy in culture with various clones and isolates of *Plasmodium falciparum* by using the tritiated hypoxanthine assay. The 13 unknown metabolites of AS were isolated and purified from free or conjugation fraction of rat plasma or urine that were collected from animals treated with injectable AS. Results showed that only metabolite 5 possessed the same antimalarial potency as artemisinin, and metabolites 3 and 12 have a similar potency as artelinic acid, whose activities are superior to chloroquine and mefloquine. However, the antimalarial potencies of the 3 metabolites were much less than AS and DHA. The 3 active metabolites of AS were highly active against both chloroquine-sensitive (D6) and mefloquine-resistant (W2 and TM91C235) clones of *P. falciparum* with 50% inhibitory concentration (IC_{50}) in the range of 0.78-4.68 ng/ml. Other 10 metabolites showed poor or no antimalarial effect in this investigation. As well, our previous study demonstrated that the mean terminal half-lives of total metabolites of AS in plasma and blood were 76 and 105 hours, respectively. In the present study, the metabolites 3 and 5 have been separated from urine collected in both time periods of 0-8 and 9-24 hours, the two metabolites may possess a longer half-life than AS ($t_{1/2}$ = 0.43h) and DHA ($t_{1/2}$ = 0.75h) in rats.

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ISOLATED MITOCHONDRIA FROM *PLASMODIUM FALCIPARUM* WITH CYTOCHROME B MUTATIONS PRESENT AN ALTERED SENSITIVITY TO ANTIMALARIAL 4-(1H)-PYRIDONES

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Antimalarial 4-(1H)-pyridone derivatives are compounds with nanomolar IC_{50} against *P. falciparum*, measured *in vitro* with the hypoxanthine incorporation assay and have also been proven efficacious in mouse models of malaria. We have shown inhibition of cytochrome c reduction by mitochondrial preparations using different electron donors, thus pointing to bc1 complex as a likely target for these compounds. When high numbers of parasites (approx 10^9 cells) were exposed to two different pyridones using two different genetic backgrounds (fully sequenced 3D7 strain and the CQ resistant K1 strain), parasite lines with reduced sensitivity to pyridones were selected. Cytochrome b gene was amplified and sequenced, showing a V284L mutation in 3D7 strain and a G133S mutation in K1 strain. Both, $3D7_{V284L}$ and $K1_{G133S}$ clones showed cross-resistance to pyridones structurally related to the selection compound, while sensitivity to standard antimalarials (Chloroquine, Atovaquone, Artemisinin and Pyrimethamine) remain unaltered. However, the $K1_{G133S}$ clone showed cross resistance to the known cytochrome b inhibitors