1

SEQUENCING THE GENOME OF *IXODES SCAPULARIS* - THE LYME DISEASE TICK

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Ticks in the family Ixodidae (hard ticks) transmit the greatest variety of pathogens of any invertebrate vector and are second only to mosquitoes as vectors of human disease. In the U.S., the black legged or Lyme disease tick, *Ixodes scapularis* transmits the causative agents of Lyme disease, babesiosis and human granulocytic anaplasmosis. The *I. scapularis* genome is currently being sequenced with funding from the National Institute for Allergy and Infectious Diseases (National Institute of Allergy and Infectious Diseases) and the National Institutes of Health (NIH). Sequencing is being undertaken by the Broad Institute and The Institute for Genomic Research (TIGR). This project is the first to sequence a medically significant tick species and a member of the subphylum Chelicerata. The *I. scapularis* sequencing plan calls for 100, 000 ESTs, complete sequencing of 60 BACs and shotgun sequencing to 6X genome coverage. Over 9 million reads representing approximately 3 fold coverage of the genome have been generated to date. All data types associated with the *I. scapularis* project including genomic sequence, ESTs, genome assemblies and annotations will be made available to the scientific community through the National Institute of Allergy and Infectious Diseases funded VectorBase at http://www.vectorbase.org/index.php. An overview of the *Ixodes* genome project will be provided and preliminary studies of *I. scapularis* genome organization will be presented.

2

A BORRELIACIDAL FACTOR FOUND IN THE SALIVA OF *AMBLYOMMA AMERICANUM* TICKS

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Previous studies, using a live/dead colorimetric assay, demonstrated that pilocarpine-induced saliva obtained from *Amblyomma americanum* ticks killed *Borrelia burgdorferi in vitro*. In contrast, *Ixodes scapularis* saliva obtained by similar protocol had no demonstrable effect on *B. burgdorferi*. Likewise, a pilocarpine control had no significant effect on the growth of *B. burgdorferi* in this assay. PAGE analysis of both *A. americanum* and *I. scapularis* saliva indicated at least (6) distinct protein diffferences between *A. americanum* and *I. scapularis* saliva, localized between 20 and 120 kDa. Further studies indicated that trypsinization could inactivate borreliacidal activity of whole saliva, indicating that borreliacidal activity was protein related. In an attempt to isolate a borreliacidal factor, *A. americanum* saliva was size fractionated by HPLC and 11 distinct molecular weight pools were produced and concentrated to approximate their original concentration in tick saliva. When tested *in vitro* by the borreliacidal assay, two pools (#3 and #4) demonstrated 35% and 98% killing of *B. burgdorferi* respectively. Chromatograms indicated that pool #3 is actually a shoulder which overlaps with the adjacent pool #4. Using comparative two-dimensional gels, overlaying the gels for pool #4 to an adjacent pool (#5) exhibiting no borreliacidal activity, at least (6) unique molecules were identified within pool #4. Subsequent two-dimensional gels were analyzed to compare sub-fractions of pool #4 which exhibited borreliacidal activity to sub-fractions which contained no demonstrable effect. N-terminal sequence analysis was obtained on several unique proteins isolated by this analysis and will be discussed.

3

PROTEINS AND PEPTIDES INDUCED IN THE MIDGUTS OF BLOOD-FED TICKS CONTRIBUTE TO CONTROL OF MICROBIAL INFECTIONS: NEW INSIGHTS FROM A CDNA LIBRARY OF MIDGUT TRANSCRIPTS IN *DERMACENTOR VARIABILIS*

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Blood feeding in ticks induces expression of numerous proteases, protease inhibitors, lectins, oxidative stress, heat shock and detoxifying proteins in the midgut. Sequencing a cDNA library from the midguts of feeding female *Dermacentor variabilis* revealed multiple copies of many of these classes of proteins. Included were 15 proteases, 6 protease inhibitors, 5 carbohydrate digestive enzymes, 2 Co-A ligases, 2 superoxide dismutases, a peroxiredoxin, 4 glutathione-S- transferases, other detoxifying proteins, two lectins (including one similar to Dorin-M), a von Willebrand factor peptide and a number of cell, lipid and protein binding peptides. Functional assignments were supported by matches using the ACARI, BLAST nr and Conserved Domain Databases. Although mostly concerned with intracellular digestion of endocytosed hemoglobin (only 6 had signal peptides), many of these peptides and proteins also inhibit microbial growth or are directly lethal to ingested microorganisms. Thus, the arsenal of antimicrobial agents is more extensive than just defensin or lysozyme, creating a hostile environment inimical to microbial growth. Moreover, several of these enzymes are secreted (e.g., cysteine proteases, cystatin and lectin) and may function in controlling microbial growth in the midgut lumen. In haematophagous insects, many of these same midgut proteins are important in upregulating both the cellular responses to oxidative stress and the innate immune response to microbial challenge. In this report, the putative antimicrobial roles of the tick's diverse midgut proteins are described and compared to similar roles in the midguts of blood feeding insects. The presence of many proteins with similar functions in these different protein classes suggests that gene duplication contributed to the successful adaptation of ticks to their blood feeding habit. This cDNA library may be useful to scientists wishing to investigate the role of the tick midgut in blood digestion, antimicrobial activity or survival of tick-borne pathogens acquired during blood feeding.

(ACMCIP Abstract)

4

NEW DEVELOPMENTS IN THE EPIDEMIOLOGY AND CONTROL OF TICK-BORNE RELAPSING FEVER IN EAST AFRICA

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Tick-borne relapsing fever (TBRF) caused by the spirochete *Borrelia duttonii* is common in central Tanzania, where it can be a substantial cause of serious illness. Although TBRF is known throughout the country, the extent and true burden of the disease in Tanzania, and indeed elsewhere in Africa, remain to be ascertained. Recent studies have shown that the epidemiology of TBRF is more complex than previously believed, with the demonstration that a newly discovered, and as yet unnamed, species is also a causal agent of disease and with the detection of *Borrelia spp.* infections in 11% of children with fever and in 4% of otherwise healthy children in a highly endemic region in central Tanzania. Further studies are revealing the difficulty of reliable TBRF diagnosis, particularly in symptom-based differentiation of TBRF from malaria, and have begun to investigate the extent and natural history of the *Ornithodoros sp.* soft tick vectors infesting households within endemic areas. An overview of new insights into the epidemiology of this neglected disease will be presented, and the realistic prospect of reduction of household tick infestations and control of TBRF by standard vector control methods will be considered in the light

of results from a new randomised-controlled trial of insecticide-treated bednets in central Tanzania.

5

EFFECTS OF A HORIZONTAL VECTOR CONTROL STRATEGY ON *TRIATOMA INFESTANS* INFESTATION AND CHAGAS' DISEASE TRANSMISSION IN RURAL NORTHWESTERN ARGENTINA

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In 1992, the Chagas' disease vector control program of Argentina shifted from a vertical and centralized structure to a horizontal strategy based on residual spraying with pyrethroid insecticides and on bug detection activities conducted by villagers and community leaders that lasted until 1999. We assessed the effect of such control strategy on *Triatoma infestans,* the main domestic vector, in a well-defined rural district based on longitudinal records from the Argentinean National Chagas Service. We determined the temporal variations in the domestic and peridomestic infestation by *T. infestans* and in the reported numbers of human acute cases during 1993-2004 in 272 rural communities of the Moreno department, one of the poorest and most endemic districts in northwestern Argentina. In Moreno, vector control actions reduced the prevalence of domestic infestation from 77% in 1993 to 4% in 1995 (the attack phase), but economic, logistic and political constraints affected the sustainability of the program during the surveillance phase, leading to an increased domestic infestation by *T. infestans* (range, 10-28% during 1996-2004), and a renewed vector-mediated transmission of *Trypanosoma cruzi* to humans between 1998 and 2004. When performed by villagers, the intensity and quality of residual insecticide spraying was not sufficient to eliminate domestic *T. infestans* populations. Several factors, such as insecticide spraying frequency, time elapsed since the last spraying round and spraying coverage, were associated significantly with increased prevalence of infestation by *T. infestans.* Periodic training workshops for the villagers and active supervision by official vector control programs are needed to consolidate the surveillance system and to achieve the changes in behavior that are required to sustain a horizontal vector control strategy over time.

6

EFFICACY OF ACTIVE UNDERGROUND RABBIT HOLES AROUND HOUSES FOR REDUCING THE INDOOR DENSITY OF *PHLEBOTOMUS PAPATASI*, VECTOR OF *LEISHMANIA MAJOR*, ETIOLOGIC AGENT OF ZOONOTIC CUTANEOUS LEISHMANIASIS IN TUNISIA, NORTH AFRICA

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Control of sand fly populations is largely based on insecticide residual house spraying and use of insecticide-impregnated bednets. These methods have met with variable success. These techniques also may, to varying degrees, negatively impact the environment and lead to development of resistance by sand flies. Therefore, alternatives to these methods are desirable. Zoonotic cutaneous leishmaniasis caused by *Leishmania major* and transmitted by *Phlebotomus papatasi* is endemic in central and southern Tunisia. Our entomological survey in the region of Sidi Bouzid showed high sand fly density in articificial underground rabbit holes compared to animal shelter or bedrooms. Villagers raise rabbits in man-made underground holes for food and for income. Raising rabbit in abondoned artificial underground holes in peridomestic areas reduced significantly the density of *Phlebotomus papatasi* in bedrooms. Digging new underground rabbit holes in peridomestic area of one house where rabbit have never been raised before significantly reduced the density of *Phlebotomus papatasi* in the bedroom. Disturbing rabbit holes made in the peridomestic area by cleaning all rabit feces induced a significant increase in the density of *Phlebotomus papatasi* inside bedrooms. In addition, the number of sand flies entering rabbit holes was significantly higher than the number of flies exiting the holes. These results strongly suggest that rabbits raised in underground holes in the peridomestic areas could be a zooprophylactic method to control zoonotic cutaneous leishmaniasis in rural areas. Large-scale community-based intervention is needed for an entomological evaluation of this approach.

7

EVALUATION OF NOVEL LONG-LASTING, INSECTICIDE-IMPREGNATED MATERIALS TO CONTROL ADULT SAND FLIES IN IRAQ, KENYA AND EGYPT

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Currently there are no vaccines or prophylactic drugs to protect military personnel against leishmaniasis. The only method available is to prevent bites from infected sand flies. An aggressive prevention and control program at Tallil Air Base in Iraq had minimal impact on sand fly populations and did little to protect soldiers from the disease. The goal of our study was to evaluate the efficacy of long-lasting, insecticide-impregnated materials (netting or plastic sheeting) to control adult sand flies. Field studies were conducted in Iraq, Kenya and Egypt using deltamethrin- or permethrin-treated materials, compared with non-treated materials. CDC light traps, unbaited or baited with dry ice (CO_2), were used to collect sand flies over insecticide-treated floorings and within treated bed nets and other barrier materials. Light traps inside treated bed nets or barriers caught fewer sand flies than non-treated bed nets or barriers, though living sand flies were still discovered in light traps 2-6 hours after passing through treated bed nets with different mesh sizes and insecticide treatments. Treated floorings did not reduce numbers of sand flies caught in light traps. The results of these studies are discussed in the context of improving methods to control adult sand flies using novel long-lasting, insecticide-impregnated netting or plastic materials.

8

MALARIA VECTOR CONTROL IN SUB-SAHARAN AFRICA: INSECTICIDE-TREATED NETS VERSUS INDOOR RESIDUAL SPRAYING

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Large-scale vector control is the best tool for primary malaria prevention. Indoor residual spraying (IRS) has been practiced successfully for over 60 years and helped to eliminate malaria transmission in much of the world. In sub-Saharan Africa (SSA), however, programmes and hence successes with IRS have been rather limited. From the 1990s onward the development of insecticide-treated nets (ITNs) has produced a new efficacious tool for large-scale transmission control. Many discussions have recently been conducted about the merits of both interventions, but few have been based on available epidemiological evidence. Here we compare both interventions in terms of their mortality and morbidity impact. In a companion paper we present comparatively operational aspects and cost. We reviewed systematically available evidence of impact of both interventions in the frame of two Cochrane systematic reviews. We included impact on all-cause mortality and key morbidity indicators. We used evidence from randomized controlled trials (n=22 for ITNs and n=5 for IRS) and historical studies. Finally, we included recent data on mortality impact from national-scale programmes. Both interventions

are highly effective in preventing mortality and morbidity. Overall, ITNs reduce child mortality by 18% in trials but their long-terms effect is likely to be underestimated. ITNs also reduce the number of clinical episodes by around 50%, contributing to reduce the burden of malaria to health services and households. Historical evidence of IRS impact is overwhelming. Comparative testing of ITNs versus ITNs suggests that their impact level is very similar. Finally, SSA countries having implemented large-scale vector control are demonstrating impressive child mortality trends. In conclusion, both ITNs and IRS are highly effective for primary malaria prevention in endemic countries. As a consequence, operational feasibility and cost become important parameters for deciding on one or the other strategy at country level.

9

ANEMIA PREVALENCE AMONG CHILDREN AFTER INDOOR RESIDUAL SPRAYING (IRS) ON BIOKO ISLAND

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Anemia is an important indicator of poor health status in children living in tropical countries such as Equatorial Guinea, whose government has a partnership with a consortium lead by Marathon Oil Company for malaria control efforts. IRS started on Bioko in early 2004, and a year later choloroquine was replaced by artemisinin-based combination therapy (ACT). The objective of this study was to assess if the introduction of IRS and ACT was followed by reduced anemia prevalence among children. Annual household surveys were conducted in a network of 18 sentinel sites covering all inhabited areas of Bioko. In urban sites, houses were randomly identified from satellite images and using a program to select random coordinates. In rural sites, houses were randomly selected from a listing of all eligible houses. Hemoglobin was measured in children ages 2-14 years with Hemocue photometers. Sample size was 2433 in 2004, 3089 in 2005 and 5314 in 2006. The same survey collected parasitemia data with ICT Malaria rapid tests. Between 2004 and 2006, anemia prevalence had a small but significant reduction from 73% (95%CI 67 to 79) to 67% (63 to 71%), p=0.016. In the same interval, parasitemia prevalence had decreased from 45% (40 to 51%) to 26% (20 to 33%). In 2006, anemia was associated with parasitemia in children 2 to <5 years (OR=3.8, 95% CI:2.6 to 5.6), with a significantly weaker (p=0.004) association among children ages 5 to <15 years (OR=1.7, 95%CI 1.4 to 3.0). An ecological analysis revealed that sites with persistently high parasitemia tend to have high persistent anemia prevalence as well. A comparison of mean Hb among parasitemic and non-parasitemic subjects with CDC normal values suggests that concurrent parasitemia explains a lesser part of the gap between parasitemic and the reference (CDC) population. In conclusion, anemia prevalence is decreasing slower than parasitemia prevalence, IRS and ACT are not sufficient to control anemia in this environment. To have a more substantial impact on anemia prevalence, the Bioko Island Malaria Control Project intends to increase efforts in an integrated, non-disease specific approach, procuring and distributing the proper drugs and supplements, training providers to promote access and utilization of iron supplements, mebendazole, and an iron-rich diet, and developing educational materials to increase adherence to iron supplements.

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MONITORING INSECTICIDE-TREATED BEDNET POSSESSION AND USE: COMPARISON OF DATA COLLECTED VIA HEALTH FACILITY AND HOUSEHOLD SURVEYS --- LINDI REGION AND RUFIJI DISTRICT, TANZANIA, 2005

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Insecticide-treated bednets (ITNs) are a proven intervention to prevent malaria, a leading cause of mortality in Africa. Data on ITN coverage collected at health facilities (HFs) may be an important monitoring tool for malaria control programs and local health planners. However, such data may not represent community-level coverage. This study compared data on bednet and ITN possession and use collected via HF surveys and representative household (HH) surveys (a "gold standard" method for measuring ITN coverage) in Lindi Region and Rufiji District, Tanzania. In Lindi Region, we collected data on 637 children <5 years old (under-5s) via a HF survey (444 well-child care [e.g., immunizations] and 193 sick-child visits), and on 305 HHs with at least one under-5 (including 354 children) via a HH survey. In Rufiji District, we collected data on 1317 under-5s via a HF survey (886 well-child care and 451 sick-child visits), and on 323 HHs with at least one under-5 (including 448 children) via a HH survey. HH possession of bednets by HHs with at least one under-5 was slightly higher using HF data versus HH data in both Lindi Region (96.9% vs. 90.7%) and Rufiji District (88.3% vs. 82.4%). Reported use of bednets was substantially higher using HF data versus HH data in both Lindi Region (79.7% vs. 46.3%) and Rufiji District (83.2% vs. 73.0%). Similarly, reported use of ITNs was higher using HF data versus HH data in both Lindi Region (38.3% vs. 21.5%) and Rufiji District (73.4% vs. 55.4%). Based on HF data, children attending well-child care and sick-child visits had similar levels of ITN coverage. HF-based data overestimated community-level ITN coverage in our study. Selection bias or social desirability bias may play a role as caretakers of under-5s who attend HFs may be more likely to use or report using bednets and ITNs. Additional studies of validity, cost, and utility are needed before recommending this monitoring strategy for widespread use, as overestimation of ITN coverage could lead to inappropriate public health actions.

11

ESTIMATING MALARIA INCIDENCE RATES USING LONGITUDINAL DATA: AN ANALYSIS OF THE GARKI PROJECT USING A MICRO-SIMULATION MODEL

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A challenge to the use of longitudinal surveys such as demographic surveillance systems (DSS) for evaluating anti-malaria public health intervention(s) is developing accurate estimates of malaria incidence rates. Direct estimation of incidence rates is not possible in these surveys as data collection takes place at widely spaced time points relative to the frequency of transition of the human population between infection states. We address this challenge by developing a stochastic process model that includes the unobserved dynamics but is compatible with the observed data on infection status at survey intervals. The Garki Project dataset is used to test our model. Our model assumes heterogeneity in risk of malaria infection between and within each of three age classes (young children, adolescents and adults) and that this risk is related to rainfall and the number of observed malaria parasite positive states for each individual. We assume that the duration of a malaria infection is longer for a young child than for an adolescent or adult and that the duration of infection within an age group varies according to the number

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of infections of each individual. Using an optimization software program we identify the parameter specifications that will reproduce the published incidence rates for the Garki Project. We compare the average of the weekly incidence rates per survey interval based on our micro-simulation to the corresponding published incidence rates from the Garki Project for the baseline period. We find that the incidence rates based on our micro-simulation are at least seven times higher for each age class than previous published rates. These results suggest that the estimated impact of indoor residual spraying on malaria transmission during the Garki Project is inaccurate and an improved methodology for evaluating the impact of the interventions used in Garki is needed.

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MINORITY VARIANT PFCRT K76T MUTATIONS IN MALAWI SUGGEST LURKING CHLOROQUINE RESISTANCE

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In Malawi, where chloroquine was replaced with sulfadoxine-pyrimethamine in 1993, chloroquine-resistant mutations at pfcrt76 seem to have disappeared. However, most malaria infections are polyclonal and standard PCR methods cannot detect small subpopulations of resistant parasites (minority variants) in a host with a predominantly sensitive infection. We have developed a heteroduplex tracking assay (HTA) that is able to detect pfcrt K76T mutations (CVIET and SVMNT) in minority variant populations representing as little as 1% of the total parasite population in a single patient. In 27 patients from an urban hospital (Queen Elizabeth Central Hospital), only 1 patient (3.7%) contained minority variant chloroquine-resistant mutants by HTA. In contrast, 21 of 60 patients (35%) from a rural health center (Mpemba) contained minority variant chlroquine-resistant mutants. All the mutant variants comigrated with the CVIET control (strain K1) and not the SVMNT control (strain 7g8). These results suggest that chloroquine-resistant minority variants are present in rural areas of Malawi, where, perhaps, the transition from chloroquine to SP occurred later than in urban centers. Surveillance for minority variant drug resistant mutations may be useful for decisions about antimalarial drug policy.

13

INTERMITTENT PREVENTIVE MALARIA TREATMENT DELIVERED ALONGSIDE ROUTINE VACCINATIONS IN TANZANIAN INFANTS: COVERAGE AND IMPACT ON INDICATORS OF MALARIA AND ANAEMIA

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Controlled efficacy trials of intermittent preventive malaria treatment in infants (IPTi) with SP suggest that this intervention is a promising new malaria control tool which is likely to have a measurable impact on malaria. As part of a five-year community effectiveness programme of IPTi in southern Tanzania, we have developed a strategy for IPTi to be put into public health practice if a policy recommendation is made. The IPTi strategy has been applied by the routine health services in 12 randomly selected divisions (of 24) in five rural districts of southern Tanzania, with a population of almost one million people. Implementation started in the first quarter of 2005. Between May and August 2006, health facility and household surveys will document availability of the intervention at health facility level, estimate coverage at household level and evaluate the effect of the IPTi strategy on indicators of anaemia and malaria in children aged 2-11 months. Results will be available from all 135 vaccination clinics in the area and 5,760 randomly selected households in clusters of 30 will be included in the survey. Coverage of the intervention in children aged 6-11 months and the effect on anaemia and malaria in children will be presented and discussed.

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COMMUNITY LEVEL ACCEPTABILITY OF INTERMITTENT PREVENTIVE TREATMENT FOR MALARIA CONTROL IN TANZANIAN INFANTS

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Controlled efficacy trials of Intermittent Preventive Treatment in infants (IPTi) have shown that the delivery of sulfadoxine-pyrimethamine (SP) at the time of selected routine vaccinations may be a useful addition to malaria control strategies. Following the demonstration of safety and efficacy in a randomised controlled trial in southern Tanzania a five year program is pursuing the development, implementation and evaluation of a strategy for the delivery of IPTi through routine health services. The success of any health intervention is dependent on its acceptability to health workers and the intervention's intended recipients. This is particularly important for an intervention linked to routine vaccinations as a reduction in vaccination coverage following the introduction of IPTi would be a highly undesirable outcome. It is also important to understand whether health-seeking behaviour is unduly influenced by a vaccination-linked malaria control tool. In this project the acceptability of IPTi is being explored at the community level by a team of social scientists working with eight community participant observers. Data is collected using a range of qualitative methods including focus group discussions and in-depth interviews to assess mothers' understanding of IPTi and their willingness to accept this new intervention. The results after 18 months of implementation will be presented and their relevance to larger-scale implementation discussed.

15

DISTRIBUTION OF TWO ESSENTIAL AMINO ACID TRANSPORTERS IN THE LARVAL ALIMENTARY CANAL OF THE AFRICAN MALARIA MOSQUITO AN. GAMBIAE (DIPTERA: CULICIDAE)

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To reach the second instar stage, the mosquito larval diet must have at least 10 essential L-amino acids (EA's), the set of which is uniform among many organisms. Lack of any EAA's in mosquito diet leads to arrested development and death. Consequently, the interference with molecular mechanisms of EAA's absorption e.g. plasma membrane Nutrient amino Acid Transporters (NATs) will be lethal to mosquitoes. In contrast to well understood amino acid roles, the knowledge of NATs is very fragmented in insects and just emerging in vector mosquitoes. Recently we identified a cluster of NATs in the Sodium Neurotransmitter symport Family (SNF = SLC6) which includes 7 transporters from the *Anopheles gambiae* genome. Heterologous expression of 2 of these NATs revealed high capacity transport mechanisms for aromatic substrates selective for tryptophan and indole-branched substrates and phenylalanine and

phenyl-branched substrates, denoted as agNAT6 and agNAT8, respectively. The branched aromatic substrates are critical for ecdysis, nerve function and immunity against pathogens. Here we report the spatial and polar localization of the 2 NATs in the mosquito gut with custom designed antibodies to agNAT6 and agNAT8 epitopes. Both transporters were found in the gastric caeca (GC), anterior midgut (AMG) and posterior midgut (PMG), the latter coinciding with the region of amino acid absorption. Within the PMG, both NATs were localized to the apical membranes although a weak basal integration was observed for agNAT6. In the AMG, agNAT8 was apical while agNAT6 entered both apical and basal membranes. In GC, agNAT6 integrated with the apical brush border, while agNAT8 was on the basal side. Intense basal labeling was seen for agNAT8 in the membranes of the cardia while labeling for agNAT6 was absent. Both transporters were basally located in the cell membranes of the salivary glands, suggesting their roles as substrate providers in the synthesis of salivary enzymes. Both these transporters were also highly expressed in the rectum. Our findings confirm important absorptive and secretory epithelial functions of agNAT6/8 and reveal a critical mechanism of mosquito nutrition that might be exploited in attempts to control this major malaria vector.

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MICROARRAY ANALYSIS OF DIFFERENTIAL GENE EXPRESSION IN THE MIDGUT OF *ANOPHELES GAMBIAE* LARVAE

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Although recent studies have highlighted the feasibility of larviciding as a cost-effective way of reducing the incidence of malaria in endemic areas of Africa, many aspects of the larval biology of the most important vector species, *Anopheles gambiae,* remain poorly understood. It has been proposed that this species presents a remarkable degree of physiological specialization among different sections of the midgut. As opposed to the common acid pH-based digestion strategy found in most insect larvae, *An. gambiae* presents an alkaline digestive strategy found only in a small number of insect taxa. Therefore, we believe that an increased understanding of the molecular and physiological aspects of this digestive process will provide new opportunities for the design of highly specific, environmentally sound control methods. By using microarray chips containing the whole transcriptome of *An. gambiae*, we sought to identify differential gene expression patterns among the main physiological sub-divisions of the larval midgut. In three replicate experiments, the midguts of 1,000 larvae were dissected and separated into gastric caeca (GC), anterior midgut (AM) and posterior midgut (PM). Total RNA extracted from the different regions was hybridized to microarray chips. Normalized gene expression data was used to select transcripts significantly ($p<0.01$) enriched at least four fold in each gut region as compared to whole larvae. The GC presented the greatest number of enriched transcripts (98), followed by the PM (66) and the AM (44). Analysis of the available molecular ontologies among these transcripts suggests that: a) genes associated with catalytic activity are up-regulated in the GC and AM, and b) genes associated with transporter activity constitute the most important up-regulated group in the PM. These results are compatible with existing models of mosquito digestion, where the GC and AM are considered to be areas of high enzymatic activity, and the PM is considered to be responsible for most nutrient absorption.

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THE DIFFERENTIAL GENE EXPRESSION OF DETOXIFICATION ENZYMES IMPLICATED IN INSECTICIDE RESISTANCE OF *AEDES AEGYPTI* USING A SMALL SCALE MICROARRAY

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The emergence of insecticide resistance is a major problem for vector control strategies. Metabolic resistance to insecticides is caused by 3 large multigene enzyme families; cytochrome P450s, carboxylesterase (COEs) and glutathione transfereases (GSTs). The availability of the *Aedes aegypti* genome has allowed us to develop a small-scale microarray for the dengue and yellow fever mosquito. This array, or 'detox chip', contains unique fragments from P450s, COEs and GSTs and is similar in design to the array which was developed for *Anopheles gambiae.* The chip is being used to address several questions related to the regulation and induction of these supergene families. The chip has been used to identify those genes which are putatively involved in conferring insecticide resistance in *Ae. aegypti*. We compared strains originating from Northern Thailand with resistance to DDT and permethrin and also monitored gene expression in both the adult and larval stages. The results and their implications will be discussed.

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DIFFERENTIAL GENE EXPRESSION BETWEEN M AND S FORMS OF *ANOPHELES GAMBIAE*

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The primary vector of malaria in sub-Saharan Africa, *Anopheles gambiae*, is undergoing speciation into two forms known as M and S. Association of the M form with irrigated agricultural sites (e.g., ricefields) allows it to remain reproductively active in arid environments that normally preclude the rain-dependent S form, and has resulted in increased malaria transmission spatially and temporally. Where sympatric and synchronous in West Africa, these forms mate assortatively. Nothing is known of the genetic or even phenotypic basis of their ecological and behavioral isolation, although the strongest sequence divergence maps to two "speciation islands" near the centromere of chromosomes X and 2L. As a first step to help gain insight on this question, we are comparing global gene expression between two geographic isolates each of M and S at key stages of development, using the Affymetrix GeneChip *Plasmodium/Anopheles* Array. We hypothesize that candidate speciation genes will include those whose expression patterns at a given developmental stage are similar between isolates of the same form but vary in the same direction and magnitude between isolates of different forms. We further hypothesize that at least some of these genes will map to the speciation islands. Geographic isolates of S form are derived from Mali and Kenya; those of M form are derived from Mali and Cameroon. The three developmental stages examined initially by microarray are late larval, three day old virgin females, and gravid females. Follow-up real-time PCR studies will expand the developmental profile of candidate genes from laboratory colonies and eventually from wild-caught specimens. Though drawing functional inferences will present a formidable future challenge, this study is an initial step toward identifying genes responsible for ecotypic differentiation and speciation in *A. gambiae*.

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GENETIC LINKAGE MAPPING AND EVIDENCE OF POPULATION EXPANSION IN THE WEST NILE VIRUS VECTOR CULEX TARSALIS

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Culex tarsalis is an important vector of West Nile Virus, Western Equine Encephalitis Virus and St. Louis Encephalitis Virus. Significant spatial and temporal differences in viral susceptibility have been detected in this mosquito. Until recently, tools have not been available to study genetic variability, population structure or genetic components of viral transmission in *Cx. tarsalis*. We examined genetic structure of *Cx. tarsalis* in five states using mitochondrial sequence data and microsatellite markers. Microsatellites reveal the presence of genetic structure and isolation by distance, while the mitochondrial ND4 marker indicates that all populations are panmictic. Microsatellite loci exhibit an excess of alleles when compared to observed heterozygosity in most populations. ND4 sequence analysis reveals an excess in haplotype number and in rare mutations, which deviate from expected values under mutation-drift equilibrium. Taken together, our data suggest that *Cx. tarsalis* has undergone a population expansion event in the western United States. Using microsatellites and ISSR markers, we constructed the first *Cx. tarsalis* linkage map, which will be useful in future studies to identify genetic loci associated with virus transmission phenotypes in this important arboviral vector.

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AGING, REPRODUCTION, AND INSULIN SIGNALING IN THE MOSQUITO *AEDES AEGYPTI*

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The mosquito's lifespan is a key component of its ability to vector parasites, since many parasites must spend up to two weeks developing within their vector hosts. In fact, the difference in time between the incubation period of the parasite and the mosquito's lifespan can be quite small. Thus, even a modest decrease in the mosquito's lifespan could have a large impact on disease transmission. In invertebrates and most likely other organisms the insulin signaling cascade (ISC) has the potential to regulate both lifespan and reproduction. Towards the goal of reducing mosquito lifespan, we have characterized several components of the ISC in mosquitoes including p110, the catalytic subunit of phosphoinositide (PI) 3-kinase and phosphatase and tensin homolog (PTEN) an inhibitor of the ISC. Orthologues of both molecules have been isolated from *Aedes aegypti* and the gene structure and transcripts have been determined. Seven splice variants of PTEN were identified and we have characterized the expression pattern of six of these variants and p110 throughout development and in various adult tissues. In addition, we demonstrated that an inverse relationship exists between lifespan and reproduction. Mosquitoes that reproduced multiple times had shorter lifespans than those that did not reproduce or those that went through only one reproductive cycle. Furthermore, egg production decreased during each successive reproductive cycle. Determining how the insulin signaling cascade regulates this balance between lifespan and reproduction is the first step towards engineering a short lived, but reproductively successful mosquito.

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QUANTITATIVE ANALYSIS OF THE *ANOPHELES GAMBIAE* HEMOLYMPH IMMUNE PROTEOME

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Mosquito hemolymph is an important organ in which proteins involved in metabolism, immune defense, signaling and transport are located. Hemolymph is the major resource base for malaria parasites developing in the vector, leading to the release of massive numbers of foreign cells when oocysts rupture and sporozoites migrate through the hemolymph before eventually invading the salivary glands. We have used isobaric tagging reagent (iTRAQ) and mass spectrometry to measure abundance levels of hemolymph proteins in response to sporozoite release. The proteins with the largest abundance changes appear to be enriched for immune functions, while others with less striking alteration include potential regulatory and transcription factors. Protein identities and functional classification will be discussed.

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ELEVATED HEPCIDIN LEVELS ARE ASSOCIATED WITH ADVERSE BIRTH OUTCOMES IN SCHISTOSOME INFECTED PREGNANT WOMEN

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This study was undertaken to assess the role of dysregulated iron metabolism in mediating *Schistosoma japonicum* associated adverse birth outcomes. Previously, we demonstrated that pregnant women with moderate *S. japonicum* infection have elevated systemic and placental pro-inflammation and give birth to babies with decreased birth weight compared to uninfected women. There are no data addressing the impact of inflammation-associated dysregulated iron metabolism, as assessed by pro-hepcidin levels, in mediating this deleterious birth outcome. We enrolled pregnant women residing in Leyte, The Philippines with singleton pregnancies in the second or third trimester. At enrollment, we collected: pre-natal history, SES, height, weight, and smoking status. In addition, three stools were collected and examined in duplicate by the Kato-Katz method to quantify the intensity of infection with *S. japonicum*, hookworm, *Ascaris*, and *Trichurius*. At 32 weeks of gestation, we obtained maternal blood for assessment of iron status. At delivery, we measured birth weight and obtained placental and cord blood for iron status assays. Multivariate models were adjusted for important confounders in assessing the relationship between *S. japonicum* intensity, iron status and birth outcomes. We obtained delivery data and samples on N=72 women. Maternal pro-hepcidin level, measured at 32 weeks of gestation, was positively associated with intensity of *S. japonicum* infection: women with moderate or high intensity *S. japonicum* infection had 38% higher pro-hepcidin levels compared to uninfected women (p=0.01). In addition, maternal pro-hepcidin level was a significant predictor of decreased birth weight (p=0.04), even after accounting for maternal height, weight and gravidity. Women with pro-hepcidin levels in the highest tertile gave birth to babies that were 388 grams lighter than babies born to mothers with pro-hepcidin levels in the lowest tertils (p=0.004). In conclusion,

these results suggest that decreased maternal bio-availability of iron, as measured by pro-hepcidin, is an important mechanism mediating low birth weight, likely through decreased availability of iron to the growing fetus.

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SCHISTOSOMA JAPONICUM REINFECTION AFTER PRAZIQUANTEL TREATMENT CAUSES ANEMIA OF INFLAMMATION

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There is a relationship between schistosomiasis and anemia, though the magnitude and exact mechanisms involved are unclear. In a cohort of 580 *Schistosoma japonicum* infected 7-30 year-olds from Leyte the Philippines we evaulate the impact of reinfection with *S. japonicum* after treatment with praziquantel on mean hemoglobin, iron deficiency and non-iron deficiency anemia (IDA and NIDA), and inflammatory markers. All participants were treated at baseline and followed-up every 3 months for a total of 18 months. At each follow-up, participants provided stools to quantify reinfection and venous blood samples for hemograms and measures of iron status and inflammation. After 18 months, reinfection with *S. japonicum* was associated with a -0.39 g/dL (95% CI: -0.63, -0.16) lower mean hemoglobin and 1.70 (95% CI: 1.10, 2.61) times higher odds of all-cause anemia, compared to no reinfection. Reinfection was associated with IDA in the high reinfection intensities only. Conversely, reinfection was associated with NIDA in all infection intensities. Reinfection was associated with serum IL-6 responses ($p<0.01$) and these responses were associated with NIDA ($p=0.019$), but not with IDA ($p=0.29$). Our results provide strong evidence for the causal relationship between *S. japonicum* infection and anemia. Rapid reinfection led to a reversal of the positive treatment effect on hemoglobin, within one year of treatment. The principle mechanism involved in *S. japonicum*-associated anemia is that of pro-inflammatory cytokine-mediated anemia, with iron deficiency playing a role in high intensity infections. Based on the proposed mechanism, anemia is unlikely to be ameliorated by iron therapy alone.

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TH2 CYTOKINES ARE ASSOCIATED WITH PERSISTENT HEPATIC FIBROSIS IN HUMAN *S. JAPONICUM* INFECTION

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Hepatic fibrosis is among the most serious consequences of chronic schistosomiasis, is more common among males and is thought to result from a dysregulated host immune response. No studies have assessed cytokine profiles that may be involved in *Schistosoma japonicum*-associated hepatic fibrosis. We conducted a prospective, community-based longitudinal treatment-reinfection study in Leyte, the Philippines, among 611 *S. japonicum* infected subjects aged 7-30 years, to evaluate the relationship between cytokine production and hepatic fibrosis independent of other predictors of fibrosis. Participants were treated with praziquantel at baseline. Abdominal ultrasound to detect hepatic fibrosis (grade I-III) was performed at baseline and 12 months post-treatment. Stool for assessment of *S. japonicum* infection was collected at baseline and at 3, 6, 9 and 12 months post-treatment. Cytokines (interleukin [IL]-4, IL-5, IL-10, IL-13, tumor necrosis factor [TNF]-α and interferon [IFN]-γ) produced by peripheral blood mononuclear cells (PBMC) in response to soluble worm antigen preparation (SWAP), soluble egg antigen (SEA) and control media were measured once 4 weeks post-treatment. Multivariate models adjusted for confounders were used in all analyses. IL-4 in response to SWAP and IL-10 in response to both SWAP and SEA were associated with presence of baseline fibrosis (all $P<0.03$). In subjects with baseline fibrosis, IL-4 to SWAP, and IL-5 and IL-13 to both SWAP and SEA were associated with persistent fibrosis at 12 months post-treatment (all $P<0.05$). Males showed consistently stronger Th2 cytokine responses to both SWAP and SEA compared to females (all $P<0.02$). In conclusion, these results suggest an independent role of Th2-biased cytokine responses to *S. japonicum* antigens in persistent hepatic fibrosis, and indicate that Th2 cytokines may contribute to the higher prevalence of fibrosis in males. Considering our previous findings of the protective role of Th2-cytokines against reinfection with *S. japonicum*, concern regarding enhanced hepatic morbidity accompanying a Th2-boosting vaccine is warranted and should be evaluated in future vaccine trials.

(ACMCIP Abstract)

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SCHISTOSOMA MANSONI DERIVED HIGH MOBILITY GROUP BOX-1 (HMGB-1) PROTEIN MAY HAVE AN IMPORTANT ROLE IN EGG-INDUCED GRANULOMA IN SCHISTOSOMIASIS MANSONI

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Recently, we identified and cloned the homologue of a human HMGB-1 protein (SmHMGB-1) from *Schistosoma mansoni*. *In vitro* studies showed that SmHMGB-1 is a potent inducer of TNF-γ from macrophages. Subsequent characterization studies revelaed that SmHMGB-1 is expressed in various life-cycle stages of the parasite, more importantly the egg

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stages. Therefore, in this study we analyzed whether SmHMGB-1 has any role in egg-induced granuloma. Results from our immunohistochemical studies showed that SmHMGB-1 protein is highly expressed in miracidia. In vitro studies confirmed that significant quantities of SmHMGB-1 are secreted by egg stages. Interestingly, circulating levels of SmHMGB-1 antigen were significantly increased in the serum of infected animals, especially during egg laying. To determine whether SmHMGB1 has any effect on granuloma cells, we then determined the cytokine profile of granuloma macrophages exposed to rSmHMGB-1. These studies confirmed that SmHMGB-1 can induce significantly high levels of TNF-γ from granuloma macrophages. This TNF-g-inducing activity of SmHMGB-1 was associated with its B box domain and this function could be blocked by anti-SmHMGB-1 antibodies. These findings suggest an important role for SmHMGB-1 in egg-induced granuloma in *Schistosomiasis mansoni*.

(ACMCIP Abstract)

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THE EFFECT OF PRAZIQUANTEL TREATMENT ON IMMUNE RESPONSES AGAINST *SCHISTOSOMIASIS MANSINI* DURING PREGNANCY: CYTOKINE AND ANTIBODY RESPONSES IN PREGNANT WOMEN AND THEIR INFANTS

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Treatment of schistosomiasis with praziquantel exposes the worms to immunologically mediated killing and leads to a boosting of anti-schistosome responses, with a 'type 2 helper T-cell' bias that may protect against re-infection. WHO policy first recommended praziquantel treatment during pregnancy in 2002, but there is no information on the immunological advantages or disadvantages of schistosomiasis treatment during pregnancy for mothers or their infants. To elucidate the effect of praziquantel treatment against *S. mansoni* infection during pregnancy on anti-schistosome immune responses of pregnant women and their infants. A cohort of 427 S. mansoni infected women was recruited within a larger trial of antihelminth treatment in pregnancy. After providing stool and blood samples women in the second or third trimester of pregnancy were randomised to receive praziquantel or placebo. Six weeks after delivery all women were given praziquantel and albendazole treatment. Maternal whole blood culture cytokine responses, plasma cytokines and antibody responses to S.mansoni worm (SWA) and egg antigens (SEA) were measured before and, six weeks after each treatment. Babies' responses were measured in cord blood and at one year of age. Preliminary analysis of type-1 and type-2 cytokine-responses at baseline show negative correlations with intensity of *S. mansoni* infection, weak negative associations with gestational age, and no association with maternal age, gravidity, or socio-economic status. In general, maternal responses appear markedly higher after delivery than during pregnancy. The last mother follow-up sample will be obtained in June 2006 and 240 babies of cohort mothers have provided samples at age one year. An unblinded analysis will be done in August 2006 when cytokine and antibody assays are completed. It is these unblinded results that will be presented at the meeting.

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SCHISTOSOMA MANSONI INFECTION INCREASES SUSCEPTIBILITY TO AIDS VIRUS INFECTION TRANSMISSION AND REPLICATION IN NON-HUMAN PRIMATES

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We sought to test the hypothesis that helminth infection will increase host susceptibility to AIDS virus infection. We utilized a rhesus monkey coinfection model involving *Schistosoma mansoni* and an R5-tropic clade C simian-human immunodeficiency virus (SHIV). Systemic viral infection could be achieved with 17 times less virus in animals with schistosomiasis compared to parasite-free animals. In addition, peak viral loads in coinfected animals were significantly higher ($P = 0.0159$) than in control monkeys. Coinfected animals demonstrated a significant decrease in their memory CD4+T cell counts, a sign of viral pathogenesis, as early as 12 weeks after SHIV inoculation ($P = 0.04$), while memory CD4+ T-cell counts in parasite-free SHIV-infected animals remained stable up to 45 weeks after SHIV inoculation. Together, these data are the first *in vivo* evidence that infection with schistosomes significantly increases the risk of AIDS virus transmission. The implications of these findings are that *S. mansoni*-infected individuals may be more susceptible to HIV infection, progress more quickly to AIDS, and have a higher risk potential to spread HIV infection.

(ACMCIP Abstract)

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RESISTANCE TO *S. JAPONICUM* REINFECTION IN MATURE WOMEN IS NOT MEDIATED BY ADAPTIVE CYTOKINE RESPONSES

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This study was undertaken to examine the role of antigen specific cytokine responses in mediating the increased resistance to *Schistosoma japonicum* reinfection observed in older women. In many endemic communities, resistance to schistosome infection increases with age and is greater in females compared to males, even after accounting for differential water contact. In previous work, we determined that increased Th2/Th1 responses to Sj97 and SWAP are associated with resistance to reinfection. Using a longitudinal treatment-reinfection study design (N=576), we evaluated the relationships among sex, pubertal development and protective Th2/Th1 cytokine ratios to SWAP and Sj97 with resistance to reinfection after PZQ treatment. We evaluated the impact of sex and pubertal development (mature vs immature) on time to reinfection using proportional hazards models. We evaluated the impact of pubertal development and sex on levels of protective cytokine responses using multivariate regression. Mature females had significantly longer times to reinfection (11.49 months) compared to immature females (8.71 months), immature males (8.14 months), or mature males (9.78 months) even after adjusting for differential water contact (all $P < 0.02$). Elevated Th2/Th1 cytokine ratios to SWAP and Sj97, which have been associated with resistance to reinfection in this cohort, were not elevated in mature

females. In conclusion, these data indicate that mature females are significantly more resistant to *S. japonicum* reinfection than males or immature females. Mechanisms other than enhanced adaptive cytokine responses, such as augmented innate immune responses, may mediate this relative resistance.

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BARRIERS TO PROMPT AND EFFECTIVE MALARIA TREATMENT IN RURAL TANZANIA: BETTER DRUGS IS NOT ENOUGH

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Appropriate treatment of malaria episodes, especially in young children, is a mainstay of the malaria control strategy in endemic countries. Unfortunately, in most sub-Saharan settings, the Abuja target of reaching 60% of those suffering from malaria with effective treatment are far from being reached.

Access to appropriate and timely treatment is hampered by inter-linked factors at different levels (household, health system, policy). Availability of an efficacious drug at a low wholesale price is an important but not sufficient element to ensure treatment effectiveness. Functioning health services, alternative treatment options, household circumstances, geographic location and policy and regulatory issues are just a few among other important factors. To effectively improve access to treatment, it is crucial to address all obstacles. Within the frame of a program to improve access to malaria treatment in rural Tanzania we assessed the relative importance of a range of potential obstacles to effective malaria treatment. Data were derived from several complementary quantitative and qualitative studies on disease perception, treatment seeking, drug availability and quality of care conducted in the area of a Demographic Surveillance Site (DSS) in south-western Tanzania. Descriptive and logistic regression analyses were used to identify factors significantly correlated with a positive treatment outcome (administration of correct dose of recommended first-line antimalarial within 24 hours of onset of symptoms). 80 children with a recent fever episode were included and dependant variables comprised among others: demographic information, recognition of symptoms, perceived severity of the illness, distance, availability and quality of nearest care or treatment providers, and socio-economic status. Risk factors are then discussed in relation to three major ongoing interventions: social marketing, improvement of quality of care and services in health facilities and commercial drug selling shops. In conclusion, large number of factors can positively or negatively influence access to treatment and their importance may vary considerably between settings. To design effective interventions to improve access to malaria treatment it is crucial to know the relative importance of these factors in a given setting so that limited resources can be invested most effectively.

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EARLY TREATMENT OF UNCOMPLICATED MALARIA WITH A COMBINATION INCLUDING AMODIAQUINE: FOLLOW UP OF 175 CLINICAL ATTACKS IN SENEGAL, 2004-2005

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Acceptability of drug in the community and early treatment of clinical attacks is a major challenge for malaria control. In 2004-05, amodiaquine + sulfadoxine-pyrimethamine (AQ+SP) combination was chosen by Senegal as first-line treatment of uncomplicated *Plasmodium falciparum* malaria. Since April 2006, it has been replaced by artesunate + AQ combination. Based on a two year follow up and 175 malaria attacks treated with AQ+SP, this work aims to draw attention to the poor tolerance and acceptability of a combination including AQ. All inhabitants of a Senegalese village with seasonal malaria transmission, who accepted to participate to a daily clinical surveillance, were included. In case of fever +/- symptoms suggesting malaria, confirmed by a positive blood film, AQ+SP was given for 3 days by the medical team. A total of 73 clinical attacks, fulfilling inclusion criteria of WHO standard protocol for drug efficacy evaluation, were followed up for a 42 day period. Patients were visited 3 times a day for the 3 day-treatment and observed in the Health Centre during 1/2 hour after each oral administration. Among 73 clinical attacks included in the efficacy follow-up, only five patients were lost to follow-up, late clinical failure occurred in one patient and late parasitological failure in two. Among 175 confirmed cases of *Pf* malaria attacks, 50 (29%) vomited before treatment. Among the other patients, 51 (41%) began to vomit after treatment, including 17 patients who vomited more than once. This high frequency of vomiting was observed even though 65 (37%) patients received anti-emetic treatment. Finally, four patients presented scratching during treatment. In conclusion, poor tolerance of AQ+SP raises the problem of its acceptability in the community. It is to be feared that poor tolerance will occur for the new combination also including AQ. Therefore complementary studies should be carried out urgently on the tolerance of the new combination, because poor tolerance will result in delayed treatment and potentially promote drug resistance.

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EPIDEMIOLOGY OF CONGENITAL MALARIA IN NIGERIA: A MULTI-CENTRE STUDY

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Contrary to earlier reports, congenital malaria is increasingly reported in endemic areas of the world. However, most reports involve small case series, thereby making it difficult to evaluate the true burden of malaria in the newborn. The objective of this study was to define the incidence of congenital malaria and the clinical consequences of malaria in the neonate in Nigeria with a view to providing a framework for its control. In a prospective multi-center study, 2,500 consecutive mother-baby pairs were enrolled over 12 months. Blood smears were prepared from mothers, placental aspirates, cord blood and neonates within 4 hour of delivery. We here report results of 1875 mother-baby pairs in the per protocol population. Patent malaria parasitaemia was detected throughout the year at all sampling sites in all centers. Parasitaemia was detected in 95 neonates (5.1%) with its occurrence varying between study centers. The mean parasite density among infected neonates was low [Mean = 48/µL, range 8 -200/µL)]. Thirty two of 95 neonates (33.7%) were symptomatic within 3 days of birth. Fever and refusal to feed were the most commonly observed symptoms. Despite reports of varying levels of chloroquine resistance in Nigeria at the time of the study, response of malaria infection to chloroquine among both symptomatic and asymptomatic babies was good with prompt clearance of parasitaemia and a cure rate of 88.4% at D 14. The infection which failed to respond to chloroquine was cured with oral sulfadoxine-pyrimethamine. In contrast, spontaneous clearance of

patent parasitaemia occurred in 59 of the neonates (62.1%) before day 2. Antepartum maternal and placental parasitaemia were the most important risk factors for patent neonatal parasitaemia. In conclusion, congenital malaria is often asymptomatic, clears spontaneously and may not warrant treatment. Newborn babies with unexplained fever and refusal to feed in malaria endemic areas should be screened for malaria and treated with effective antimalarial drugs. Possible augmentation of neonatal response to chemotherapy by transplacentally transferred immunity by semi-immune mothers make chloroquine and sulfadoxine-pyrimethamine viable therapeutic options to consider in the treatment of congenital malaria in the delicate neonate especially as the safety of artemisinin containing combination therapy has not been clearly established in infants and children less than 5kg.

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USE OF ULTRASOUND TECHNOLOGY TO INVESTIGATE THE TEMPORAL RELATIONSHIP BETWEEN MATERNAL MALARIA INFECTION AND *IN UTERO* FETAL GROWTH

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Malaria infection during pregnancy is associated with adverse perinatal outcomes, including small-for-gestational age (SGA) and low birth weight (LBW). Little information exists, however, regarding the *in utero* effects of malaria or the temporal relationship between infection and fetal growth, including intrauterine growth restriction (IUGR). To better understand the *in utero* effects, we conducted a prospective cohort study between May 2005 and May 2006 in Kinshasa, Democratic Republic of Congo. Women seeking first antenatal care were eligible to participate if they were ≥18 years old, had no evidence of pre-eclampsia and had an ultrasound confirmed pregnancy of ≤22 weeks gestation. Participants were monitored monthly for parasitemia and longitudinal sonographic assessment of fetal growth. Patient demographics, pregnancy history, and longitudinal measurements of nutrition, anemia and pre-eclampsia were ascertained. At delivery, newborn biometry and maternal peripheral and placental malaria were assessed. IUGR and SGA were defined as estimated fetal weight and birthweight, respectively, at <10th centile for gestational age. 182 of 1,111 first antenatal care attendees met all enrollment criteria and consented to participate. The median number of ultrasounds per women was five (Range 2-8) and delivery outcomes were obtained for 178 of 182 women (97.8%). At enrollment, the mean ultrasound confirmed gestational age was 18 weeks (SD 3 weeks) and mean maternal age was 27 years; 25.8% were primigravid and 2.7% HIV positive. Sixty percent of women had at least one antenatal malaria infection and 28.5% had ≥2 infections. Nearly one-third of fetuses (30.3%) had at least one ultrasound examination that indicated IUGR and 27.2% were SGA at delivery; 9.2% of infants were LBW and 4.6% were delivered preterm (<37 weeks). In bivariate analyses, 2nd trimester malaria was associated with a higher risk of both IUGR and SGA than 3rd trimester infection. Fetuses that were chronically exposed (≥3 maternal infections) were three times as likely to have an IUGR event as fetuses with ≤2 exposures (RR= 3.16, 95%CI 1.73, 5.77). Multivariate random-effects models that account for correlated, repeat measures data will also be presented. This research will help identify crucial times during pregnancy when malaria infection is most harmful, highlighting windows when presumptive antimalarial interventions may have the greatest impact.

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CHANGING EPIDEMIOLOGY OF *PLASMODIUM* BLOOD-STAGE INFECTIONS IN THE WOSERA REGION OF PAPUA NEW GUINEA

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In Papua New Guinea (PNG), complex patterns of malaria commonly include single and mixed infections of *Plasmodium falciparum*, *P. vivax*, *P. malariae*, and *P. ovale*. Here, we assess recent epidemiologic characteristics of *Plasmodium* blood-stage infections in the Wosera region through four cross-sectional surveys (August 2001 to June 2003). Whereas previous studies performed here have relied on blood smear / light microscopy (LM) for diagnosing Plasmodium species infections, we introduce a newly developed, post-PCR, semi-quantitative, ligase detection reaction-fluorescent microsphere assay (LDR-FMA). A direct comparison of the two methods for over 1,100 samples showed that diagnosis was concordant for >80% of the analyses performed for *P. falciparum* (PF), *P. vivax* (PV) and *P. malariae* (PM). Greater sensitivity of the LDR-FMA accounted for 75% of the discordance between diagnoses. Based on LM, the prevalence of blood-stage PF, PV and PM infections was found to be markedly reduced compared to an early 1990s survey. In addition, there were significant shifts in age distribution of infections with PV becoming the most common parasite in children under 4 yrs of age. Consistent with previous studies, prevalence of all Plasmodium species infections increased significantly in samples analyzed by the PCR-based LDR-FMA. This increase was most pronounced for PM, PO and mixed infections and in adolescent (10-19 years) and adult age groups, suggesting that light microscopy may lead to under-reported prevalence of less common Plasmodium species, infection complexity, and a skewed distribution of infections towards younger age groups. This study shows that the application of LDR-FMA diagnosis in large epidemiological studies or malaria control interventions is feasible and may contribute novel insights regarding the epidemiology of malaria.

(ACMCIP Abstract)

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TOPOGRAPHY, LAND-COVER, AND ELEVATION PREDICT AREAS AT RISK FOR MALARIA WITHIN COMMUNITIES IN A HIGHLAND REGION OF WESTERN KENYA

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Although highland regions of East Africa appear to be experiencing increased transmission of *Plasmodium falciparum*, higher elevation is a protective factor for the estimated 34 million people living in these areas, in part because lower temperature slows the development of both parasites and mosquitoes. Other aspects of the terrain, however, such as topography and land-cover, also affect habitat suitability for *Anopheles* breeding and thus risk of malaria transmission. In this study, we apply GIS and very high-resolution remotely sensed imagery to investigate the role of these factors in determining malaria risk at small, sub-community scales. A case-control study was conducted to identify environmental variables associated with malaria within two subdistricts in the western Kenyan highlands using hydrologic techniques to model the predicted

flow of water across the surface of the landscape. These surface analyses, derived from digital terrain models, were used to generate indices describing predicted water accumulation in the fields, pastures, and vegetated regions surrounding the 1,341 households in the study area. Cases were comprised of the 1,230 individuals presenting to clinics with slide-confirmed malaria from 2001-2004, while all 7,391 non-presenting individuals were considered to be controls. Case households were located an average distance of 498m from regions with very high wetness indices, compared to an average distance of 539m for controls ($t=3.28$, $p<0.01$). Distance to high wetness indices remained an independent predictor of risk when controlling for household elevation in multivariate regression ($\chi\eta\iota^2=17.9$, 1 df, $p<0.0001$). Additionally, associations were demonstrated between land-cover composition (e.g. area of forested or farmed land) around households and malaria incidence at the household level. The strength of these associations was modified by predicted water accumulation in these regions. Combining hydrologic flow models with remotely sensed imagery appears to offer a valuable tool in predicting highland regions at risk for malaria.

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THE USE OF PERSONAL DIGITAL ASSISTANTS FOR DATA ENTRY AT THE POINT OF COLLECTION IN TROPICAL MEDICAL RESEARCH

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Advances in information technology have created new opportunities for data collection and processing in tropical medical field research. In the last two years electronic handheld devices, or Personal Digital Assistants (PDAs) have been used for a variety of applications in rural southern Tanzania. Interviewees' responses are entered directly into the PDA instead of being written on paper. The PDAs are able to ensure that data entry fields are not left blank, that data entry values are in acceptable pre-defined ranges, that responses are internally consistent with each other and that skip patterns are properly handled. The chore of double data entry and reconciliation is no longer necessary. We present experiences with PDA use for data capture in (i) a cross-sectional survey of over 21,000 households (ii) recruitment and follow-up of participants in clinical trials following a standard anti-malarial drug efficacy testing protocol and (iii) the documentation of activities in a time and motion study. In each situation the data capture form was loaded onto the PDAs using Pendragon Forms v4.0 and then installed on Palm m130 PDAs. Interviewers, who typically had only four years' secondary school education and no prior experience with computers, had no difficulty learning to use PDAs. Each interviewer had a PDA and a solar charger, and team supervisors were given access to a laptop for backing-up and monitoring data. Data completeness was very high, eg 99.7% (n=21,529) of intended households. Inclusion, exclusion and withdrawal criteria were adhered to throughout the clinical trials and summaries of trial status were available immediately and on demand during the course of follow-up. The logistics of the time and motion study were eased and accuracy of timings enhanced by use of PDAs. In each setting, data were available for cleaning and analysis within 24 hours of completion of data collection. The PDAs were well accepted by interviewers, supervisors and interviewees alike. Electronic handheld devices can be useful tools even in rural, tropical, resource-poor settings.

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PUBLIC HEALTH ISSUES AND FIRE ANT ENVENOMATION: NEW PERSPECTIVES ON AN AGE-OLD PROBLEM

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Insects from the Family Formicidae in the Order Hymenoptera comprise some of the most ubiquitous of all insects, the ants. Species in this family are universally social with varying versions of a caste system. Most possess a painful bite, sting or ability to emit other deterrent chemicals (e.g., formic acid for which the family is named). In particular two imported fire ant (IFA) species, *Solenopsis richteri* (Black Imported Fire Ant, BIFA) and *S. invicta* (Red Imported Fire Ant, RIFA) are of immense importance. Both species have painful, venomous stings, strong defense instincts and attack en masse when disturbed. Throughout the Americas, they represent an important factor in human morbidity and mortality. Geographic information, so skillfully pioneered by epidemiologists such as John Snow a century and a half ago, provides an invaluable link between epidemiologic and temperospatial variables. Such a synthesis should provide a useful method to mesh population-based health, epidemiology, environmental health, economic concerns and entomology to reveal relationships between these disciplines allowing for better control. With the improvements in desktop geographic information systems (GIS) and global positioning systems (GPS) investigators can probe relationships between the epidemiology of ant envenomations and other factors that to improve population health. As this study will demonstrate, fire ants--long considered in the tropics as primarily agricultural pests--have a public health significance that may well be equally important. As reported previously IFA morbidity and mortality notes that in endemic areas the incidence of anaphylaxis to IFA stings exceeds that of all other Hymenoptera combined. Public health issues, combined with insects that can be as aggressive in their population dynamics as their individual behavior, emphasize the need for research into the public health-IFA nexus. Future research, should synthesize further the interface of medical entomology, agriculture, environmental science and epidemiology from a wide range of sources.

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CUTEREBRA CUTANEOUS MYIASIS - NEW HAMPSHIRE, 2004

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Cutaneous myiasis, or skin infestation by fly larvae, is uncommon among humans in North America, with <60 cases reported in the literature since 1941. However, *Cuterebra* (rodent or rabbit botfly) species are known to cause cutaneous myiasis among small animals in North America, particularly the northeastern United States and southeastern Canada. In the rare instance of human myiasis, the inflammatory response resolves upon spontaneous extrusion or extraction of larvae. Usually no antibiotics are needed, although secondary bacterial infections can occur. Four residents of New Hampshire with *Cuterebra* cutaneous myiasis are described. A chart review of the four patients was performed. Diagnosis was confirmed by entomologist review of extracted larvae. Mean age of affected persons was 27 years (range: 4-55). Two patients were male. All illness onsets occurred during the summer. Body areas affected included neck, cheeks, and thigh (2). All patients had a single, erythematous, indurated, nodular lesion measuring 7-15 mm; two lesions had a central pore. Two patients described an itching, burning sensation at the site. A botfly larva was extracted from the lesions in three patients, and spontaneously extruded from the lesion of the fourth. Diagnosis was made after the larvae were noted, a mean of 7 days after initial health-care evaluation (range: 4-11). All four patients were prescribed antibiotics,

two before the diagnosis of myiasis was made, and all continued to take antibiotics after the diagnosis. In conclusion, physicians in areas where *Cuterebra* species are endemic should maintain a high index of suspicion for myiasis among patients who present with a typical clinical picture as observed among these patients. Such awareness might aid in more rapid and accurate diagnosis and treatment.

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RESPONSE OF *AEDES ALBOPICTUS* TO SIX TRAPS IN SUBURBAN SETTINGS IN NORTH CENTRAL FLORIDA

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Aedes albopictus (Skuse), a proven disease vector and severe nuisance mosquito, has in the past 20 years, become established in over 1,000 countries throughout 32 southern and Midwestern states. In a nation-wide survey, *Ae. albopictus* was recently ranked the second most troublesome mosquito in the U.S. by vector control and public health service personnel. We tested six adult mosquito traps in suburban Gainesville Florida for effectiveness in collecting *Ae. Albopictus*. Two commercially available traps, the Mosquito Magnet Professional and Liberty traps, designed for homeowner premises control of mosquitoes, were compared against a standard surveillance CDC 512 light trap, a Mosquito Magnet MM-X prototype surveillance trap, and two traps designed specifically to capture *Aedes* (*Stegomyia*) mosquitoes: the Wilton trap and the Fay-Prince omnidirectional trap. Traps were rotated through six residential properties around Gainesville in a 6X6 Latin square design during the peak adult *Ae. Albopictus* season, July, August, and September (2004). Mosquito Magnet traps were optimally baited with octenol and Lurex™ cartridges. Those traps not generating CO_2 were supplied with compressed CO_2 at the rate of 500 ml/min. Significantly more *Ae. Albopictus* ($p < 0.0001$) were captured in commercial traps (MM Liberty, Pro and MM-X) than in standard surveillance (CDC) or *Aedes* (*Stegomyia*) traps (Fay-Prince, Wilton traps). Order of rank and trap means were: MM Liberty (88.4) > MM-X (81.6) > MM Pro (65.8) > Fay-Prince (26.3) > CDC 512 (18.1) > Wilton trap (13.2). Results indicate new, commercial traps are a suitable substitute for older, routinely used surveillance traps.

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TICK INDUCED TH2 POLARIZATION OF HOST INTRACELLULAR CYTOKINES BY INFESTATION WITH *IXODES SCAPULARIS* NYMPHS

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Tick saliva inhibits host hemostasis, pain/itch and immune responses to facilitate blood feeding and transmission of infectious agents. In order to determine if Ixodes scapularis infestation can cause Th2 polarization of antigen specific CD4 T-cells, 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 tick infested recipients. BALB/c mice were exposed to a primary infestation with Borrelia burgdorferi strain B31 infected nymphs or an infected tick challenge occurred after one to three infestations, each separated by a tick-free period of 14 days, with pathogen-free nymphs. Each mouse was infested with fifteen nymphs on days -4 and -1 prior to adoptive transfer of 1 $x10^6$ clonotypic T-cells on day 0 and intradermal injection of 200 µg HA at the tick feeding site at the same time. Clonotypic T-cells were collected from lymph nodes draining the bite site on day +4 and re-stimulated for 5 hours with 100 µg HA. Cells were immunophenotyped, their division assessed by CFSE dilution, and intracellular cytokine staining performed. Infestation with pathogen-free ticks caused a small but significant increase in the percentage of clonotypic T-cells producing IL-4, while infestation with Borrelia burgdorferi infected ticks resulted in an increase of approximately 50% of that induced by pathogen-free tick infestation. One to four infestations with pathogen-free ticks reduced IL-4 (and TNF-) polarization and T-cell proliferation. Similar findings were obtained if an infected tick challenge was given after one to three infestations with pathogen-free ticks. Taken together, these results suggest that ticks promote host CD4 cells specific for associated antigens to express IL-4, perhaps because IL-4 facilitates tick feeding and/or pathogen transmission, and that the host adapts over multiple infestations by producing less IL-4.

(ACMCIP Abstract)

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GENETIC CHARACTERIZATION OF *TRYPANOSOMA CRUZI* ISOLATES FROM *TRIATOMA* SPP. IN THE UNITED STATES BASED ON SSU RIBOSOMAL RNA GENE SEQUENCES

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Trypanosoma cruzi (Euglenozoa; Trypanosomatidae), the protozoan pathogen of Chagas disease, and its triatomine (Hemiptera: Reduviidae) insect vectors have been discovered in diverse ecological niches throughout North and South America. Eleven species of triatomine bugs have been reported in the United States. Seven *Triatoma* species have been reported from the state of Texas with prevalence of *T. cruzi* infection ranging from 17-48%. Locally-acquired *T. cruzi* infections have been documented in a wide range of mammalian hosts in Texas including humans, non-human primates, domestic dogs, and other wildlife. Since the discovery of the pathogen in U.S. triatomine bugs in 1916, little work has been done to characterize the genetic variability of native strains of the parasite. In the present study, nuclear-encoded small subunit ribosomal RNA gene sequences from more than 40 Texas *T. cruzi* isolates were analyzed. Parasite DNA was extracted, amplified and sequenced from the hindgut of four *Triatoma* species (*T. gerstaeckeri*, *T. sanguisuga*, *T. protracta*, and *T. indictiva*) collected in 12 counties across Texas. Variation is present among isolates from within and across triatomine host species. The isolates do not cluster based on geographic location within Texas. However, sequences from Texas isolates vary significantly from non-U.S. isolates. Additional comparisons are currently being made with Texas canine and non-human primate *T. cruzi* isolates.

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MOLECULAR QUANTIFICATION OF FECAL ANAEROBIC FLORA IN HEALTH AND IN ACUTE DIARRHEA

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The human colon normally harbors a large (1012 bacteria/g feces) and diverse bacterial flora. This flora interacts with the host at both local and systemic levels, resulting in a broad range of immunological, physiological and metabolic effects. The status of the colonic anaerobic bacterial flora in diarrhea has been poorly characterized, because these organisms are often difficult to culture. Real time PCR with SYBR Green detection was used to quantitate common fecal anaerobic bacteria, by using primers targeted at the 16S rDNA of six common genera of fecal anaerobes, which together make up approximately 85% of the fecal anaerobes. Fecal DNA was extracted using QIAGEN stool extraction kits. Subjects providing feces for study included healthy volunteers of different ages derived from a rural community, healthy babies attending the Well Baby Clinic, and patients with diarrhea presenting to the Emergency Services. Real-time PCR amplification was established for the following bacterial genera and species: *Bacteroides-Prevotella* genus, *Bifidobacterium* genus, *Lactobacillus*

gp, *C. coccoides* gp, *R. productus* gp, and *Eubacterium* gp, which together form the major anaerobic flora. Bacterial numbers were expressed as a fraction of total bacteria (amplified using a universal primer set). In health and in acute diarrhea, there was wide variation in the composition of the fecal anaerobic bacteria. In conclusion, real time PCR amplification of 16S rDNA can provide quantitative data on the patterns of fecal anaerobic bacterial flora. This technique is potentially useful in examining alterations in colonic bacterial flora in diarrheal disease.

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COMMUNITY PERCEPTIONS OF BLOODY DIARRHEA IN THE URBAN SLUMS OF KAMALAPUR, BANGLADESH: IMPLICATIONS FOR A *SHIGELLA* VACCINE

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In Bangladesh, shigellosis causes an estimated 35,000 child deaths annually; mortality rates can reach 75,000 during epidemic years. Understanding local perception of disease causation would help formulate better strategies to prevent shigellosis. A cross-sectional survey to describe perceptions of bloody diarrhea (BD) was conducted in the slum of Kamalapur, Bangladesh. Using available census data, households within Kamalapur were randomly selected for interview. Between March and June, 2003 we interviewed persons > 15 years of age in selected households using a standardized questionnaire addressing perceptions of BD. We interviewed 541 persons. Tubewells were the main water source for 325 (60%) households; 428 (79%) reported using a latrine. Exposures that respondents believed increased the risk of BD included eating food with chili (502, 93%), standing sewage (424, 78%), drinking dirty or unboiled water (391, 72%), and not washing hands after defecating (357, 66%). Strategies that were believed to prevent BD included improving the water supply (259, 48%), sanitary disposal of feces (253, 47%), and avoiding chili (219, 41%). Washing hands before handling food was reported by 157 (29%), and 191 (35%) reported washing hands after cleaning a child after defecating. Only 134 (25%) households believed there was medical treatment for BD. Overall, 507 (93%) respondents perceived that a vaccine could prevent BD; 533 (99%) would take a vaccine. If the vaccine provided lifetime protection, 445 (83%) households, whose median monthly income was $105 (range, $60 - $175), reported they would pay a median of $0.05 (range, $0.01 - $0.15) to get the vaccine. Community perceptions regarding causes of BD may represent important obstacles to deployment of effective preventive interventions. Educational campaigns to improve hygiene should address local beliefs of disease causation. Despite biomedical misconceptions, a vaccine to prevent shigellosis was perceived as beneficial and was highly acceptable to the community, if priced low enough.

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SPATIO-TEMPORAL DISTRIBUTION AND ECOLOGICAL DETERMINANTS OF ENTERIC DISEASES IN VIETNAM, 1991-2001

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In Vietnam, shigellosis/dysentery, typhoid fever and cholera are enteric diseases of public health concern. To better understand their epidemiology, we determined the spatial and temporal distributions of each disease and explored potential ecological risk factors. From national surveillance data, 1991-2001, annual and monthly incidence rates were calculated for each province, and mapped using geographical information systems. To identify factors influencing spatio-temporal trends, data related to living standards, topography, climate, vaccine campaigns and trade policies were examined. Shigellosis/ dysentery was found to be the most prevalent disease, and increased 3-fold with the highest annual rates recorded in the central highlands (177-371/ 100,000). Typhoid fever was endemic in the Mekong River delta (33-170), and emerged in the far north western provinces in the mid-1990s. In contrast, cholera was epidemic and most prevalent in the central coastal regions (3-8/100,000) until 1997, and then decreased significantly nationwide prior to vaccine introduction. High incidence rates were associated with poverty, inadequate water and sanitation facilities, high rainfall and vapor pressure, and coincided with border openings and freer trade with neighboring countries. Understanding the dynamics of these diseases, and factors associated with their incidence, spread, emergence and/or decline is crucial to optimize interventions, maximize their cost-effectiveness and subsequently improve health outcomes.

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FIRST ISOLATION AND INITIAL MOLECULAR CHARACTERISATION OF *RICKETTSIA AFRICAE* IN GERMANY FROM A PATIENT RETURNING FROM SOUTH AFRICA

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Rickettsia africae is the causative agent of African tick bite fever (ATBF), an acute febrile illness frequently accompanied by inoculation eschars, regional lymphadenitis, myalgia and severe headache. It is transmitted by Amblyomma ticks in large areas of sub-Saharan Africa and some Carribean islands. ATBF has been recognized as an emerging health problem for international travellers to Africa.

We describe the first isolation of *R. africae* in Germany from a patient returning from South Africa:

A 35-year-old male German patient fell ill with fever, headache, and myalgia one day after returning from a trip to South Africa in January 2006. Clinical examination showed low-grade fever (38.2°C), a macular rash at the chest and the legs, and a necrotic black lesion surrounded by an erythematous halo identified at the right calf. A skin biopsy from the presumed eschar, serum and whole blood were sent to the Bundeswehr Institute of Microbiology for confirmation of a suspected rickettsial infection by molecular and cultural methods. The patient was treated with doxycycline (200 mg once a day for 7 days), and his condition improved rapidly. DNA was extracted from crushed skin material, plasma and isolated peripheral leucocytes and examined by PCR. *Rickettsia* spp. was demonstrated in the biopsy by using a generic real-time PCR with primers targeting the citrate synthase gene. Shell vial cultures were done on samples of biopsy material, serum, and peripheral leucocytes using Vero and L-929 cells. Growth of rickettsia (determined by a decrease in real-time PCR ct-values) was detected in Vero cells 5 days after the cells were inoculated with skin biopsy material. It was confirmed by staining of infected Vero cells according to Romanowsky and an immunofluorescence assay. The analysis of sequences of the *rO*mpA gene derived from the shell vial isolate showed a 100% identity with a French *R. africae* isolate in 3900 nucleotides investigated. Seroconversion determined by indirect immunofluorescence test was detected in the patient with titers to *R. conorii* of <1/8 and 1/128 in acute- and convalescent-phase sera (sampled four weeks later), respectively.We herein describe the first isolation of *Rickettsia africae* from a human sample in Germany. Cases of ATBF are suspected in many travellers returning from certain parts of Africa with fever and exanthema. Further studies are indicated to identify the prevalence of this disease in German tourists.

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STUDIES ON THE RELATION BETWEEN ARSENIC IN SURFACE WATER AND BURULI ULCER DISEASE

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Buruli ulcer (BU) caused by infections with *Mycobacterium ulcerans* is a serious debilitating disease usually associated with water bodies. In Ghana, approximately 6000 cases were recorded in a national survey in 1999, with the majority of cases being in agricultural and mining areas. Infection is acquired through *M. ulcerans* in the natural environment. The mode of transmission however remains unclear and the bacterium has been found in biofilms, fish, aquatic insects and crustaceans. Studies have hypothesized several modes of transmission including aerosol and direct transmission. Despite these, there are knowledge gaps concerning factors that pre-dispose to infection. Arsenic is implicated in several types of skin diseases including skin cancers. It has immunosuppressive affects and therefore enhances susceptibility to infection. Arsenic concentrations in surface water were used in this study and were determined for abstraction points used by inhabitants of settlements in the study areas. Samples were collected from 12 endemic and 10 non-endemic areas, grouped into artisanal and non-artisanal mining areas. The prevalence of BU per community, where samples were collected, was then related to the arsenic levels in an exposure-response analysis using SPSS (Version 12.0 for Windows). The mean arsenic level in the artisanal mining area was 7.63 mg/l while that in the NMA area was 2.36 mg/l, both greater than the maximum recommended values of 0.01mg/l by the WHO. The preliminary results showed no evidence of significance between arsenic concentrations in surface water and BU endemicity (r=-0.053, p=0.815) and prevalence (r=-0.283, p=0.27). These results need however to be evaluated on a much larger scale.

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THE EPIDEMIOLOGY OF SYPHILIS CASES IN THREE SOCIALLY MARGINALIZED POPULATIONS OF LOW-INCOME, URBAN, COASTAL PERU

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Syphilis infection in Peru is not well documented. We describe the epidemiology of syphilis in socially-marginalized populations in low-income, urban, coastal Peru. Socially-marginalized heterosexually-identified men, women, and MSM completed an epidemiologic survey evaluating sexual risk behavior and syphilis prevalence. Syphilis testing was conducted using RPR-nosticon II Rapid Plasma Reagin kits (Shield Diagnostics, Dundee, UK); reactive titers were confirmed by Serodia-TPPA (Fujirebio Diagnostics Inc, Toyko, Japan). The prevalence of syphilis in MSM was 28.9% (48/166), 1.4% in heterosexually-identified men (13/917), and 4.7% (5/107) in women, significantly different across populations (p<0.001). All further results refer to the MSM only. In univariate analysis, increased syphilis prevalence was associated with higher rates of HSV-2 infection (odds ratio [OR]: 13.68, 95% CI, 3.16 - 59.13), increased age per year (OR: 1.07, 95% CI, 1.00 - 1.14), and increased number of sexually active years (OR: 1.10, 95% CI, 1.03 - 1.16). Syphilis prevalence was not associated with unprotected sex acts with stable or non-stable partners, relationship duration or partner type, despite extensive analyses and comparisons across multiple sub-groups. Unexpectedly, the only group with marginally different syphilis prevalence (p=0.097) was MSM reporting their live-in boyfriend as their only partner (43%, 10/23) compared to all other MSM (23%, 28/120). Syphilis prevalence among MSM is high and not strongly associated with sexual risk behavior. Although the lack of association between syphilis and unprotected sex may be due to small sample size, it could also indicate re-infection from stable partnerships consistent with previous findings of high syphilis re-infection rate among this population. In the socially-marginalized women, syphilis prevalence is significantly higher than that found in two previous studies of general population women (1.1%) and comparable to the prevalence found in CSWs (3.9%); indicating potential high-risk for congenital syphilis due to the limited preventive efforts targeting this population.

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BUBONIC PLAGUE IN THE CITY OF LOS ANGELES: "FINDING AN EPIDEMIOLOGIC NEEDLE IN A HAYSTACK OF CA-MRSA"

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Despite presence of sylvatic plague in the Western US, human infection in an urban setting without evident risk factors is very significant. The last case of *Yersinia pestis* (YP) infection in Los Angeles County (LAC) occurred in 1984 in a veterinarian with known exposure. In April 2006, an urban case of bubonic plague occurred with no clear risk factors. A 28-yo female was admitted with a 3-day history of fever and a painful right axillary mass, with no other skin lesions noted. Despite initial sepsis requiring fluids and pressors, she then improved with empiric addition of levofloxacin. Suspecting CA-MRSA abscess, the admitting team attempted axillary drainage twice, without facemasks. All chest radiographs were negative. The laboratory reported presumptive YP in her admission blood cultures 3 days later. Physician review of the blood smear showed bipolar staining gram-negative bacilli. ID consultants queried the patient regarding exposures and initiated infection control measures. Beyond noting domestic incursions by rodents and feral cats, she firmly denied direct animal contact or travel outside of her highly urban locale. Given a lack of risk factors for YP, LAC Department of Health Services was notified immediately. Antibiotic prophylaxis was given to 7 hospital employees, due to a theoretical potential for infectious aerosolization. 1) without relevant history, omission of plague as a diagnostic possibility is very likely, 2) use of universal precautions remains a vital safety practice, 3) early diagnosis is possible if blood smears are reviewed by skilled workers, 4) crucial risk factors were not quickly elicited, stemming largely from socio-cultural barriers. A close alliance between clinicians and public health is optimal for managing such difficult cases.

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OPTIMAL DIAGNOSIS OF ENTERIC FEVER IN CHILDREN IN PAKISTAN

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Enteric fever is endemic in Pakistan, where the most common cause of pediatric bacteremia is *Salmonella* Typhi or *S.* Paratyphi. Accurate microbiological diagnosis currently depends on cultures of blood, since culture of bone marrow is rarely done. Therefore, we undertook a controlled evaluation of a widely used aerobic pediatric blood culture medium containing resins (PP) versus an anaerobic lytic (AL) medium for the recovery and time to detection of *S.* Typhi or *S.* Paratyphi (facultative

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intracellular pathogens) in the blood of children (ages 1-15 years) with suspected enteric fever (fever >3 days). Samples of blood obtained by venipuncture were distributed equally between PP and AL bottles that were weighed before and after filling to accurately assess the volumes of blood cultured. Statistical analysis was performed with McNemars modified chi-square test for yield and the Wilcoxon matched-pairs signed rank test for time to detection. Of 817 paired blood cultures submitted over 12 months, 46 (5.6%) grew pathogens (39 *S.* Typhi and 7 *S.* Paratyphi) and 36 (4.4%) grew contaminants. Both media were comparable for the recovery of *S.* Typhi or Paratyphi; most (36) grew in both, 7 in PP only, and 3 in AL only (P=0.34). The median blood volumes were 1.86 ml (PP) and 1.96 ml (AL); when only sets with equal volumes (within 20%) were considered, 17 grew in both and 2 each in PP and AL. When cultures were positive in both PP and AL, the median time to detection was 16.4h (interquartile range 11.8 to 22h) for PP and 10.7h (interquartile range 8.8 to 16h) for AL, respectively (P<0.0001). All multi-drug (ampicillin, chloramphenicol, and co-trimoxazole) resistance (36% of isolates) was observed among isolates of *S.* Typhi. All isolates were susceptible to ceftriaxone and ciprofloxacin by current CLSI (NCCLS) interpretive criteria, although resistance to nalidixic-acid was common (36% of isolates). *S.* Typhi and Paratyphi were detected significantly sooner in the anaerobic lytic medium; however, the total yield was not improved versus the pediatric aerobic resin medium.

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MICROBIAL ETIOLOGIES OF DISSEMINATED PNEUMONIA AND SEPSIS IN SA KAEO PROVINCE, THAILAND

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Pneumonia, sepsis, and other severe infections are leading causes of death in developing countries. Definitive laboratory data describing microbial etiologies and antimicrobial resistance are lacking, impacting clinical management and public health planning. As part of a multi-year project to improve capacity and treatment, a state-of-the-art automated blood culture system was implemented in Sa Kaeo, a rural province of Thailand near the Cambodian border. Specimens were collected from inpatients at all all 8 public hospitals in Sa Kaeo Province from 7 May 2005 - 6 May 2006. Isolates were identified by conventional methods in Sa Kaeo and confirmed by reference laboratories of the Ministry of Public Health. Antibiotic susceptibility profiles were done by disk-diffusion with minimum inhibitory concentrations determined for resistant isolates. Of 4,143 specimens submitted, 535 (12.9%) were positive for a pathogen after exclusion of presumed contaminants. In culture positive patients, pneumonia was diagnosed in 26%, sepsis in 14% of those <5 years of age and in 29% of those ≥5 years of age, with 30% having other severe disease. Over 23% of positives were from children under the age of 5. Single infections comprised 97% of positives. Pathogens included *Escherichia coli* (30.6%), nontyphoidal *Salmonella* (8.2%), *Klebsiella* species (7.9%), *Mycobacterium* species (4.6%), *Cryptococcus neoformans* (4.3%), *Burkholderia pseudomallei* (2.8%), *Streptococcus pneumoniae* (2.8%), and *Haemophilus influenzae* (0.4%). Antibiotic susceptibility testing is ongoing. In conclusion, greatly enhanced clinical laboratory system was implemented, leading to improved identification of pathogens from blood cultures. Many agents rarely identified in blood cultures from Western nations were found, including *B. pseudomallei* and nontyphoidal *Salmonella*, suggesting that these agents play significant roles in community acquired pneumonia and sepsis.

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OUTBREAK OF LEPTOSPIROSIS AMONG ADVENTURE RACE PARTICIPANTS - TAMPA, FLORIDA, 2005

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Extreme sports are gaining popularity worldwide. Exposure to environments contaminated by animal urine may put athletes at risk for leptospirosis, a bacterial zoonosis of worldwide distribution with 10% case-fatality. Recently, several large leptospirosis outbreaks have occurred following extreme sporting events. On November 21, 2005, a 32 year-old male New York resident was hospitalized with suspected leptospirosis. He participated in an endurance-length swamp race on November 4-5, 2005 in Florida. We interviewed racers to assess illness, medical care, and race activities. A suspect case was defined as fever plus two or more signs/symptoms of leptospirosis occurring in a racer after November 4, 2005. Suspect case-patients were referred for treatment as needed, and asked to submit blood and urine for culture, and serum for microagglutination testing (MAT) and Dip-S-Tick IgM immunoassay (DST). A titer of ≥ 400 or a positive DST confirmed leptospirosis. We interviewed 192 (96%) of 200 racers from 32 states and Canada. 43 (22%) met the suspect case definition. Median age was 37 years (range 19-66) and 128 (67%) were male. 14 (33%) of 43 were serologically-confirmed, including the index case. *Leptospira* were isolated by culture in the urine and blood from one case-patient. Mean incubation time from the start of the race until onset of illness for the confirmed cases was 13.5 days (range 2-22). Three case-patients were hospitalized. The most common signs and symptoms reported by suspect case-patients were: fever (100%), headache (91%), chills (69%), sweats (68%), and muscle/joint pains (68%). Factors associated with increased risk of leptospirosis among suspect case-patients included swallowing river water (Odds Ratio [OR] 3.4, 95% Confidence Interval [CI] 1.6-7.0), swallowing swamp water (OR = 2.4, 95% CI = 1.1-5.2) and being submerged (OR = 2.3, 95% CI = 1.1-4.7). Having severe cuts on the legs and wearing shorts during the race were prevalent but not associated with an increased risk of infection. In conclusion, this outbreak occurred in an area not previously considered endemic for leptospirosis and resulted in a high rate of symptomatic infection. In the absence of modifiable risk factors, targeted chemoprophylaxis should be employed to reduce the risk of leptospirosis in adventure races with extreme water exposure.

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EVALUATION OF IGG-ELISA USING *CYSTICERCUS CELLULOSAE* SOMATIC AND EXCRETORY SECRETORY ANTIGENS FOR DIAGNOSIS OF NEUROCYSTICERCOSIS REVEALING THE BIOLOGICAL STAGE OF THE PARASITE

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Neurocysticercosis (NCC) is a disease of high prevalence in tropical developing countries including India. The present study describes the development and evaluation of ELISA using Cysticercus cellulosae somatic and excretory secretory (ES) antigens for detection of anti-Cysticercus IgG antibodies in serum and CSF for the diagnosis of NCC. Results of the ELISAs in a group of cases with a definitive diagnosis of NCC are correlated with the biological stages of the parasite such as live vesicular or degenerated stage. A higher frequency of obtaining a positive IgG-

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antibody titer in the ELISA using C. cellulosae somatic antigen could be observed among patients with dead or degenerated stage of the parasite in brain (p=0.036 in either serum or CSF). Similarly, a higher frequency of obtaining a positive IgG-antibody titer in the ELISA using C. cellulosae ES antigen could be observed among patients with live stage of the parasite in brain; a significant association was observed in case of CSF (p=0.0007) whereas, the association was statistically insignificant in serum (p=0.118). From the observations in the present study, it is assumed that the ELISAs using C. cellulosae somatic and ES antigens may be able to discriminate the individual cases of NCC with either live vesicular or degenerated stage of Cysticercus in human CNS. The demonstration of serum IgG antibodies against C. cellulosae ES antigen only may be indicative of the live stage of the parasite where a detectable level of antibodies specific to the somatic antigen of Cysticercus is not yet developed. Demonstration of serum IgG antibodies against C. cellulosae somatic antigen only may be suggestive of the late degenerated stage of the parasite where there is no detectable limit of transient antibodies specific to ES antigens. The IgG antibodies demonstrated against both C. cellulosae somatic antigen and ES antigen might be an indicative of an early stage of degeneration of the parasite where still a detectable antibody level specific to the parasite ES antigens are circulated along with a detectable level of antibodies specific to the degenerated somatic antigens. Otherwise, there may be parasites possibly of multiple stages. Results of the present study open up an avenue for screening and diagnosis of NCC in either individual case studies in hospitals or in disease eradicating programs in areas endemic for cysticercosis.

(ACMCIP Abstract)

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USE OF RT24H QUICK ELISA ASSAY IN DIAGNOSIS OF CYSTICEROSIS

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Cysticercosis in human and pigs are caused by the infection with the larval or cysticercus of the pork tapeworm *Taenia solium*. Neurocysticercosis refers to this infection in the brain and other nerve tissues, it is prevalent wherever pigs are allowed to roam for food and sanitation facilities are inadequate. High prevalence of this disease are reported in Central and South America, Sub-Saharan Africa, Southeast Asia, India and China. Diagnosis is mainly by neuroimaging and through immuno-diagnosis using the enzyme-linked immunoelectrotransfer blot (EITB). The EITB has proven to be the best immunological test available today. It is based on seven lentil lectin-bound glycoproteins (LLGP), affinity purified from cysts dissected from infected pigs. The LLGP contains a mixture of antigens that belong to three main families of proteins namely 50-kDa, 24/42-kDa and 8-kDa. The 42-kDa protein is a dimmer of the 24 kDa protein. We had purified, sequenced and cloned this membrane-bound protein. A hydrophilic stretch of 92 amino acids from the 24-kDa sequence was expressed in Tni cells using a Baculovirus expression system, and was purified to homogeneity (rT24H). The effectiveness of rT24H as a potential diagnostic antigen was evaluated in Quick ELISA assays format by studying the sensitivity and specificity of the protein with a large collection of human sera obtained from neurocysticercosis patients, individuals with other parasitic infections, and individuals who were residing in non-cysticercosis endemic areas or from non-travelers residing in the US. The rT24H Quick ELISA has a sensitivity of 96.3% and specificity of 99.2%.

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DIFFERENTIAL EXPRESSION OF CALRETICULIN DURING THE DEVELOPMENTAL STAGES OF *TAENIA SOLIUM*

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Taenia solium, a cestode that causes neurocysticercosis and taeniosis in humans, has a complex life cycle. Recently, we cloned and expressed *T. solium* calreticulin (TsCRT) as a functional Ca^{2+}-binding protein. Calreticulin is a ubiquitous protein involved in cellular Ca^{2+} homeostasis and protein folding. In mammalian and plant cells, calreticulin is up-regulated as a result of stress, extracellular and intracellular Ca^{2+} modification, differentiation, mitosis, meiosis, and embryogenesis. Up-regulation of this protein in some parasites has been observed in their different life stages. To begin exploring the biological importance of TsCRT, we have used a specific polyclonal antibody raised against the recombinant TsCRT to locate this protein in the different stages of *T. solium*. In invaginated and evaginated cysticerci and in 8-day tapeworms, TsCRT was preferentially detected in the subtegumentary cells, muscle cells of the suckers and rostellum and in some cells associated with the excretory canals. In mature and gravid proglottids obtained from infected humans, spermatogonia, ovogonia, uterine epithelium, and cells of the vans deferens gave a positive signal. In the gravid uterus, multinucleated structures were highly positive to TsCRT. We propose these cell cumuli are fertilized ova that have undergone mitosis. In contrast, TsCRT was not detected in the mature spermatozoa present in the lumen of the vas deferens. These data indicate that calreticulin is ubiquitous in *Taenia solium*, supporting its importance in the physiology of living organisms. Nonetheless, expression of TsCRT is not homogeneous; since up-regulation in some cell types was evident, mainly during maturation of germ cells and in embriogenesis.

(ACMCIP Abstract)

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GENETIC POLYMORPHISM IN *TAENIA SOLIUM* CYSTICERCI RECOVERED FROM EXPERIMENTAL INFECTIONS

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Random amplified polymorphic DNA technique (RAPD) showed that *Taenia solium* cysticerci recovered from naturally infected pigs from Mexico, Honduras and Tanzania have a clonal structure and local lineages with probable events of genetic recombination without genetic flow within them. To evaluate the genetic polymorphisms from cysticerci recovered from experimental infections in pigs, using *T. solium* eggs obtained from human carriers, four 2 month old- piglets from a certified farm were infected with eggs obtained from three *T. solium* adult worms: A: 10 year old female, B: 25 year old female, C: 44 year old male, the 4th pig was infected with a mixture of eggs from the three tapeworms. Each pig was inoculated *per os* with 50,000 eggs. After 16 weeks pigs were humanely euthanized and cysticerci were excised. Ten parasites recovered from each pig were analyzed by RAPD using commercial primers OPB11; 14 and 18. Proportion of polymorphic alleles, mean heterozygosity, Nei´s genetic distances *D* and the UPGMA dendogram were obtained with the TFPGA program. Percent establishment was: 2.2, 4.2, 0.2 and 0.6 (tapeworms A, B, C and the mixture, respectively); 36 loci were identified, 83% polymorphic, average heterozygosity was 0.016; the dendogram clustered the cysticerci into two main clades: one included cysticerci from

tapeworm A and some parasites from the mixture and from tapeworm B, while the other had most cysticerci from tapeworm B, the mixture and all from tapeworm C. Our data of polymorphic loci (P= 0.14-0.55) and heterozygosity (H= 0.06-0.22) were slightly higher than those published for natural infections (P= 0.18-0.40 and H= 0.03-0.16). The small number of cysticerci used may account for these results, alternatively, cysticerci recovered from experimental infections might have a higher polymorphic genetic pool than those coming from natural infections, because environmental and genetic selection forces present in nature, such as tolerance to desiccation and natural selection for some genotypes, influence natural infections but do not participate in experimental ones

(ACMCIP Abstract)

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KINETICS OF THE IMMUNE RESPONSE IN THE INTESTINAL MUCOSA OF HAMSTERS INFECTED WITH *TAENIA SOLIUM* ADULT WORMS

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Taenia solium is a cestode that infects humans. We developed experimental models in rodents to study the host-parasite relationship. Inflammatory reactions in the mucosa of the small intestine of infected hamsters are seen. Eosinophils, goblet cells and lymphocytes increased at 10-16 days post-infection and remained abundant until tapeworms were eliminated. The present study aimed to detect IL-5, IL-13 and IFNγ. Hamsters were infected with *T. solium* cysticerci and necropsied at different times post-infection; 1 cm biopsies were obtained from the jejunum around the scolex implantation site, fixed in PBS-pformaldehyde, 5 µm sections were obtained and adsorbed onto slides covered with silane. Digoxigenin labeled oligonucleotides complementary to Il-5, IL-13 and IFNγ RNA were used as probes for in situ hybridization. Probes for α-tubuline and for HPV were used as positive and negative controls respectively. Anti-digoxigenin antibodies coupled to alkaline phosphatase were used to detect the probes in tissues. Positive reactions for mRNA cytokines were observed as follows: for IFNγ at 2 to 12 days, IL-5 at 8 to 24 days and IL-13 at 16 to 24 days post-infection. Intense reactions were seen in infected hamsters as compared to non-infected. These data were compared with tapeworm recovery, more worms were observed from 6 to 16 days post-infection, after that tapeworms began to be eliminated, few tapeworms were recovered at 24 days and no hamster remained infected at 28 days.

(ACMCIP Abstract)

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ZOONOTIC FILARIASIS IN THE ARABIAN PENINSULA: AUTOCHTHONOUS ONCHOCERCIASIS AND DIROFILARIASIS?

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Zoonotic filariasis occurs worldwide, even in countries where no indigenous human infection is reported. We describe three cases of zoonotic filariasis; one of *Onchocerca* sp in a child and two of *Dirofilaria* sp; all in patients from Kuwait, which is not endemic for human filariasis. A female Kuwaiti who visited Saudi Arabia, presented initially with ocular problems. Later, a nodule that appeared in the suprapubic area was resected; a worm *in situ* was identified as an *Onchocerca* sp. She was diagnosed as a case of ocular toxoplasmosis too. An adult male Kuwaiti presented with a mass in the abdominal wall. Excision of the subcutaneous nodule identified a worm; the anatomic features in section were those of *Dirofilaria repens*. A female Indian, resident of Kuwait City had traveled to India for a fortnight, a month before presentation. She complained of a moving object in the eye. The live worm extracted was diagnosed as an immature female *D. repens* on the basis of the cuticular ornamentation, size and other features which we discuss. All the patients had a history of travel outside Kuwait. However, these visits were of short duration, rather than a prolonged stay when a sustained series of bites leading to infection is more likely. Hence, the infections are suspected to be autochthonous to the Arabian Peninsula rather than Kuwait *per se*. This report illustrates that even in countries considered non-endemic for human filariasis, a high index of suspicion is necessary. The vectors of the filarial parasites in the Arabian Peninsula, have yet to be identified. Specifically, *Simulium* sp. and *Culicoides* sp., which transmit onchocerciasis, are known from this geographic area. Mosquitoes such as *Aedes* sp. and *Culex* sp., intermediate hosts of *Dirofilaria* sp., occur seasonally in the country. These are the first reports of zoonotic filariasis in the Arabian Peninsula and indeed, in the Arabian Gulf region.

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RISK FACTORS FOR CLINICAL PROGRESSION OF PRE-ULCERATIVE AMERICAN CUTANEOUS LEISHMANIASIS IN NORTHEASTERN BRAZIL

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Most individuals with American Cutaneous Leishmaniasis (ACL) present 30-60 days after the appearance of an ulcerated lesion and little is known about risk factors for clinical progression and therapeutic failure in early stages of infection. We evaluate risk factors and immune responses associated with the development of ulcerative disease and treatment failure in patients in the pre-ulcerative phase of ACL caused by *L. braziliensis*. This is an observational cohort study of 25 patients with ACL in an endemic area. Cases are 13-60 years of age with less than 30 days of pre-ulcerative cutaneous leishmaniasis confirmed by intradermal skin test and/or culture. Patients with previous or non-cutaneous forms of leishmaniasis, or chronic diseases were excluded. Follow-up was at 15-30 day intervals for 150 days, and stool samples and peripheral blood were collected at 0, 30 and 60 days to determine helminth co-infection and cytokine levels of IFN- , TNF- , IL-10 and IL-5, respectively. All participants were treated with antimony (20mg/kg/day x 20 days). Helminth co-infection was treated at day 60. Outcomes are lesion activity and cure by 90 days without recurrence. In preliminary analysis of 17 patients, 71% failed treatment and the majority (75%) developed ulcerated lesions. Treatment failure was associated with lower body-mass-index, fewer lesions, older age, and helminth co-infection. There was no correlation with outcome and size of primary lesion or lymphnode at presentation or follow-up. IFN-γ and TNF-α are elevated at day 0 and peak at day 30 ($p<0.01$). Treatment failure is associated with lower IFN-γ at day 0 and 30. Elevated TNF-α at day 0 was associated with lesion ulceration and treatment failure ($p<0.01$). There was no association with cytokine levels and outcome at day 60 for either IFN-γ or TNF-α. Analyses of IL-10 and IL-5 are pending. Preliminary results show high levels of ulceration and treatment failure despite early treatment for ACL. Level and timing of immune response are related to lesion activity and treatment response.

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ACUTE HEPATITIS A IN A YOUNG RETURNING TRAVELLER FROM KENYA DESPITE IMMUNIZATION BEFORE DEPARTURE

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Immunogenicity of hepatitis A aluminium-adsorbed vaccine is excellent with a very high seroconversion rate (> 95%) after one single dose. All studies have demonstrated an excellent response 14 days or more after one single injection but little is known on the effectiveness during the first days following the immunization. We present here the case of a young man who was vaccinated against Hepatitis A with an aluminium-adsorbed vaccine (Havrix 1440®) 11 days before leaving for Kenya and who contracted an acute symptomatic hepatitis A. A 25 years old man visited our Travel Clinic on the 8th of January 2004 for a trip to Mombassa (Kenya) from January 18th to February 2nd. He got a booster for diphtheria, tetanus, poliomyelitis, rubella, mumps and measles and a first dose of an aluminium-adsorbed hepatitis A vaccine (Havrix 1440®). On February 18th (14 days after return), he presented with fever, myalgia and light headache but no clinical jaundice. Malaria was ruled out with microscopy. Biochemistry tests showed a moderate elevation of liver transaminase (ASAT=208 U/L and ALAT=320 U/L) on February 22nd (highest level). Hepatitis serologies revealed a protective titre of antiHBS antibodies (Ab) (from previous HBV vaccination), anti-HCV Ab negative, and positive IgM Ab against HAV. Hepatitis A was thus confirmed. The HIV test was negative. There are few reports of acute symptomatic hepatitis A after immunization. We know from previous studies (during epidemics) that the time from immunization to exposure should be at least 14 days for the vaccine to confer full protection. On the other hand, we also know that protection against symptomatic hepatitis can be achieved earlier, even at the time of exposure because of the long incubation period of the disease. In our case, the time period between immunization and departure date was quite short (11days) and may provide the explanation for the failure if he has been exposed early during the travel. When doing last-minute vaccination against hepatitis A, physicians need to inform the traveller about the suboptimal protection provided by the vaccine given in these conditions. This case highlights also the need for the physician to keep in mind a broad differential diagnosis in febrile returning travellers. It is especially important with hepatitis A because of the potential spreading of the disease among family members.

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SOLAR DISINFECTION OF DRINKING WATER IS AN EFFECTIVE INTERVENTION AGAINST WATERBORNE DISEASE IN DEVELOPING COUNTRIES OR IN THE AFTERMATH OF NATURAL (OR MAN-MADE) DISASTERS

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In Sub-Saharan Africa ~769,000 children under 5 years of age, died annually from diarrhoeal diseases in 2000-2003. Solar Disinfection (SODIS) is a technique for making contaminated drinking water safe where transparent bottles are filled with biologically contaminated water and placed in direct sunlight for 6 hours. SODIS reduces faecal contamination levels from 1 million bacteria per ml to zero in < 1.5 hours and is completely effective against the pathogens responsible for cholera, dysentery, typhoid, giardiasis, salmonella, gastroenteritis, and polio. Previous studies have reported a dramatic reduction in incidence of diarrhoea among those children who drank water exposed to direct sunlight compared with a control group that drank water not exposed to sunlight. The biocidal effect of sunlight is due to optical and thermal processes and a strong synergistic effect occurs at temperatures exceeding 45°C. SODIS was approved by the WHO in January 2005 after the Asian Tsunami, however many relief organisations remain reluctant to use the technique. In this presentation we review the state-of-the-art in this field of research and briefly describe the scientific and sociological challenges that remain for this effective, appropriate and sustainable intervention against waterborne disease.

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LABORATORY-BASED SURVEILLANCE FOR ACUTE FEBRILE ILLNESS IN EGYPT: A FOCUS ON LEPTOSPIROSIS

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Recent surveillance for acute febrile illness (AFI) in Egypt revealed that 30% of cases were due to brucellosis and typhoid fever. A previous serologic retrospective analysis of a subset of acute serum samples revealed that leptospirosis may account for about 15% of the unexplained cases. This prospective laboratory-based surveillance was conducted to determine the proportion of AFI due to leptospirosis and other causes in 4 large governorate hospitals in Egypt. Eligible patients were enrolled from Jun 2005-Apr 2006 in hospitals located around the country. A case of AFI was defined as any individual with fever for at least 2 days or temperature on admission of 38.5°C or greater, age ≥ 4 yrs and no identified cause of fever such as diarrhea, pneumonia, or a clinical diagnosis of typhoid fever or brucellosis. Acute (admission) and convalescent (discharge) serum specimens and information about demographic characteristics and possible exposures were obtained from all patients. Leptospirosis diagnosis was conducted by blood culture (using EMJH *Leptospira* media), serological tests (ELISA, microscopic agglutination test [MAT] using a panel of 20 serovars) and/or PCR using *lig*A primers. A total of 981 patients were enrolled; typhoid fever and brucellosis were confirmed by blood culture or serology (tube agglutination or ELISA) in 139 (14.2%) and 182 (18.6%) of all cases respectively. Acute leptospirosis was confirmed in 45 (4.6%) patients by culture, PCR or 4-fold rising titer with MAT. Median age of these patients was 33 yrs, 53% male, 62% reported animal contact, and 65% had rodent contact which was significantly associated with positivity OR 2.0 (95% CI 1.04-3.76). Also, 149 (15.2%) of the samples were positive by IgM ELISA. MAT titers were positive for 17 serovars with 5 predominant ones (72%): Georgia 19.8%, Celledoni 17.4%, Ballum 12.8%, Australis 11.6% and Mankarso 10.4%. In conclusion, *Leptospira* spp. are likely to be important causes of AFI in Egypt. Case control studies are needed to better define risk factors and develop prevention strategies. Furthermore, it is important to increase physician awareness about leptospirosis in Egypt and strengthen the laboratory infrastructure to enable its diagnosis.

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[14C]ARTESUNATE TISSUE DISTRIBUTION IN PREGNANT RATS FOLLOWING A SINGLE INTRAVENOUS DOSE WITH WHOLE-BODY AUTORADIOGRAPHY

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This study was conducted to address concern that artesunate (AS) administration at doses not toxic to adult animals may be related to embryonic lethality and resorption. [^{14}C]AS with a specific activity of 45.86 μCi/mg was applied at a single iv bolus of 5 mg/kg to embryonic day 11 pregnant SD rats. At 0, 0.5, 3, 8, 24, 48, 96 and 192 hours after the dose, 2 rats were euthanized for sagittal sections of 40 μm, followed by whole-body autoradiography. The radioactivity in selected tissues was quantified using an image analysis system and expressed as μg equivalents

of artesunate per gram sample. Concentrations of radioactivity in tissues were then fitted against time points for PK parameters. The highest concentrations in most tissues were observed at 0.5-1 h post-dose (66% of the tissues sampled). The reproductive tissues and fetuses showed similar patterns of distribution where the highest concentrations were observed at 0.5 -1 h post-dose. Fetal development was shown to be insufficient. At all time points fetal tissues appeared pale in color. At 96 and 192 h post-dose fetuses appeared underdeveloped. PK parameter analysis for reproductive tissues demonstrated that the highest [^{14}C] AS AUC was found in the placenta, followed by the fetal body. The [^{14}C] AS AUC in placenta was even higher than the dam's blood. The $AUC_{0\text{-}96\ h}$ for blood, amnion, amniotic fluid, fetal body and placenta were 142.92, 29.18, 12.09, 91.51 and 353.67 µg*hr/ml, respectively. The Cmax for these tissues were 1.77, 0.97, 0.67, 2.37 and 3.40 µg/ml, respectively. The t1/2s of elimination phase were 116, 69, 119, 234 and 279 hr, respectively. The AUC ratio of the placenta vs the blood (dam) was 2.47, and the t1/2 ratio of fetal body and placenta vs blood (dam) were 2.02 and 2.41. Taking the PK profiles, life-time observation of the current study and our previous results into the consideration, the embryotoxicity of AS is related to the concentration distribution in the placenta and its penetration into fetal tissues. This is the first experimental evidence to date showing the distribution of AS in fetal development related tissues.

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HEMATOLOGICAL COMPLICATIONS IN PATIENTS WITH IMPORTED MALARIA HOSPITALIZED AT THE MARGARITA ISLAND, VENEZUELA, 2001-2004

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Imported malaria constitutes an important public health problem in many countries, even in those with endemic zones, where disease could be acquired in these areas and then seen in non-endemic regions. Non-immune populations are susceptible to complications due to malaria infection. In addition, *P. vivax* the predominant *Plasmodium spp.* in Venezuela can cause severe malaria. We describe the clinical features of patients diagnosed with imported malaria at our institution in Margarita Island (a non-endemic area), Venezuela, in a 4-y period. We conducted a retrospective observational study to identify the clinical and epidemiological features among hospitalized patients at HCLO with imported malaria acquired at various endemic locations in Venezuela, 2001-2004. During this period, 7 patients with imported malaria were hospitalized at our Institution. Diagnosis was confirmed by thin and thick peripheral blood smears. Mean age was 28 years old; 71% were males; 86% corresponded to *Plasmodium vivax* infection (acquired in Sucre, northeastern Venezuela) and 14% to *P. falciparum* (acquired in Bolivar, southern Venezuela). All patients presented fever, malaise; 86% myalgia, 71% chills, 57% headache, 42% jaundice and coluria, 27% hematemesis and epistaxis, and 14% hematuria, among others. Mean Hb levels on admission were 8.0g/dL (100% <12 g/dL); platelets: 66,000cells/mm^3 (100% had platelets below 150,000; but 29% <60,000); total leukocyte count: 3.1x10^{3}cells/mm^3 (85% had leukopenia). Laboratory values after discharge included a Hb: 8.6g/dL (t=-2.065; p=.084); platelets: 72,142cells/mm^3 (t=-1.153; p=.293); and total leukocyte count: 4.9x10^{3}cells/mm^3 (t=-4.333; p=.005). Fifty seven percent of patients required blood transfusions. All patients with *P. vivax* received CQ+PQ and with *P. falciparum* Q+PQ. Mean hospitalization stay was 10 days. One fatality was identified (CFR=14%) associated to severe malaria due to *P. falciparum.* Imported malaria due to *P. vivax* and *P. falciparum* in our population are associated with significant hematologic complications such as severe thrombocytopenia and leukopenia (observed in 100% and 86% of our patients). Our findings illustrate the importance of educating non-immune populations about malaria risk; and from a pubic health perspective, the need to develop malaria prevention strategies at a national level to avoid cases of imported malaria.

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MONKEY BITES IN TRAVELERS

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With the increase of international travel and import of exotic pets, the incidence of monkey bites in humans is becoming more frequent in the US. When bitten or scratched by a monkey, other than the chance of bacterial infection (cellulitis, osteomyelitis and tenosynovitis), there is a small but serious risk of contracting viral infections like Rabies, Herpes B, monkey pox and perhaps even Ebola or Marburg virus. The data was collected on patients consulting a hospital travel clinic at between 08/03/03 to 04/01/06. The majority of patients had sought initial medical care in the country of injury. A total of 9 monkey bites presented during this time and the data included: age, sex, country where bitten, location of bite, situational circumstances, time to treatment, initial treatment and outcome. Nine cases are reported: case 1:19, female, South Africa, face, taking photo, 36 hours, ABX, no infection; case 2: 32, male, Senegal, right hand, feeding monkey, 3 days, RIG, RV, ABX and acyclovir, no infection; case 3: 23,male, India, right arm, playing with monkey, 6 hours, RIG, RV, ABX, acyclovir, no infection; case 4: 39, male, Kenya, right hand, feeding monkey, 12 hours, dT and sutures needed, cellulitis requiring IV ABX; case 5: 47, male, India, right foot, kicked monkey, 6 hours, RIG, RV, ABX, acyclovir, no infection; case 6: 27, female, Thailand, right hand, playing with monkey, 24 hours, RIG, RV, ABX, no infection; case 7: 22, female, South Africa, right arm, unprovoked attack, 24 hours, dT plus sutures required, After 96 hours headache and fever developed and patient was treated with RV, IV ABX and Acyclovir. LP was negative and pt. recovered without sequelae; case 8: 32, female, Costa Rica, left hand, fighting with monkey who was trying to take camera, 2 hours, RIG, RV, ABX, no infection; case 9: 17, male, Nigeria, right hand and right shoulder, carrying monkey, 4 hours, ABX, sutures required, no infection. In conclusion, in the light of the seriousness of a possible bacterial infection and the potential outcome of an untreated viral infection, it should become routine practice for travel medicine physicians to educate patients seeking travel prophylaxis on how to minimize the risk of getting bitten by monkeys and appropriate post-bite measures and treatment. It's advisable for travelers to avoid any direct interaction with monkeys: feeding, petting, and antagonizing them. It should be remembered occasionally attacks may be unprovoked.

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DIFFERENTIATION OF POST-TRAVEL FEVER IN A 25 YEAR-OLD MEDICAL STUDENT

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A 25 y/o male medical student presented at a community emergency room with fevers, malaise, chills, headache, nausea, vomiting and profuse diarrhea. He also traveled to remote villages, frequently ate local food and swam in both fresh and saltwater. Vital signs demonstrated a temperature of 103.9° F, blood pressure of 90/48, pulse of 140, and respirations of 70. Physical exam showed a weak appearing patient with normal mental status, no lymphadenopathy, no oral lesions, no neck stiffness, clear chest and normal heart sounds. There was no hepatosplenomegaly, abdominal tenderness, guarding or rebound. Joint tenderness was absent. There were no skin rashes. Initial lab values were significant for thrombocytopenia (78K) and anemia (Hgb 10). Coagulation

study abnormalities included a d-dimer of 9.24 and fibrinogen of 460. Chest x-ray was negative for abnormalities as was his abdominal x-ray. Abdominal ultrasound and computed tomography showed an edematous gallbladder, right pleural effusion, trace ascites, fatty infiltration of the liver with mild hepatosplenomegaly, and no hydronephrosis. Blood, stool and urine cultures returned negative, as did antibody testing for West Nile, HIV, hepatitis A, B and C, typhus, and rocky mountain spotted fever. The patient was resuscitated with intravenous fluids and hospitalized for six days. Six consultations were obtained. Antibiotics administered empirically included IV levofloxacin, IV metronidazole and IV vancomycin. Creatinine elevated to a level of 2.0, but lowered to 1.2 by discharge. During his hospital stay the patient gradually improved with normalization of lab values. AST and ALT remained elevated. Despite advances in diagnostic tools, post-travel fevers remain a challenging clinical problem. We report an unusual case of post-travel fever, profuse diarrhea, extreme headache, and clinical sepsis. Lessons learned in this patient's care will be discussed in the context of current methods of diagnosis and treatment. Implications for travel to remote locales will be discussed.

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CUTANEOUS LEISHMANIASIS CAUSED BY *LEISHMANIA MAJOR* IN THE FORESTED VOLTA REGION OF GHANA

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We report the first proven human cases of cutaneous leishmaniasis (CL) originating from the forested Volta Region of Ghana and attributed to *Leishmania major*. Polymerase chain reaction (PCR) was applied to detect and identify *Leishmania* DNA in skin biopsies taken from 4 patients with active lesions. Primers specific for the ribosomal internal transcribed spacer region (LITSR) of *L. major* were used to amplify a 336 bp product. Amplicons were sequenced and interrogated by BLAST analysis against a range of Old World *Leishmania* species from diverse geographic locations. Bootstrap analysis and evolutionary distances revealed that the four infections from Ghana were identical and shared nearly complete ITS-1 identity with a Kenyan isolate of *L. major*. All four Ghanaian patients were permanent, non-traveling residents of the Ho District in southeastern Ghana, an area that receives abundant rainfall (2,100mm) and is ecologically atypical as a focus of *L. major* transmission. Ghana Health Service records cite more than 2,426 cases of CL from Ho District during 1999-2002 and 6,450 new cases during 2003. From Senegal to Kenya, *L. major* transmission is maintained in a cycle that principally involves the sand fly species *Phlebotomus duboscqi* and the multimammate rat, *Mastomys natalensis*, in an arid sahel-savannah environment. *Mastomys natalensis* is reportedly abundant in the Volta Region, but intensive entomological sampling in the Ho district, and elsewhere in Ghana, has found *P. duboscqi* to be near least abundant of 19 different sand fly species caught. Efforts to elucidate the transmission dynamics of CL in Ghana are underway.

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PRAZIQUANTEL BINDS SCHISTOSOMA MANSONI ADULT WORM ACTIN

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Praziquantel (PZQ) is widely used for treatment of schistosomiasis. It induces worm muscle contractions and tegumental disruption, followed by exposure of parasite surface membrane antigens to the host immunological defense mechanisms. It may be assumed that PZQ, like cholesterol, is too hydrophobic to traverse the schistosome outer lipid bilayers by passive diffusion, and likely needs binding to a surface membrane protein carrier for distribution throughout the worm. However, PZQ binding site on the schistosome surface and precise mechanism of action are not known as yet. The Claisen's condensation reaction was used to bind PZQ on cellulose acetate membranes. Triton-insoluble surface membrane antigens of Schistosoma mansoni adult worms were allowed to bind to the PZQ column. The binding molecules were examined for identity by amino acid micro sequencing and immunogenicity in outbred and inbred mice. PZQ column was found to selectively bind molecules of 45 kDa from the Triton-insoluble surface membrane antigens of S. mansoni adult worms. Amino acid microsequencing revealed that the 45-kDa species consist predominantly of schistosome actin. This finding was supported by the poor immunogenicity of the 45 kDa molecules in outbred and inbred mice. PZQ was also shown to bind bovine actin, but not bovine serum albumin. However, pre-incubation with bovine actin did not impair PZQ effect on adult worms *in vitro*. The study represents an attempt to understand how PZQ distributes across schistosome outer lipid bilayers.

(ACMCIP Abstract)

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AUDIT OF ANTIMALARIAL DRUG PRESCRIBING PRACTICE IN PRIVATE AND PUBLIC HEALTH FACILITIES IN SOUTH-EAST NIGERIA

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This study was undertaken to assess the prescribing practice for treating uncomplicated malaria in both government and private health facilities in Cross River State, South-eastern Nigeria. Clinical records of 665 patients recently treated for uncomplicated malaria in 6 private and 7 government health facilities were retrospectively assessed to determine the prescribing practice of the attending clinicians and the drug use pattern in the study area. Data was collected in standard forms by postgraduate doctors trained in the research methods. Private sector clinicians were less likely to record history or physical examination than those in public facilities. Diagnostic blood slides were performed in 45% of patients, with no difference between private and public sector. Monotherapy was 77% of all prescriptions; commonest drug prescribed was chloroquine (30.2%), followed by sulphadoxine-pyrimethamine (22.7%) and Artemisinin alone (15.8%). There was little difference between private and government services. Combination therapy was 20.8% of all prescriptions, and commonest combination treatment was chloroquine with sulphadoxine-pyrimethamine; only 20 patients (3.0%) received Artemisinin-based combination drugs. In conclusion, monotherapy with chloroquine, sulphadoxine-pyrimethamine and artemisinin were the first, second and third commonest prescriptions respectively for uncomplicated malaria in Cross State, Southeastern Nigeria

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CLINICAL UTILITY OF MONTENEGRO SKIN TEST IN THE DIAGNOSIS OF CUTANEOUS LEISHMANIASIS IN NORTHCENTRAL VENEZUELA

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PCR-based test is the ideal method of parasitological confirmation of cutaneous (CL) and mucocutaneous leishmaniasis (MCL). However, its cost limits its wider clinical implementation in resource-constrained settings. Montenegro skin test (MST) represents an alternative complementary diagnostic test for CL and MCL in endemic regions. We evaluated the clinical utility of MST compared to indirect immunofluorescence detection (IIF) in the diagnosis of CL in a cohort of patients in Northcentral Venezuela. From all patients evaluated at our referral center with a diagnosis of CL confirmed by different diagnostic methods between 1997-2005 (MST, IIF, and smear among others) 200 of them were randomly selected to test both MST and IFI to confirm the diagnosis of CL. Sensibility, specificity, positive and negative predictive values, probability coefficients and the Kappa concordance index ( ) were calculated between the two tests. Mean age: 33 years old; 74% were from Miranda State, with a mean clinical evolution of 5 months. Most patients presented just one lesion (76%), which were located mostly in extremities (43% in legs). From the total, 98% patients were positive by IIF and 92.5% were positive by MST. Just 15 patients were not concordant between MST and the IIF (13 false-negative and 2 false-positive) (crude concordance rate: 92.5%; χ^2=10.627, p=0.029; κ=0.185, p<0.01). The sensibility of the MST was 93.4% (95%CI 89.9-96.9%), specificity 50% (95%CI 1-99%), PPV 98.9%, NPV 13.3%, PPC 1.87% and NPC 0.13%. The results of our study suggest that MST is a clinically useful test in the diagnosis of more than 90% of individuals diagnosed by IIF with a significant concordant rate and a high level of sensibility, although a low level of specificity. We conclude that the MST can be used as a screening test in endemic areas which should be complemented with tests such as IIF.

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PUBLIC HEALTH IMPACT OF A RURAL UNIVERSITY IN HAITI

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Haiti is the most impoverished country in the Western Hemisphere and one of the poorest in the world. Considering all social and economic indicators, this troubled island nation ranks 153 out of 177 countries listed in the United Nations Development Program Human Development Index, 2003. Decades of political instability and economic crisis have led to overall deterioration of the healthcare system, extreme environmental decline and widespread public health crisis. A shortage of safe drinking water, inadequate sanitation and widespread malnutrition are major reasons why Haiti has the highest infant mortality and lowest life expectancy in the Latin American/Caribbean Region. Nearly two thirds of Haiti's population resides in rural areas and while international experts agree that rural development is critical to stemming the tide of economic and environmental decline, most financial, material and educational resources are concentrated in a few large cities. The University of Fondwa (UNIF) is a model of international cooperation with a goal to provide education to the sons and daughters of Haitian peasants so that they may become agents of change in their own rural communities. UNIF opened in a rural mountain community in Haiti in 2004 with programs in agronomy, veterinary medicine and management. The philosophy and curriculum are based on principles of integrated rural development. UNIF addresses many of the Essential Public Health Services most notably informing, educating and empowering people about health issues, diagnosing and investigating health hazards in the community and mobilizing community partnerships. UNIF also serves as a nidus for research and innovative solutions to public health problems such as zoonotic disease surveillance, prevention and recognition of disasters. By integrating principles of public health into the core curriculum, placing students in internship experiences with NGO's working on public health issues, community outreach seminars and community based participatory research practices, the University of Fondwa has considerable potential for impact on public health in Haiti.

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REDUCED SERUM CONCENTRATIONS OF RETINOL AND A-TOCOPHEROL AND HIGH CONCENTRATIONS OF HYDROPEROXIDES ARE ASSOCIATED WITH INTENSITY OF *S. MANSONI* INFECTION AND LEVELS OF SCHISTOSOMAL PERIPORTAL FIBROSIS IN ETHIOPIAN SCHOOL CHILDREN

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Communities with comparable levels of *Schistosoma mansoni* infection often exhibit differential morbidity levels, and micronutrient malnutrition has been suspected to contribute to these disparities. We studied the association of vitamin A, antioxidant micronutrients and oxidative stress with *S. mansoni* infection and schistosomal peri-portal fibrosis (PPF). This cross-sectional study involved 421 students (245 males and 176 females; mean age 12.5 $\pm$ 3.6 years) of which 333 were from two *S. mansoni* endemic villages (Workemado and Sille) while 88 were non-endemic healthy controls from Sheno. Detection of *S. mansoni* infection was based on examination of quintet Kato-Katz thick smears. Schistosomal PPF was diagnosed according to the WHO's guideline on ultrasonography in schistosomiasis. Serum retinol, α-tocopherol and hydroperoxides were used as biomarkers for vitamin A status, antioxidants and oxidative stress, respectively. Prevalence of *S. mansoni* infection in Workemado and Sille was comparable (90.6% vs. 95%, respectively), while the prevalence of PPF in Workemado was significantly higher than in Sille (7.0% vs. 0.6%, P < 0.001). Serum retinol concentrations were significantly lower and hydroperoxides were significantly higher in subjects from *S. mansoni* endemic areas compared to healthy controls. Serum α-tocopherol concentrations in subjects with high prevalence of PPF were significantly reduced compared to the group with low prevalence of PPF and non-endemic healthy controls. In conclusion, iomarkers for vitamin A status, antioxidants and oxidative stress are associated with intensity of *S. mansoni* infection and levels of schistosomal PPF.

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DISSEMINATED BCG INFECTION, A CASE REPORT

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BCG vaccination remains the best method of preventing severe *Mycobacterium tuberculosis* infections in developing countries. It has numerous complications; most are self-limiting. However, in immunocompromised patients, it may lead to disseminated mycobacterium infection, which is almost always lethal. We report an infant with disseminated BCG infection and CD2 deficiency and similar affected sister, both of whom died at the age of 4 months.

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WHAT IS DELUSIONAL PARASITOSIS?

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We have been seeing an increasing number of patients with pathogenic bacterial and fungal infections associated with recurrent open skin sores/lesions and with crawling tingling (pin prick) sensations, often interpreted as and confused with presence and movement of parasites under the skin and in body cavities. The presence of parasites could not be substantiated upon thorough testing. Patients were classified by health care practitioners as delusional. They were found to represent, however, genuine clinical cases but not of parasitic infections. Our studies of a few hundred patients over the last seven years have led to the description of a new disease, "Neuro-cutaneous Syndrome" (NCS), a dental toxicity disorder caused by the use of toxic sealants (liners, bases) during routine dental procedures, e.g., root canals, fillings, etc. NCS is an epidemic in disguise. Patients will show variable degrees of hyper reactivity depending on their level of sensitivity to the toxicity of dental material(s) used. The symptoms, toxic dental materials used and mode of their action, and associated opportunistic infections are discussed. Testing and treatment protocols are presented along with 24 case histories highlighted with photos of patients before and after rehabilitation. All patients who have followed and completed our treatment protocols have invariably recovered.

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CASE-CONTROL AND RETROSPECTIVE-COHORT STUDIES IN OUTBREAK INVESTIGATIONS, 1986-2005: AN UPDATE OF A CLASSICAL STUDY

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Case control and retrospective cohort studies are important analytical tools during epidemiological field investigations. We reviewed the scientific literature to assess trends in their use in outbreak investigations. Potential outbreak investigation reports were identified through a MEDLINE search for the word "outbreak" in the paper with the words "outbreak", "outbreaks", "cluster" or "epidemic" in the title, but without the words "outbreak news". The search was limited to publications about humans between 1986-2005, excluding reviews, historical articles and commentaries. Two hundred potential outbreak reports published in English, Spanish or Portuguese were then randomly selected, 50 from each five-year period, and assigned in alternated-fashion to one of four reviewers. Review assessed if publications were original outbreak reports and if case-control or retrospective cohort studies were used. As of May/08/2006, 9821 potential outbreak investigations were found in the 1986-2005 period, and 8660 were in English, Portuguese or Spanish (95.6% in English). Potential outbreak reported in these languages increased 291% from the 1966-85 period, more than the 185.1% increase in overall MEDLINE records. On average, 20 additional reports were published annually between 1986-2005 (R^2=97%). Of the 200 sampled articles, three were unavailable on time and 116 (57%, weighted %) were original outbreak reports (31, 31, 31 and 23 in each five-year period, respectively). Case control or retrospective cohort studies were used in 43 outbreak investigations (37%, weighted %), more frequently than reported for the 1980-5 period (17%, p<0.001) but without differences over time (86-90: 35%, 91-95: 42%, 96-00:39%, 00-05:30%, p=0.845). Case-control studies were used in 21 outbreak investigations, retrospective cohort studies were used in 20, and both study designs were use in two investigations. We observed increased publication of outbreak-related manuscripts and more frequent use of analytical studies in outbreak investigation in the last 20 years. Sound, prompt outbreak investigations are key for the response to emerging global pandemics, particularly in settings with high epidemic potential. Continued efforts in field epidemiology training are needed, particularly in non-English speaking countries where outbreak investigations are published less frequently.

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EPIDEMIOLOGY OF AMERICAN CUTANEOUS LEISHMANIASIS IN CHILDREN AND ADOLESCENTS FROM NORTHCENTRAL VENEZUELA

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In the Americas, Cutaneous Leishmaniasis (CL) is caused primarily by members of *Leishmania mexicana* and *Leishmania braziliensis* complex. CL comprises a broad range of cutaneous manifestations which may produce severe and chronic sequelae in adults. However, it has been suggested that CL has different clinical and epidemiological features in children and adolescents that need to be further elucidated. We evaluated the epidemiologic features of CL in a cohort of pediatric patients from Northcentral Venezuela between 1997-2005. A total of 43 children and adolescents with the clinical diagnosis of CL were evaluated at our referral center (IMT). Different diagnostic methods were used to confirm the diagnosis (Montenegro Skin Test, MST; Indirect Immunofluorescence Test, IIF; and smear). Clinico-epidemiological features of ACL among these patients were studied and described. Mean age: 9 years old; 35% were adolescents and 35% school-age children (6-12 y-old); 81% were from Miranda State, with a mean clinical evolution of 3 months. Most patients presented just one lesion (86%), which were located mostly in extremities (45% in legs, 19% in arms, 13% in hands; 4 cases were in the face). Of the 43, 40 (93%) patients were positive by MST, 97.7% were positive by IIF and 48.8% were positive by smear. With the combination of these tests, CL was confirmed in 97.7% patients. MST values tended to be related to patients' age (r^2=.0867, F=2.184, p=.153), mean of 9 mm in the group of 13-24 months, 10.4 mm in the 2-5 y-old group, 12.1 mm in the 6-11 y-old and 10.5 mm in the 12-18 y-old. There is a growing interest in defining the clinical and epidemiological features of CL in children and adolescents compared to adults. In our study, we identified that most disease tend to affect extremities but also the face; and we found that skin testing for leishmaniasis vary according to age in an endemic population in Northcentral Venezuela.

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THE INTEGRATION OF NEGLECTED DISEASE PROGRAMS: PHASE 1 OF AN ONGOING EXERCISE LED BY MINISTRY OF HEALTH PROGRAM COORDINATORS, TOGO

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The integration of neglected disease programs is currently considered essential for achieving an effective and sustainable public health impact. Though frequently discussed at the supranational level, there is limited field experience with integrating more than 2 vertical programs. In Togo, a West-African country with 5.7 million inhabitants, programs for malaria, lymphatic filariasis, onchocerciasis and guinea worm are recognized as successful while the schistosomiasis, geohelminth and trachoma programs are facing difficulties due to lack of funding. This project let the national program coordinators from Togo decide for themselves which activities could be integrated, while giving them some financial and technical support. The program consists of 2 phases: a preparatory phase (phase 1) and an implementation phase (phase 2). Phase 1 started in January 2006. The main activities are defining integration, identifying program coordinators who want to join the integration team, deciding which

activities can be integrated and developing practical guidelines and tools. In addition, challenges which similar integration projects could face will be identified and solutions proposed. The Ministry of Health is committed to the project. Though a lot of programs (e.g., HIV/AIDS, EPI and tuberculosis) were interested in the process, the team decided to start phase 1 with the above mentioned neglected disease programs. Integration was defined as the integration of community based services which can be executed by a village volunteer. Consequently integration and cooperation of all levels of the health system is necessary. Detailed inventories of all activities currently conducted and which would be conducted in case resources were available were made. During a consensus meeting, activities which could be integrated were identified, such as health education, distribution of services and items, training, supervision, monitoring and evaluation and budget. An update of the program will be given during the presentation.

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COMPARATIVE NEUROPATHOLOGY OF THE ARTEMESININ COMPOUNDS ARTEETHER, ARTEMETHER, ARTELINATE, AND ARTESUNATE IN *RATTUS NORVEGICUS*. I. CYTOPATHOLOGY OF THE AUDITORY NUCLEI

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Artesunate is being developed by the U.S. Army for the treatment of severe falciparum malaria. The neurological safety of AS was demonstrated when no evidence of neuropathology could be observed in dorsal column, tegmental, precerebellar, vestibular, auditory, visual, cerebellar and midbrain nuclei in Rhesus monkeys following cumulative doses of 28, 56, 112, 224 and 896 mg/kg of AS given intravenously, as reported previously. Unlike AS, brainstem neurotoxicity was demonstrated in rats, dogs and Rhesus monkeys following arteether administration, as reported previously. The current study compares the potential neurotoxic effects of arteether (AE), artemether (AM), artelinate (AL), and artesunate (AS) on brainstem auditory nuclei of male Sprague-Dawley rats. The intramuscular dose regimens utilized were: i) AE 6, 12.5, 25 and 50 mg/kg for periods between 7-16d; ii) AM 12.5 and 25 mg/kg/d for periods of 7-13d; AL 4.25, 34.25, 38.5 and 68.5 mg/kg ranging between 10-14d; AS 15 and 30 mg/kg/d x 14d. Evidence of neuropathology was studied using several cellular stains: methods of Nissl, Klüver-Barrera and H&E. The cumulative doses were: i) AE at 42, 87.5, 125, 175, 250, 325, or 350 mg/kg; AM at 87.5, 175, or 300 mg/kg; iii) AL, at 59.5, or 479.5 mg/kg; and iv) AS at 59.5, 210, or 420 mg/kg. Matching vehicle control rats were prepared for each artemisinin dose group. The auditory structures studied were i) cochlear nuclei (DCN, PVCN, AVCN, ICN); ii) superior olivary nuclei (LSO, MSO); iii) trapezoid nuclear complex (VNTB, LNTB); iv) periolivary nuclear complex and the v) nuclei of the lateral lemniscus (VNLL, INLL, DNLL). These nuclear groups were not injured when cumulative doses were: i) AE at 42, 87.5, 125 mg/kg; ii) AM at 87.5 mg/kg. Auditory injury was present when cumulative dose of AE or AM reached 175 mg/kg. The affected nuclei included the VNTB, LNTB, LSO and MSO. Cumulative dose above 175 mg/kg of AE or AM resulted in more extensive auditory injury. Matching vehicle controls did not show evidence of neuropathology. In summary, the cumulative no injury dose for the auditory system was: i) AE at 42, 87.5, and 125 mg/kg; ii) AM at 87.5 mg/kg; iii) AL at 59.5 mg/kg; iv) AS at 59.5, 210, and 420 mg/kg. Artesunate appears to have the widest margin of neurological safety while AM or AE may also be beneficial. Recent clinical trials in Asia and Africa indicate the usefulness of AM for the initial treatment of falciparum malaria.

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BURDEN OF MORBIDITY AND MORTALITY ASSOCIATED TO LEISHMANIASIS AND ITS IMPACT ON CHILDHOOD HEALTH IN VENEZUELA

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Morbidity and mortality due to leishmaniases are on increase. American visceral leishmaniasis (AVL) is of higher priority than American cutaneous leishmaniasis (ACL) as it is a fatal disease in the absence of treatment. But ACL morbidity and mortality has been understudied in many epidemiological settings, one of them in the childhood age. For these reasons in this report we analyzed the trends in mortality due to infections caused by *Leishmania spp*. in Venezuela, between 1995-2002, and its impact on childhood health and disease. A systematic review of AVL and ACL mortality in Venezuela, between 1995-2002, was done, from mortality records from Ministry of Health and regional offices of Environmental Health, using descriptive and analytical statistics, focusing on the impact of disease in childhood age groups. From 1995 through 2000, 242 cases of AVL were reported from 12 states, in various sections of Venezuela, with a relatively stable national incidence rate of 0.2 cases per 100,000 pop/year; 26.0% were from Margarita Island. From 1995 through 2002, 18,448 cases of ACL were reported from 22 states of the country, with a national incidence rate of 27.6-74.6 cases per 100,000 pop/year; 19.3% were from Lara state. For this period, 64 patients died from leishmaniasis, 39.1% were <20 y-old (12.5% were <1 y-old, 10.9% 1-2 y-old, 7.8% 2-5 y-old and 7.8% 5-19 y-old). From total death patients, 62.5% (40) were AVL, 18.8% (12) were ACL and 6.3% (4) were mucocutaneous leishmaniasis. As has been seen by us and by others, the significant impact of leishmaniasis on childhood morbidity and mortality is an issue of increasing importance in the recent years in many endemic regions. Many issues, currently, deserved to be studied in regard to this morbidity and mortality, as the impact of new treatments, the prevention with the use of impregnated insecticide nets and curtains, among others, which could lead to improve and reduced the leishmaniasis-associated morbidity and mortality in the childhood age.

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THE TRADITIONAL KINSHIP SYSTEM ENHANCES COMMUNITY INVOLVEMENT AND IMPROVES IVERMECTIN COVERAGE FOR ONCHOCERCIASIS CONTROL IN SOUTHEAST NIGERIA

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Onchocerciasis (river blindness) can be controlled by annual mass treatment with ivermectin, the delivery of which must be sustained indefinitely. Community-directed treatment with ivermectin (CDTI), using community voluntarism and decision making, is the approach for sustained delivery that the African Programme for Onchocerciasis Control (APOC) promotes to governments and involved NGOs. In three Carter Center-assisted states in southeastern Nigeria we modified CDTI to include traditional kinship systems in 21 rural villages. We compared performance in 2004, before the traditional kinship system was employed, to 2005, after it was employed. The objective was to determine if using the kinship system could improve performance and sustainability of CDTI, which had stagnated, and improve integration of health care services in these

villages. Performance improved dramatically. The period of distribution was reduced from a mean of 32 days in 2004 to 8 days in 2005. The percentage of villages treated improved from 77% in 2004, to 100% in 2005, while therapeutic coverage improved from 72% to 81%. In addition: 1) demand for monetary or other incentives was eliminated, 2) more women became more actively involved, and 3) attrition of voluntary distributors dropped from 26 % to under 2%. However, training and supervision requirements increased, as distributors increased from an average of one per village in 2004 to 20 per village in 2005. The availability of many trained distributors in each kinship group resulted in a smaller workload with the convenience of mobilizing family members and neighbors in a smaller area within the village. This indeed reduced the attrition rate of CDDs and promoted involvement of female CDDs. This approach can help to deliver other health care services promptly and efficiently, and should be promoted for other community based initiatives.

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RICKETTSIOSIS IN A TRAVELER RETURNING FROM HONDURAS

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While tick-borne rickettsioses are known to occur in many parts of the world, including Africa, Asia, Europe, the Americas, and Australia, the occurrence of rickettsioses in Central America is poorly documented and rarely described. An outbreak of rickettsial illness was reported from Costa Rica in 1974. Exanthematous typhus was reported from Guatemala in 1977. We report a case of tick-borne rickettsiosis in a traveler returning from Honduras. The patient is a 51 year-old man who traveled to Roatan, Honduras in 2005, where he was bitten by a tick. He reported erythema and induration with necrosis at the site of the tick bite, as well as headache, fever, weakness, dizziness, abdominal discomfort, diarrhea, flu-like illness, and respiratory symptoms. Evaluation in Honduras led to diagnoses including malaria, respiratory infection, and parasites, followed by treatment with chloroquine, primaquine, an antibiotic "Bacillin", and mebendazole with some relief. He returned to the US two months after the tick bite and presented with residual symptoms. His physical examination was unremarkable. Rickettsial serologies performed by a commercial laboratory and the CDC were markedly elevated for Rickettsia rickettsii, Rickettsia conorii, and Rickettsia africae. He improved after treatment with doxycycline This is the first reported occurrence of a tick-borne rickettsiosis in Honduras. There could be at least two potential explanations for the lack of published reports of tick-borne rickettsioses from Honduras: 1) absence of infection; 2) absence of published reports (diagnosis not being made or infections not being reported in published literature). The distribution of tick-borne rickettsioses and intensity of transmission can change over time. Given the documented occurrence of rickettsioses in North America including Mexico, the Caribbean, South America, Costa Rica, and Panama, it is not surprising that tick-borne rickettsioses can also occur in Honduras.

Rickettsioses are emerging infectious diseases increasingly reported from around the world. Recognition is important because rickettsial infections are treatable and can be serious or fatal. The identification of rickettsioses in travelers has served to document their presence in some countries or regions, and has helped to define the global distribution of rickettsial disease. Our case demonstrates the valuable role of travelers as sentinels for infectious diseases.

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A SURVEY OF ZOONOTIC PATHOGENS CARRIED BY NORWAY RATS IN BALTIMORE, MARYLAND, USA

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Norway rats are reservoirs for several zoonotic agents, including hantaviruses, and are implicated in the transmission of pathogens to humans in urban environments. Because the resulting human diseases range in their degree of severity, the prevalence of rodent-borne illnesses is hypothesized to be underreported. To survey the pathogens carried by Norway rats (*Rattus norvegicus*), rats were live-trapped in alleys in Baltimore, Maryland, USA from April 2005 - April 2006. Following trapping, rats were brought to the laboratory where they were sexed, weighed, and bled. Cecum and fecal samples were collected for microscopic determination of nematode and cestode egg burden. Serum was used to assess antibody production against several viruses, bacteria, and parasites by ELISA or IFA. Our preliminary data reveal that Norway rats are infected with several zoonotic pathogens and were seropositive for Seoul virus 57.7% (116/201), *Calodium hepatica* 87.9% (176/201), *Hymenolepis* spp. 34.0% (55/162), *Trichuris* trichuria 14.8% (24/162), Rickettsia typhi 7.0% (14/201), Ehrlichia spp. 4.4% (4/91), Bartonella *elizabethae* 32.7% (16/49), and *Leptospira icterohemorrhagiae* 29.7% (11/37), but were seronegative for Lymphocytic choriomeningitis virus (0/48). Results for Hepatitis E virus and *Cryptosporidium parvum* are pending. In addition to zoonotic pathogens, Norway rats were screened for exposure to rodent-borne pathogens associated with laboratory colony outbreaks. We determined that wild-caught rats were seropositive for several rodent-associated pathogens, including Rat coronavirus 91.6% (44/48), Rat parvovirus 47.9% (23/48), Rat parvovirus H-1 10.4% (5/48), Killer rat virus 10.4% (5/48), Sendai virus 4.2% (2/48), Theiler's mouse encephalomyelitis virus 5.4% (2/37), Cilia associated respiratory bacillus 52.1% (25/48), *Mycoplasma pulmonis* 72.9% (35/48), *Nippostrongylus brasiliensis* 71.6% (116/162), and pinworms 28.4% (46/162), but were seronegative for Rat reovirus (0/47) and Pneumonia virus of mice (0/47). Norway rats are potential vectors for a range of zoonotic and rodent-borne pathogens and may contribute to outbreaks in human populations as well as laboratory rodent colonies.

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RENAL TRANSPORTERS IN LEPTOSPIROSES-INDUCED ACUTE RENAL FAILURE AND CHALLENGE OF REVERSAL AFTER ANTIMICROBIAL TREATMENT

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Tubulo-interstitial nephritis is the main manifestation of acute renal failure (ARF) in leptospiral infection. Pathogenesis may be related to the presence of virulent toxin/s produced by the microorganism. Acute renal injury is associated with a special form of tubular dysfunction that leads to polyuria, hypokalemia, and sodium wasting, involving renal transporters. Related to treatment, we hypothesized that antibiotic administration could improve renal damage. This syudy was undertaken to evaluate the

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expression of two of the major Na+ renal transporters: type 3 Na+/H+ exchanger (NHE3) in proximal tubular cells, and bumetanide-sensitive Na+-K+-2Cl- cotransporter(BSC-1 or NKCC2) in thick ascending limb, in untreated and treated hamsters infected with *Leptospira interrogans*. Twenty-four hamsters were inoculated intraperitoneally with a lethal dose of 10^8 *Leptospira interrogans* serovar Copenhageni strain Fiocruz L1-130 (Experiment 1- E1-Weil's disease acute progressive illness) and 10^3(Experiment 2-E2-Weil's disease - biphasic illness). Animals were divided in control(placebo) and treated groups (Ampicillin, 100 and 80mg/kg/day bid,E1 and E2 respectively) with mean duration of 4 days. Treatment started on the second (E1) and eight day of illness (E2). Animals were sacrificed on day 5 (E1) and day 11 (E2) post-infection. In renal tissue we analyzed the presence of antigen/s by immunohistochemistry (IH). The degree of tubulo-interstitial dysfunction, was evaluated by determining the level of NHE3 and NKCC2 expression by IH and Immunoblot in control and treated animals. Experiments 1 and 2 showed that in controls, the presence of Leptospira antigen/s were distributed in renal tissue, with NHE3 and NKCC2 expression decreased (with respect to normal animals), as demonstrated by IH and Immunoblot. In treated animals, there was minimal or no detection of Leptospira antigen/s. Furthermore, NHE3 and NKCC2 expression was observed to be equivalent to that seen in normal animals as showed both by IH and Immunoblot. In conclusion, tubular dysfunction related to Na^+ transporters could be caused by leptospira antigen/s or their products, and transporters expression were reestablished after both early and late antibiotic treatment. Antibiotic treatment in animal models of leptospirosis-induced ARF might change renal transporters.

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DEFINING CHRONIC CIGUATERA ILLNESS BY ABNORMALITIES IN INNATE IMMUNE RESPONSES: FINAL COMMON PATHWAYS OF CHRONIC, BIOTOXIN-ASSOCIATED ILLNESS

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Ciguatera, a biotoxin-associated illness acquired following consumption of contaminated piscivorous reef-dwelling fish, remains difficult to diagnose. Some cases of ciguatera show self-healing; chronic illness can be marked by significant disability and refractory symptoms. Diagnosis of chronic ciguatera illness relies on history, with only one reliable diagnostic test noted in the world's literature - visual contrast sensitivity (VCS). No commercially available, reliable assay for ciguatoxins in human blood or tissues exists. No objective biomarker that classifies patients as cases or non-cases has been confirmed. We present here a series of 50 patients with chronic ciguatera diagnosed using a two tier approach used in other biotoxin-associated illnesses, including exposure to other toxigenic dinoflagellates, including *Pfiesteria* and *Chattonella*; exposure to toxigenic cyanobacteria; and to resident toxigenic fungi found inside water-damaged buildings. A sequential treatment protocol for chronic ciguatera, beginning with cholestyramine, provided the ability to study changes in inflammatory markers found in cases but not found in controls. Correction of these abnormalities resulted in symptom reduction exceeding 75% in patients whose illness duration averaged over 4 years. Compared to controls, cases of chronic ciguatera show increased total symptoms; VCS deficits; increased incidence of particular haplotypes of HLA DR analyzed by PCR; MSH deficiency; elevation of C4a and C3a; increased IL-1B, IL-10 and MMP9; reduction of VEGF; dysregulation of simultaneously measured ACTH/cortisol and ADH/osmolality; increased nasal colonization by multiply antibiotic resistant coagulase negative staphylococci; and increased incidence of antibodies to gliadin, cardiolipin and myelin basic protein. Taken together, these patients do not demonstrate abnormalities in measures of acquired immune response, but do show multiple abnormalities in innate immune response. The abnormalities in innate immune responses show no significant differences in chronic ciguatera from any of the other biotoxin-associated illnesses, suggesting that a common mechanism creates chronic biotoxin illness.

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EIGHT YEAR FOLLOW-UP OF PATIENTS WITH POSSIBLE ESTUARINE ASSOCIATED SYNDROME (PEAS): SYMPTOM REDUCTION DIDN'T RESULT IN CURE

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Beginning in 1997, 50 patients with acute toxigenic illness termed PEAS that met the CDC case definition and 127 age/gender-matched controls were reported from one site in a series of papers. PEAS was a biotoxin-associated illness not previously reported in the wild, but had occurred in laboratory staff exposed to cultures of toxigenic *Pfiesteria piscicida.* Despite an intense research effort the mechanisms of human illness remained poorly defined. Some patients with exposure acquired no symptoms; others exhibited an acute PEAS illness that resolved; others demonstrated persistent multi-system symptoms. For most patients with persistent symptoms the use of cholestyramine (CSM) provided symptom relief and correction of their deficits in visual contrast sensitivity (VCS). Several patients developed a chronic fatiguing illness that resulted in profound disability despite use of CSM. Thirty-six patients from the original cohorts of PEAS cases were recalled for follow-up evaluation, VCS and laboratory testing. Eleven PEAS patients were dead (22%) and 3 were lost to follow-up. 119 controls were contacted; 2 were dead (1.6%) and 8 were lost to follow-up. 11 PEAS (31%) and 2 control patients (1.7%) had more than 12 symptoms; 11 PEAS patients (31%) and 15 controls (13%) had more than 6-12 symptoms. 6 PEAS patients (17%) and 22 controls (18%) had 3-6 symptoms. 8 PEAS (22%) and 80 controls (67%) had less than three symptoms. PEAS patients with more than 6 symptoms had profound abnormalities in innate immune response, with MSH deficiency; elevation of C3a and C4a; reduction of VEGF; elevation of MMP9, IL-1B and IL-10; and dysregulation of ACTH/cortisol and ADH/osmolality. VCS deficits were not demonstrated. Marked differences between cases and controls in specific haplotypes of HLA DR by PCR were seen. Treatment of biotoxin associated illnesses, including PEAS, should include identification and correction of abnormal innate immune responses.

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TOWARDS TB CONTROL IN NIGERIA TARGETING DELAY IN TB TREATMENT CARE SEEKING

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Delay in seeking TB care is a major issue and concern in the epidemiology and control of TB in Nigeria and realization of the Millennium Development Goal target of reversing the incidence of TB. In spite of the Government policy of free treatment for TB, people affected by TB report late to health facilities, and most often when they have developed complications and might have infected many people. This concern is heightened by the present ravaging HIV/AIDS /TB confection in Nigeria. The objective of the study was to investigate delays in seeking TB care among patients attending a regional hospital in Nigeria and to design strategy to reduce the delays. Two hundred TB patients attending Mile 4 Hospital Abakaliki. an old mission centre reputed for TB care, were studied using qualitative and quantitative methods between March - August 2005. Four types of delay were identified: 1) The first delay was within the family circle in taking decisions to seek care. Typically there was a family "therapeutic" group which decides on where and when to seek therapy. 2) The second delay was in accessing funds /transportation and family accompanying attendant to the chosen health facility. 3) The third delay was within the periphery of health providers - who have not the competence to manage TB. These health providers- traditional and

orthodox, delay in providing accurate diagnosis, appropriate treatment and prompt referral. 4) The fourth delay was within the competent hospital or health facility due to attitude of health workers, and logistics in releasing test results and initiating treatment. Fifty-five % had a delay of over 3 months while 28% had delays of over 6 months. About 71.5% of patients had received inappropriate care in peripheral centers before commencement of appropriate TB therapy. In conclusion, delay in seeking care is a pervading issue in TB control in Nigeria TB control efforts should target delays through appropriate health education messages both to the community and health providers for early and prompt report, diagnosis and referral to competent TB centers.

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USE OF MALARIA PREVENTIVE MEASURES IN PREGNANCY AND PLACENTAL/NEONATAL PARASITAEMIA: A MULTICENTRE BASELINE EVALUATION IN NIGERIA

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Malaria in pregnancy has been a topical issue in recent times and most of its effects in newborns have been widely reported in the literature except for congenital malaria. This was previously reported to be very rare but has been increasingly reported in recent times in Nigeria, although based on results from small population studies. Clinicians have found this situation very worrisome, thus raising the need to examine the existing practices in the country in relation to the use of malaria preventive measures in pregnancy. This study was undertaken to evaluate on a large scale the current status of use of malaria preventive measures (anti-vector measures, chemoprophylaxis, and intermittent presumptive treatment with sulphadoxine-pyrimethamine (IPTp-SP) and their relationship with prevalence of placental and congenital malaria in Nigeria. This was a multi-centre prospective and descriptive study conducted between April 2003 and March 2004.Two thousand five hundred mother infant pairs were recruited from four geopolitical zones with varying epidemiological characteristics regarding malaria. The majority of babies (89.8%) were delivered at term and only 6.1% had low birth weight. Proportion of use of malaria preventive measures were: mosquito screens (44.8%), insecticide sprays (35.5%) while only 2.5% used insecticide treated nets. Overall, 88.5% of the mothers received some form of medications for malaria prevention comprising of pyrimethamine (76.8%), proguanil, (1%), IPTp-SP (11.7%), Chloroquine (4%). A small proportion (0.1%) of the women used herbs. Babies of mothers on IPT_p-SP were least likely to have parasitaemia (2%) (*$P<0.05$*). The combination of chemoprophylaxis/ IPTp-SP and anti-vector agents was found to be most effective in reducing parasitaemia in the newborns (*$X^2 = 5.85; P=0.016$*).

Given the efficacy of IPT_p-SP in this study and others done elsewhere in sub-Saharan Africa, there is a need to promote the use of IPT_p-SP throughout Nigeria and its combination with anti-vector agents should be emphasized. The campaign to promote the use of insecticide treated nets for prevention of malaria in pregnancy should also be intensified in Nigeria as the usage is still very low.

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PERIPARTUM MALARIA IN NIGERIA: CURRENT STATUS AND PREGNANCY OUTCOME

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Malaria in pregnancy is an important cause of a variety of maternal and perinatal adverse consequences. In Nigeria, several studies focusing on malaria in the peripartum period have been conducted but with variable findings that has militated against a focused policy on prevention. This study was undertaken to evaluate the current status of peripartum malaria and its impact on neonatal outcome in Nigeria. A descriptive study was conducted in four centers located in four different geographical zones in Nigeria over a 12month period. Focused clinical and laboratory examination were done in the subjects within 2 hours of parturition. Maternal peripheral and placental blood samples were taken for determination of malaria parasitaemia using Giemsa stain of the thick and thin blood films. Maternal and baby's haematocrit were also taken at birth. There was a quality assurance procedure for the study. A total of 2500 subjects were recruited. A total of 625 of the data sets were excluded due to breach in the study protocol. The women were aged between 14-50years. The proportion of preterm deliveries was 10%. There were a total of 404 positive smears, giving a peripartum prevalence of malaria of 21%. Those with positive peripheral smears were 319 (17%) while those with positive placental smears were 267 (14.2%). There was a significant decrease in proportion of women with placental parasitaemia with increasing parity (p=0.04). Maternal age less than 20 years was significantly associated with both peripheral (p=.016; OR=2.3; CI=2-4.9) and placental parasitaemia (p=0.01; OR=2.6: 1.2-5.4). However after adjusting for covariates only the age of the women <20years was significantly associated placental parasitaemia. Peripheral parasitaemia in the women was associated with a lower mean haematocrit 0.34±0.5 versus 0.37±0.54 (p=0.0001); lower mean birth weight (p=0.001), and a significantly higher proportion of low birth-weight babies; 24.2% versus 16.5% (p=0.025, OR = 1.65 [1.1-2.5]). In conclusion, in Nigeria 1 in every 5 women have malaria parasitaemia at delivery. Maternal age, less than 20years, was the most important predisposing factor. Reduction in maternal haematocrit, mean birth weight and a higher proportion of low birth weight babies were the major outcomes of malaria in the peripartum period.

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MALARIA SITUATION: RISK AND CONTROL IN TSUNAMI-AFFECTED AREAS, PHANG NGA PROVINCE, THAILAND

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In December 2004 the Asian tsunami caused more than 200,000 deaths. The WHO and other health organizations warned of possible communicable disease outbreaks in all tsunami affected areas. We

conducted a study comparing malaria incidence in Phang Nga, the coastal Thailand province most severely affected by the tsunami. Relative to the annual pre-tsunami incidence the annual incidence immediately following the tsunami increased in the five inland provincial districts but was stable in the three coastal districts directly sustaining tsunami damage. The increase in the non tsunami-affected districts might be attributable to an influx of foreign workers, the diversion of malaria control resources from non tsunami-affected to tsunami-affected districts; or other factors. Interestingly, an entomological survey conducted in a tsunami affected area noted to have a minor malaria outbreak detected the presence of *Anopheles sundaicus,* a species complex with a predilection for brackish coastal areas. Continued active malaria control measures and long term surveillance are recommended.

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AN INVESTIGATION INTO THE POTENTIAL CARDIOTOXIC INTERACTIONS OF QUININE AND ARTEMETHER/ LUMEFANTRINE (COARTEM®) WHEN USED SEQUENTIALLY IN THE TREATMENT OF MALAWIAN CHILDREN WITH SEVERE MALARIAL ANEMIA

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Severe malaria is a major cause of childhood morbidity and mortality in African children. Currently, the common treatment is parental quinine followed by oral sulfadoxine/pyrimethamine (SP). Due to fast development of SP resistance many African countries are in the process of replacing SP with artemether/lumefantrine (Coartem®). Lumefantrine is structurally related to quinine, which is known to give marked QTc interval prolongation on electrocardiogram (ECG). Studies so far have shown that Coartem alone does not cause clinically relevant QTc prolongation. But, the potentially important cardiotoxic consequences of sequential quinine and Coartem treatment in children have not been studied. We have evaluation, in an open labeled study, ECG changes in 42 Malawian children who received parental quinine followed by Coartem as part of their severe malaria anemia management. The patients received a blood transfusion and were started on parental quinine (total of 5 doses) followed by a 6 dose Coartem course. A twelve lead ECG was recorded just before the 1st (0 hrs) and 6 hours after the 1st (6 hrs) and 6th (78 hrs) Coartem dose and after 28 days. ECG analysis was done by automated software package and manual reading. One hundred and eight ECG recordings were available for analysis. None of the children, at any time point, had a QTc interval measure of more than 550msec (primary endpoint). QTc intervals of >450 but less then 480msec were found in 2.2%, 4.9%, 7.7% and 8.1% of ECG recordings at 0, 6 and 78 hours, and 28 days respectively. None of the patients was found to have a >60ms increase in QTc interval when compared to base line at any time point. No arrhythmia or syncope was observed in any of the children. In the first 42 children with severe malaria anemia studied, we did not find an indication of a cardiotoxic effect when quinine and Coartem were given sequentially. This is an encouraging finding, since cardio toxicity would have posed serious limitations for the use of Coartem in the final stage of antimalarial treatment following a severe malaria episode. In order to improve the power of the study, recruitment is continuing. At the conference ECG findings from a considerably larger study population will be available for presentation.

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TOXOPLASMA GONDII INFECTION IN THE UNITED STATES, 1999-2004

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Toxoplasma gondii infection can be responsible for congenital or acquired disease that leads to neurologic and ocular illness. To determine the recent prevalence of *T. gondii* infection in the U.S. population, we tested sera collected from the National Health and Examination Survey (NHANES) in 1999 through 2004 for *T. gondii* specific immunoglobulin G antibodies (Platelia Toxo-G EIA, BioRad, Hercules, CA) on persons age 6-49 years. We also compared the results to those obtained in NHANES III (1988-94). Of 18,433 persons 6-49 years old selected and examined in NHANES 1999-2004, 15,960 (90%) had sera tested for *T. gondii* antibodies. The age-adjusted *T. gondii* seroprevalence among those 6-49 years old was 10.8% (95% confidence limits [CL] 9.6%, 11.9%), and among women of childbearing age (15-44 years old), 11.0% (95% CL 9.5%, 12.4%). Seroprevalence increased with age; the age-adjusted seroprevalence was higher among persons below the poverty level (14.5%) than those at or above the poverty level (9.9%) (p<.001), and higher among non-Hispanic black (12.1%) and Mexican American (13.7%) than among non-Hispanic white persons (8.7%) (p=0.01 and p<.001, respectively). However, among U.S.-born persons age 6-49 years the age-adjusted seroprevalence was lower in Mexican Americans (4.6%) than non-Hispanic blacks (10.4%) or whites (8.1%) (p<.001 and p<.001, respectively). When comparing U.S.-born persons in the overlapping 12-49 year age group, from NHANES III (1988-1994) to NHANES 1999-2004 there was a reduction in the age-adjusted *T. gondii* prevalence from 14.1% to 9.0% (p<.001). Although *T. gondii* still infects many persons in the United States, the prevalence has decreased in the past decade.

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MALARIA ELIMINATION IN HISPANIOLA: A REALISTIC GOAL?

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Malaria remains a problem in Hispaniola, the Caribbean island shared by Haiti and the Dominican Republic. In Haiti (pop. 8 million), more than 20,000 cases of confirmed malaria were reported in 2005. This statistic is, however, unreliable due to under-reporting and inadequate quality of microscopic diagnosis in many health facilities. Two countrywide health facility surveys in Haiti in 1995 and 2005 showed that, among patients with clinically suspected malaria, 4.0% and 3.4%, respectively, were infected with *Plasmodium falciparum*. In the Dominican Republic (pop. 9 million), 3,098 cases of confirmed malaria were reported in 2005. In the Dominican Republic, most cases occur in persons aged 10-49 years (74%) and in rural areas (75%); in 2004, 28% of the cases were in Haitian nationals, mostly migrant workers in sugarcane plantations or construction projects. Malaria is concentrated in defined areas of the island, with most cases found in the Haitian departments of Grande Anse, Nippes, Artibonite, and South, and in the Dominican provinces of Bahoruco, Barahona, Azua, and La Altagracia. In addition to its health impact, malaria also affects the economy of the island; the tourism industry in the Dominican Republic reported a loss of 200 million USD after 18 tourists developed malaria in late 2004 following a visit to the coastal resorts of

Punta Cana and Bavaro. Malaria elimination in Hispaniola is a realistic objective because: a) malaria has been eliminated from all other Caribbean islands; b) malaria in Hispaniola is not highly prevalent and tends to be focal; c) *P. falciparum*, the only species found in the island, does not relapse and remains chloroquine sensitive to date; and d) the main vector, *Anopheles albimanus*, is relatively inefficient. To achieve elimination, Haiti and the Dominican Republic need to coordinate their control efforts and adopt jointly a comprehensive package of interventions including surveillance, early treatment of infections, insecticide-treated bednets, treatment of mosquito breeding sites and residual insecticide spraying.

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DETECTION OF *LEISHMANIA* PARASITES IN AN OUTBREAK SITE IN GHANA USING POLYMERASE CHAIN REACTION

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A recently recognized outbreak of Leishmaniasis in Ghana has prompted research into the epidemiology of the disease in this region. Initial published findings indicated that the causal agent was *Leishmania major*. To further characterize this outbreak, we used a real time PCR method to identify *Leishmania*-infected individuals in Ghanaian villages, and have sought to identify the local vector and mammalian reservoir which complete the disease cycle. Our study will encompass the 2006 calendar year during which human, mammalian and sand fly samples will be obtained on a monthly basis from field collections from the outbreak foci around Ho, Volta Region, Ghana. This sampling scheme attempts to elucidate the disease cycle and any seasonality to the outbreak. To date, 88 samples have been assayed including nine tissue samples from humans, 33 rodent tissue samples, 43 tissue and blood samples from domestic animals, and 25 pools of sand flies (~250 flies). Using a primer-probe set recognizing all members of the genus *Leishmania*, we determined that all nine human samples (from five humans) contained *Leishmania* DNA, but domestic animal, rodent, and sand fly samples were all negative. Real time PCR assays using species-specific primer-probe sets could not confirm either *L. major* or *L. tropica* as the infectious agent in the human samples. This result is contrary to published information and may indicate several scenarios, including infection by multiple *Leishamania* species or even a novel species. To resolve this question, we are sequencing a fragment of the 16S ribosomal gene (using a primer set that amplifies from all *Leishamania* species). These data are part of an ongoing study and will be updated accordingly.

(ACMCIP Abstract)

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DETECTION OF HISTAMINE IN FISH SOLD IN MARKETS IN LIMA, PERU

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Histamine is produced by decarboxylation of histidine in some spoiled or bacterially contaminated fish due to inappropriate storage. Ingestion of high histamine levels often produces a generalized allergic reaction and is occasionally associated with the consumption of fish like mackerel, tuna, bonito and mahi-mahi. The Food and Drug Administration defines a hazard action level in these fish as 50ppm.

After a case of histamine-related allergy experienced by a coworker after ingestion of bonito, we evaluated the presence of histamine in fish sold in Lima, Peru. The two wholesale seafood markets that supply all Lima and 15 general public markets were visited during five Mondays between 4 AM and 4 PM in April-May 2006. Fish from three species were sampled: bonito (*Sarda chiliensis chiliensis*), mackerel (*Scomber japonicus peruanus*) and mahi-mahi (*Coryphaena hippurus*). Wholesale markets were visited twice each, all other markets were visited once. One whole, uncut fish was bought per seller, and sellers were not told that fish would be tested. Histamine levels between 0-50 ppm were measured by a quantitative ELISA (Veratox®) at Naval Medical Research CenterD in Lima, Peru. Dilution methods with the same kit were used to estimate approximated concentrations above this range. We tested 38 fish (17 bonito, 16 mackerel, 5 mahi-mahi), 13 fish (32%) from wholesale seafood markets, including all mahi-mahi. Six fish had histamine levels 1-5ppm (3 mackerel, 3 bonito) and four had > 5ppm (3 mackerel, 1 bonito), all from general markets except for one mackerel with 2.8 ppm. Three mackerels bought between 2 and 4 PM had 35, 83 and 86 ppm, respectively. Fish from general markets had histamine levels > 0ppm more frequently than fish from wholesale markets (44% vs 8%, p=0.03). Higher histamine counts correlated with later time of purchase (Spearman's = 0.37, p=0.024). A sample of the bonito ingested by the original case showed over 800 ppm of histamine. Food safety is an important concern in Peru, where fishing is an important industry and seafood is a key element of local cuisine. This pilot study highlights the risks associated with seafood related intoxication. Inappropriate freezing procedures in the transport and selling of fish may be allowing production of histamine. Although this preliminary evidence precludes us from making broader conclusions, it clearly emphasizes the need for further research on seafood safety in Peru.

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SURVEILLANCE OF ACUTE RESPIRATORY INFECTION IN CHILDREN FROM DIFFERENT REGIONS OF PERU: COMPARISON OF PERUVIAN NAVY AND MINISTRY OF HEALTH DATA

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Since January 2003, the Peruvian Navy has collected weekly health data from military personnel and their dependants through an electronic surveillance system called Alerta. Acute respiratory infections (ARI) are a significant cause of morbidity. Information on the epidemiology and seasonality of these infections are crucial in planning health care services and preventive measures. This study was undertaken to describe the distribution of ARI cases in the population of children from the Peruvian Navy and compare it with Ministry of Health data. We reviewed all ARI cases in children younger than 5 years old from Jan 2004 to Dec 2005 collected by Alerta. Data from the General Direction of Epidemiology (DGE) were obtained from the same population during the same period. The data were grouped in four geographic regions: North coast, Central coast, South coast and rainforest. No data were available from the highlands. Data were described through number of cases, number of cases per health setting and number of cases per health setting per week. Time series correlation between Alerta and DGE and between Alerta regions was performed by means of linear regression analysis. From 2004-2005, Alerta collected 6406 cases. 578 (9%) corresponded to the North coast, 4684 (73%) to the Central coast, 257 (4%) to the South coast and 887 (14%) to the rainforest. The mean number of cases per health setting per week according to the Alerta system was 2.95 for the North coast, 15.71 for the Central coast, 1.89 for the South coast and 3.82 for the rainforest.

For DGE, the rates were 15.44 for the North coast, 16.40 for the Central coast, 15.61 for the South coast and 9.46 for the rainforest. There was a significant correlation between Alerta and DGE data for the North coast (β 0.322, p=0.0079, r2 0.07), for the South coast (β0.193, p<0.0001, r2 0.26) and for the rainforest (β 0.939, p=0.0001, r2 0.14). No correlation was found for the Central coast or between Alerta regions. In conclusion, the data on ARI cases collected through the Alerta system showed that each region correlated with the DGE data, except for the Central coast. There was no correlation between different geographic regions according to this system. This analysis validates Alerta data and highlights the need for surveying different populations.

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VALIDATION OF THREE DIFFERENT ALGORITHMS FOR OUTBREAK DETECTION IN ACUTE DIARRHEAL DISEASE IN PERU

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Surveillance systems are powerful tools for gathering baseline data on incidence of disease and detection of outbreaks. Automated algorithms allow the rapid use of data generated by surveillance systems. "ALERTA" is an electronic surveillance system in the Peruvian Navy that collects health data from multiple sites and transmits them to a central hub for analysis. We evaluated the usefulness of several algorithms to automatically detect outbreaks of acute diarrheal disease (ADD), the most common illness reported. We reviewed the ADD reports from Aug03-Jun05 for 2 settings: the Ancon Navy Base and the more complex Callao Base Hospital (HOBACA). Three algorithms were applied to calculate maximum expected values based on the ten previous weeks. The X-bar chart uses the average of the previous number of cases. The Moving Range chart uses the average of the variations between weeks. The Cummulative Sums chart (CUSUM) adds the differences between frequencies and their expected means. Three standard deviations were used as upper limits for an outbreak signal. Sensitivity, specificity, Positive Predictive Value (PPV) and concordance values were calculated for each algorithm and setting. Data from 96 weeks were evaluated. The Ancon Base reported 3 outbreaks with an incidence of ADD of 8.86 cases/week per 1000 persons. At HOBACA, 8 outbreaks were reported with an incidence of 1.43 cases/week per 1000 persons. In the Ancon base, all the algorithms had 100% sensitivity, while the specificity was 95% for the X bar, 98% for CUSUM and 99% for the Moving Range. PPV was 43%, 60% and 75% respectively. At HOBACA, all the algorithms had 75% sensitivity and the specificity was 87% for the X bar, 92% for CUSUM and 97% for moving range. PPV was 38%, 50% and 75% respectively. Concordance was lower between X bar and Moving Range chart (94.2% with a κ=0.517 for the Ancon base and 88.2% with κ=0.524 for the HOBACA base). In conclusion, all 3 algorithms were useful for the detection of ADD outbreaks. While there were essentially no differences in sensitivity between algorithms, the specificity and PPV were higher for the Moving Range chart. The sensitivity and specificity were lower in a more complex surveillance setting, like HOBACA. High concordance between algorithms was observed.

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RELAPSING MALARIA INFECTION IN AN ADOLESCENT FOLLOWING TRAVEL TO MOZAMBIQUE

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Travelers to Africa presenting with malaria typically are infected with *Plasmodium falciparum*. Infection with P. ovale is uncommon and seldom occurs outside of West Africa. A case of relapsing malaria acquired in Mozambique by an adolescent traveler is reported. A 16-year old male presented with a ten-day history of fever to 102.9F, rigors, malaise and diarrhea 60 days after returning from a 2-week trip to Mozambique. He reported full compliance with malaria chemoprophylaxis, mefloquine 250MG weekly beginning 2 weeks pre- and ending 4 weeks post-trip. His primary care physician started atovaquone 250/proguanil HCL100mg BID for 3 days after a positive blood smear. Species identification was not done at that time. After completing a course of atovaquone/proguanil, the patient's symptoms resolved. He then presented 45 days later with a 2-week history of fatigue and one day of fever, chills, nausea and vomiting. He was hospitalized for concern of recurrent *P. falciparum* malaria and given quinine and doxycycline for 7 days. His blood smear was positive for Plasmodium, suspect ovale. The patient was discharged 3 days after admission, afebrile and clinically improved. His specimen was later confirmed ovale species by PCR testing. He was treated with primaquine 26.3 MG daily for 14 days without further recurrence of symptoms. In conclusion, malaria in former travelers may not present until months after the trip. In patients with recurring symptoms, ovale malaria must be considered even in travelers to areas in East Africa such as Mozambique that historically have been thought to have a low prevalence of the ovale parasite. Diagnosis by PCR can be useful in these patients since accurate microscopic species identification is not always possible.

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PREVALENCE OF ANAPLASMA PHAGOCYTOPHILUM AND BORRELIA BURGDORFERI SS IN TWO HIGH RISK HABITATS IN NORTHWESTERN CALIFORNIA

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Landscape level conversion of forest may have contributed to emergence of tick-borne diseases including Lyme borreliosis (LB) and human granulocytic anaplasmosis (GA) in the eastern US and Europe and may be relevant in California as well. Human cases of GA commonly occurred in people who lived adjacent to coast redwood forests, although ticks are rarely recovered from redwood forests. In California, both LB and GA occur in hyperendemic foci with a high degree of variation in prevalence across different landscapes. Both pathogens are transmitted by the same tick vector, *Ixodes pacificus*. In this study we measured tick density and diversity, wild rodent diversity and abundance, and prevalence of *Anaplasma phagocytophilum* from four sites in northwestern California. At each site, twelve 50m transects were evenly distributed between old-growth redwood and oak forests and second-growth forests. Ticks were collected directly from wild-caught rodents two times/year and by flagging every 2 months along each transect and evaluated for *A. phagocytophilum* infection using Taqman PCR. Wild rodents were trapped by placing 10 Sherman live traps on each transect for 3 trap nights. Rodents were bled and analyzed for *B. burgdorferi* and *A. phagocytophilum* infection using both serology and PCR. Preliminarily, 8 species of rodents and six species of hard ticks, including cosmopolitan *I. pacificus* and 2 *Dermacentor* spp., as well as 3 rodent-specialist ticks, were captured and analyzed for infection of tick-borne disease. Prevalence of GA in rodents varied across sites from 1-12% and among rodent species from 0-75%. *A. phagocytophilum* PCR-positive test results were obtained for dusky-footed woodrats and tree squirrels, but not in other rodents or in ticks, while *B. burgdorferi* PCR-positive test results were detected in dusky-footed woodrats. Tick and rodent density appeared higher in oak, compared with redwood, communities, but a clear pattern of increased tick density in second-growth forest has not been detected.

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PREVALENCE OF *BORRELIA BURGDORFERI*, *BARTONELLA* SPP., *BABESIA MICROTI*, AND *ANAPLASMA PHAGOCYTOPHILUM* IN *IXODES SCAPULARIS* TICKS REMOVED FROM HUMANS

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Ixodes scapularis parasitizing Department of Defense (DOD) personnel and their dependents were received by the DOD Human Tick Test Kit Program in 2005 and tested by PCR for *Borrelia burgdorferi*, *Babesia microti*, and *Anaplasma phagocytophilum*. Most of these *I. scapularis* were acquired in the upper Midwest, mid-Atlantic, and New England regions of the U.S.; a very few were acquired in the southern U.S. Of a total of 389 I. scapularis, 78/389 were infected with *B. burgdorferi*, 3/389 were infected with *A. phagocytophilum*, 6 were co-infected with. *B. burgdorferi* and *A. phagocytophilum*, 6 were co-infected with *B. burgdorferi* and *B. microti*, and one was co-infected with *A. phagocytophilum* and *B. microti*. Because of concerns about tick-borne Bartonella infections voiced by patient groups and physicians, and because of evidence of *Bartonella* sequences amplified from *I. scapularis* ticks, further PCR of these 2005 ticks is being conducted using primers for the gltA gene of *Bartonella* spp. and will be presented. Infection rates from the 2006 tick season will also be presented.

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JUVENILE TICK SURVIVAL ESTIMATION AND APPLICATION TO A PREDICTIVE MODEL FOR ANAPLASMA PHAGOCYTOPHILUM PERSISTENCE IN NATURE

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Granulocytic anaplasmosis (GA) is an emerging tick-borne disease caused by infection with *Anaplasma phagocytophilum* and maintained throughout the Holarctic in sylvatic cycles involving Ixodes spp. ticks. In the western US, the most important bridge vector to humans and domestic animals is *Ixodes pacificus*, with nidicolous rodent-specialist ticks playing a role in enzootic cycles. To some extent, infection over-winters in ticks during tick hibernation; in California, there is no obvious reservoir host the host with the most prolonged infection, the dusky-footed woodrat, *Neotoma fuscipes*, typically experiences infection for a few weeks to as long as 6 months. A vector-SIRS model of transmission of *A. phagocytophilum* among apparently poorly competent hosts such as woodrats and several tick species was constructed to explore critical parameters driving disease dynamics, emergence, and persistence. Sensitivity analysis indicated that the single most important model parameters for predicting disease persistence was survival probabilities of juvenile ticks. Juvenile *I. pacificus* survival was experimentally determined in situ, by placing larvae and nymphs in tubes in leaf litter at several field sites that differed in microhabitat features (soil temperature and humidity, substrate), that would modify tick survival. Using these survival estimates with parameter values derived from an *A. phagocytophilum*- enzootic community in northern California, the model yielded critical survival thresholds for disease persistence, corresponding to experimental results where microhabitats had mild temperatures and high humidity.

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DENGUE VIRUS TYPE 3 IN CUBA: EVOLUTION FROM A SMALL OUTBREAK IN 2000 TO A MAJOR EPIDEMIC IN 2001

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After an absence of 17 years, DENV-3 re-appeared in Latin America in 1994. Cuba was first affected in September 2000 when a small outbreak occurred in Havana City. The infection was brought under control within six weeks using enhanced mosquito eradication measures. In June 2001, dengue transmission was again detected, this time the virus spread rapidly across the city causing a major epidemic. To understand the phylogenetic relationships of the viruses isolated from these outbreaks, the E gene sequence of three Cuban isolates and the first DENV-3 strain isolated in Nicaragua, 1994 were determined. Maximum Likelihood phylogenetic analysis incorporating global DENV-3 sequences showed that the Cuban isolates are closely related to strains belonging to genotype III and formed a distinct cluster with recent Latin American strains that have evolved in the Caribbean region. Analysis of Cuban isolates obtained in consecutive outbreaks revealed several nucleotide changes, some of them associated with non-conservative amino acid substitutions. These data are therefore consistent with the idea that a second introduction of the virus occurred in 2001, rather than *in situ* evolution. The functional significance of amino acids changes that were observed remains to be determined. Moreover, it is noteworthy the amino acid change observed among isolates obtained during the same outbreak from patients with different disease severity as well as from different biological sample (serum or spleen).

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DENGUE HEMORRHAGIC FEVER CAUSED BY SEQUENTIAL DENGUE 1 - 3 INFECTIONS AT A LONG INTERVAL: HAVANA EPIDEMIC, 2001-2002

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A DENV-3 epidemic occurred in Cuba in 2001-2002 which included cases of dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS). Here we report neutralizing antibody studies on sera from 54 of 78 DHF/DSS patients that provide evidence of infections occurring in the sequence DENV-1 followed by DENV-3. No sera evidenced infection in the sequence DENV-2 followed by DENV-3. Some sera showed a pattern of infection in the sequence DENV-1 followed by 2 then 3, however definitive categorization of a tertiary infection was not possible because of broadly reactive antibodies which could have been raised by infections in the sequence DENV-1 then DENV-3.Dengue Hemorrhagic Fever has been associated with secondary infection in individuals who experienced a primary dengue infection 3-5 years earlier. In this manuscript two important observations are reported.a. secondary dengue infection is demonstrated as an important risk factor for severe disease occurring 24 years after a primary dengue infection and b.The infection sequence,dengue 1 followed by dengue 3 was associated with severe disease.There was no evidence that dengue 2 followed by dengue 3 infections resulted DHF/DSS, although infections in this sequence leading to milder illnesses were observed.These two observations are new and of importance to understanding the pathogenesis of this disease and in vaccine safety issues.

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SPECIFIC IMMUNOGLOBULIN M, A AND E IN PRIMARY AND SECONDARY DENGUE INFECTION FROM CUBAN ADULTS AND SALVADORIAN CHILDREN

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Dengue IgM antibody detection in a single acute-phase serum by ELISA has become one of the most important and useful methods for the diagnosis of this disease. Currently, this system has become an invaluable tool for the surveillance of Dengue Fever and Dengue Hemorrhagic Fever. The usefulness of other serological markers such as IgA and IgE in serum has been less studied. One hundred twenty seven serum samples from adult patients of the Cuban dengue 3 epidemic of 2001-02 and seventy one serum samples from children patients collected during the dengue 4 epidemic of El Salvador (2002) with clinical picture of dengue fever or dengue hemorrhagic fever and with primary or secondary infection were studied. All samples were tested by capture ELISA in order to detect dengue IgM, IgA and IgE antibodies. Significant differences were observed in the IgM, IgA and IgE response in each studied group. Higher OD ratios for IgA and IgE antibodies in secondary dengue cases than primary cases were found. The usefulness of serotype specific IgM antibody detection is also analyzed and discussed. The role of these immunoglobulins in terms of protection, recovery of infection and immunopathogenesis is a priority in future dengue investigations. Cross reactivity of IgM among dengue serotypes both in primary and secondary cases should be carefully studied.

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IMPROVED DENGUE PLAQUE VIRUS FORMATION ON BHK21 AND LLCMK2 CELLS: EVALUATION OF SOME FACTORS

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Neutralizing antibodies play a key role in the prevention of dengue infection. It is important that dengue virus plaque titration and plaque reduction neutralization tests (PRNT) be highly reproducible using standardized methods. To evaluate factors that have influence in the PRNT for dengue viruses, neutralizing antibodies were determined in 24 serum samples and 12 blood samples collected on filter paper, obtained through Cuban national dengue surveillance. The influence of pH in the overlay medium, percentage of ambient CO_2, the use of two different cell lines and of rapid centrifugation on dengue plaque formation were evaluated. The efficiency of the plaquing system was optimal when overlay medium was buffered to pH 7.5. The rapid centrifugation of virus on confluent cells increased the virus titres. Higher virus titres were obtained on BHK21 rather than $LLCMK_2$ cells when viruses were added to cell suspension. Under optimal conditions, PRNT was highly reproducible and is recommended for seroepidemiological and vaccine studies using either BHK21 or $LLCMK_2$ cells. This communication also highlights the infection of $LLCMK_2$ cell suspensions for measuring neutralizing antibodies.

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ANTIBODIES DEPENDENT CELL CYTOTOXICITY IN DENGUE INFECTION

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The role of humoral immunity and neutralizing antibodies in dengue virus disease has been studied. In addition to direct interference with viral entry achieved by neutralizing antibodies, antibodies *in vivo* mediate another important function less explored in dengue infection: the antibodies depending cell cytotoxicity (ADCC). Like CTL activity, ADCC could eliminate infected cells and thereby reduce viral burden. Unfortunately, only scarce reports on ADCC during a dengue infection are currently available, and they do not clearly define ADCC role in the prevention or progression to DHF/DSS. The exceptional epidemiological circumstances in Cuba allow us to maintain a homogeneous sample with a similar history of natural infections. In this study we explore the ability of human anti-dengue antibodies to mediate ADCC using acute or convalescent serum samples from individuals who suffered secondary infection to dengue 2 virus in the epidemic of Santiago de Cuba in 1997 with different clinical pictures. All these individuals had been primarily infected by the dengue 1 virus 20 years before. According to the clinical picture, ADCC activity was detected at fifth day after clinical onset, in sera from DHF/DSS but not in sera from DF patients. However, one year after illness, ADCC activity was observed in all cases. We also measured the activation of PBMC mediated by antibodies by detection of IFN γ. In order to do so, we used serial serum samples from these patients collected every two days after fever onset. In DF samples taken between the first and third day, a higher number of IFN γ positive cells was detected, and a decrease on the fifth day. However, for DHF cases there was no detection of IFN γ positive cells in the first samples after clinical onset, but there was an increase towards the fifth day. The analysis of specific anti-dengue IgG subclasses in the acute serum samples studied showed an IgG_3 major contribution to the IFN γ production. Our results suggest that the ADCC antibodies present during the earlier stages acute infection could play a role in determining the viral spreading, and consequently avoid the progress to the severe disease. Then, ADCC could be implicated in dengue prevention.

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A DENGUE VIRUS VACCINE BASED ON ALPHAVIRUS REPLICONS INDUCES PROTECTIVE IMMUNE RESPONSES IN CYNOMOLGUS MACAQUES

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A candidate vaccine (D1ME-VRP) expressing dengue virus type 1 pre-membrane (prM) and envelope (E) proteins from a Venezuelan equine encephalitis virus (VEE) replicon system was previously shown to elicit both anti-dengue-1 neutralizing antibodies and dengue-1 specific CD4+ and CD8+ T cells in a murine model. In this study, three vaccination regimens (D1ME DNA vaccine, D1ME-VRP, and a heterologus prime boost with D1ME DNA prime and D1ME-VRP boost) were compared for immunogenicity and protection against dengue-1 virus challenge in a non-human primate model. Groups of 3 or 4 cynomolgus macaques were immunized with three doses of D1ME DNA vaccine (DDD), D1ME-VRP (VVV), or with two doses of DNA priming and a third booster dose of D1ME-VRP (DDV). A control group of animals was inoculated with PBS. Virus neutralizing antibody was measured by plaque reduction neutralization test (PRNT) and 50% neutralization titers (PRNT-50) were determined by probit analysis. T cell responses were measured by γ-IFN ELISPOT. Measured 4 weeks after final immunization, the DDV group produced the highest virus neutralizing antibody titers (PRNT-

50=2265±238) followed by VVV (1822±263) and DDD (1339±382) groups. However, moderate T cell responses were demonstrated only in DDD and DDV vaccinated animals. Six months after the final dose, all animals were challenged with live dengue-1 virus and viremia was determined by infecting vero cells with sera collected from daily bleeds. All 3 control animals became viremic for 6-7 days (mean=6.3 days). All vaccination regimens showed significant protection from viremia. DDV immunized animals were completely protected from viremia (mean=0 days). DDD and VVV vaccinated animals had mean days of viremia of 0.66 and 0.75 respectively. Thus, the antibody response and protection elicited from D1ME-VRP was comparable to those elicited from D1ME-DNA vaccine. However, the prime-boost approach resulted in higher antibody responses and complete protection.

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DIFFERENTIAL ENHANCEMENT OF DENGUE IMMUNE COMPLEX INFECTIVITY MEDIATED BY SIGNALING-COMPETENT AND SIGNALING-INCOMPETENT HUMAN FC Γ RIA (CD64) OR FC Γ RIIA (CD32)

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Fcγ receptor (FcγR)-mediated entry of infectious dengue immune complexes into monocyte/macrophages is hypothesized to be a key event in the pathogenesis of complicated dengue fever. FcγRIA and FcγRIIA, Fc receptors that predominate on the surface of such dengue-permissive cells, have previously been shown to facilitate antibody-mediated dengue enhancement in human macrophage-like cells using surrogate plaque assays to measure virus replication since dengue virus does not form plaques in such cells. We have examined the relative efficiency with which each of these receptors individually enhances dengue immune complex infectivity and have inquired whether Fc receptor signal transduction plays a role. Our strategy to answer these fundamental questions surrounding the immune enhancement phenomenon involved expression of native and mutant forms of the human γ-chain / FcγRIA complex or FcγRIIA, in dengue-permissive COS cells in which dengue virus immune enhancement was directly measured by conventional plaque assay. We found that both receptors mediated enhanced dengue immune complex infectivity, but that FcγRIIA appeared to do so far more effectively. Abrogation of signaling competency significantly diminished the capacity of FcγRIA transfectants to phagocytose opsonized large particles and to enhance dengue immune complex infectivity. Abrogation of Fc RIIA signaling competency was also associated with equally impaired phagocytosis, but had no discernable effect on dengue immune complex infectivity. These findings point to fundamental differences between FcγRIA and FcγRIIA with respect to their immune-enhancing capabilities and suggest that different mechanisms of dengue immune complex internalization may operate between these FcγR.

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PRIMARY AND SECONDARY INFECTIONS BY ASIAN AND AMERICAN GENOTYPES OF DENGUE 2 VIRUS IN *MACACUS IRUS* MONKEYS

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The Asian genotype of dengue 2 virus (DENV-2) has been associated to DHF/DSS epidemics while the American genotype has only been associated to the mild disease. Few studies have focused the secondary homotypic DENV infection including the same or different genotype of the primary infection. The present work aimed to compare the immune response and the protection capacity induced by the DENV-2 American and Asian genotypes in *Macacus Irus* monkeys after primary and secondary homotypic infections. Animals were primary infected with 4 $\log_{10}$ PFU of either American or Asian DENV-2 strains. The kinetics of antibody induction was monitored by ELISA, neutralization and hemaglutination inhibition (HAI) tests. Viremia was estimated by viral isolation and RT-PCR. After primary infection the antibody development and viremia duration were similar among the strains, but slight differences in the antibody cross-reactivity in terms of HAI and neutralization test were found. After challenge with 4 $\log_{10}$ PFU of the A15 strain, no virus was isolated while RT-PCR showed a late positive reaction. The HAI and neutralizing antibody titers notable increased after secondary infection and the levels of cross-neutralizing antibodies were different among the strains. Two main observations were done: a) The American and Asian DENV-2 genotype strains induced a similar response in terms of antibody development and lasting viremia after first inoculation b) The strong anamnestic response after viral challenge suggested that the previous immunity did not confer sterile protection to the homologous secondary infection.

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GENE POLYMORPHISMS OF IMMUNOREGULATORY CYTOKINES IN DENGUE VIRUS INFECTION

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Dengue virus infection has emerged as one of the most important arthropod-borne diseases. Some dengue infected individual's progress to the severe, life-threatening form of the disease, dengue hemorrhagic fever. Host genetic factors may be relevant and predispose some individuals to the severe dengue disease. The unique history of Dengue outbreaks in Cuba is extremely advantageous for genetic studies of dengue disease resistance or susceptibility. Little is known about predictive value of cytokine genotype for the development of clinical output of dengue infection. The -1082IL-10, -819IL10, -592IL-10 and -308TNF-α gene single nucleotide polymorphisms (SNP) were studied in individuals who suffer from different clinical pictures or subclinical dengue virus infection by polymerase chain reaction-sequence specific primer (PCR-SSP). Significant association of the tumor necrosis factor-α (-308) GG genotype was found when comparing asymptomatic and dengue haemorrhagic fever cases. No associations of interleukin-10 polymorphisms with any studied groups were detected. We failed to observe significant differences in cytokine genotype distribution between dengue fever and dengue haemorrhagic fever patients.

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DIFFERENTIAL VIRUS-SPECIFIC T-CELL RESPONSE IN DENGUE VIRUS IMMUNE CUBAN INDIVIDUALS

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Dengue virus (DV) infections play an increasing role in the world. The rapid activation of serotype cross-reactive dengue memory T cells that release shock-provoking inflammatory mediators has been suggested to explain some aspects of the severe clinical syndromes. How T cells contribute to this process, however, is incompletely defined. We take advantage of the unique history of dengue in Cuba, where the population has been exposed to the identical previous dengue infection in each outbreak to study how the rapid activation of serotype cross-reactive memory T cells that release shock-provoking inflammatory mediators contribute to the development of the severe clinical syndromes by mean of quantifying

mRNA of IFNγ, IL10, TNFα, Tbet and GATA 3 from PBMC of individuals with clearly defined dengue immune background using Real Time PCR. Comparing the *ex vivo* immune response to DV in D3V immune individuals with different DV-immunity background (DV1+/DV3+, DV2+/DV3+, and only DV3+) we observe a stronger inflammatory Th1 response to heterologous DV challenge (simulation of secondary infection) than the former groups (simulation of tertiary infection).

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CIRCULATING DENGUE VIRUS SEROTYPES SINCE THEY ENTERED PERU IN 1990 UNTIL 2006

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This study was undertaker to identify dengue virus serotypes circulating in Peru since 1990 up to 2006. The information available regarding dengue fever virus isolates in the Virology Laboratory of the Peruvian National Institutes of Health (PNIH) was reviewed, comprising the time period since 1990 to the first quarter of 2006. Viral isolation at the PNIH is performed using VERO and C6-36 cell cultures, as well as inoculating the material in brain tissue of lactating mice. Samples are collected from laboratories located in different endemic regions for dengue fever in Peru. Viral identification is performed using direct immunofluorescence with monoclonal antibodies. When necessary, PCR or genotyping techniques are used. Dengue virus serotype identification corresponded to dengue (DEN) 1 between 1990 to 1995; 1996: DEN2; 1997: DEN1; 1998: DEN2; 1999: DEN1, DEN2; 2000: DEN1, DEN2; 2001: DEN1, DEN2, DEN3, DEN4; 2002: DEN3; 2003: DEN2, DEN3; 2004: DEN3; 2005: DEN1, DEN3 (Lima); 2006: DEN 3. Circulating DEN2 in 1996 corresponded to DEN2 American genotype. Serotype 3 entering Lima corresponded to genotype 3. Dengue virus entered Peru by the Amazon Region and spread through all the Northen Coast, from Tumbes to Lima, as well as in the Amazon Region In conclusion, dengue virus serotype 1 entered Peru in 1990; and since then, the 4 serotypes of dengue fever virus have been circulating; and there is a constant risk for the occurrence or re-emergence of hemorrhagic dengue fever in endemic areas in Peru.

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STUDYING CROSS NEUTRALIZATION OF DENGUE VIRUSES WITH A PANEL OF DENGUE IMMUNE-SERA FROM TRAVELERS

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A central tenet in dengue immunology is that following natural infection with a particular serotype, one develops long term protective immunity to the infecting serotype but not to the other serotypes. Recent studies indicate, however, that genetic differences between viruses belonging to the same serotype can influence the extent of neutralization. Studies by Kochel et al. have demonstrated that people exposed to primary dengue serotype 1 (DENV1) developed cross-reactive immune responses that neutralized the American but not the Asian DENV2. We are performing a comprehensive analysis of DENV3 neutralization, asking whether homotypic immunity that develops after a primary DENV3 infection neutralizes all DENV3 strains to the same extent irrespective of genetic differences within this serotype, and whether homotypic immunity that develops after a primary DENV1 or DENV2 infection cross-neutralizes some strains of DENV 3 better than others. A major obstacle to studying cross-neutralization of dengue viruses is the scarcity of monotypic dengue immune sera. We have attempted to overcome this problem by screening American travellers who developed dengue-like symptoms during a visit to a dengue endemic country. Unlike people living permanently in dengue endemic areas who have repeated dengue infections, traveller's sera are likely to remain monotypic for many years after the infection. The travel histories can be used to determine when and where the person was exposed to the virus. We obtained serum and PBMC from 33 people who were likely to have been infected with dengue virus. 16 of the 33 subjects had high levels of neutralizing antibody to at least one serotype, whereas the remaining subjects were dengue naïve or had very low levels of neutralizing antibodies. Of the 16 positive subjects, seven had monotypic responses and the remaining nine had responses indicative of secondary infections. Experiments are currently underway with these sera to measure neutralization of 7 strains of DENV3 representing the genetic diversity within this serotype.

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IMPROVEMENT IN HOSPITAL INDICATORS AFTER CHANGES IN DENGUE CASE MANAGEMENT IN THE NATIONAL PEDIATRIC HOSPITAL IN NICARAGUA

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Dengue is a major problem in Nicaragua, as in many tropical and subtropical countries worldwide. Improving dengue case management and quality of care are a significant priority. Via a collaboration with Thai colleagues at the Queen Sirikit National Institute of Child Health, changes in management of suspected dengue cases were introduced in the National Pediatric Reference Hospital in Managua, Nicaragua. These modifications consisted of oral liquids rather than IV fluids upon admission, continuous monitoring of clinical and laboratory signs, introduction of a microhematocrit centrifuge on the ward for frequent surveillance to detect increased vascular permeability, use of IV fluids prinicipally during the critical phase and for shorter periods, and introduction of colloids in management of shock. To assess the impact of these measures, two periods were compared, representing the 2003 and 2005 dengue seasons, before and after the implementation of these new practices. Apart from these specific changes, there were no other differences in case definition, management, or disease severity between the two periods. For instance, 29% of hospitalized dengue patients were classified as dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS) in 2003 versus 26% in 2005. A number of outcomes were evaluated, including nosocomial infections, intensive care, duration of hospitalization, number of patients receiving IV fluid, and day of inititiation of IV fluid. In 2003, 239 hospitalized laboratory-confirmed dengue cases 0-14 years of age who presented at the hospital $\leq$ 4 days after onset of symptoms were included in the study, versus 46 in 2005. Some of the most important outcomes were a dramatic reduction in nosocomial infections, from 25 in 2003 to 0 in 2005 ($p=0.04$) and in admissions to the intensive care unit, from 8 in 2003 to 0 in 2005 ($p=0.44$). Other significant findings included reduction in (i) the days of IV fluid administration ($p=0.001$), (ii) the number of patients receiving IV fluids ($p<0.0001$), and (iii) duration of hospitalization ($p=0.003$) Overall, this study demonstrates concrete gains in dengue patient care and case management.

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IDENTIFICATION OF CONTINUOS B-CELL EPITOPES IN THE ENVELOPE GLYCOPROTEIN OF DENGUE VIRUS TYPE 3

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Dengue virus infection is a growing global public health concern in tropical and subtropical regions of the world. The virus is a single-stranded RNA virus that belongs to the *Flaviviridae* family with 4 antigenically distinct serotypes (DENV-1 to DENV-4). There is no antiviral therapy available and

development of a dengue vaccine has proved to be elusive due to the requirement of the vaccine to elicit protection against all four serotypes simultaneously. One possible strategy to avoid pathogeny associated with a dengue vaccine would be to construct a chimeric vaccine composed of selected critical epitopes of the four serotypes. The majority of the epitopes involved in dengue neutralization are on the envelope (E) glycoprotein, wich is the major surface protein of the viral particles. The aim of the present investigation is to identify B cell epitopes in the E-glycoprotein elicited by natural dengue virus type 3 infections. For mapping immunodominat epitopes, ninety five peptides (each with 15-mers, overlap of 10) were synthesized (Synpep, California-USA), covering the 490 amino acids (aa) of the E-protein sequence deducted from the genome of a Dengue 3 isolate from Brazil. These peptides were tested by ELISA against a pool of positive and negative dengue patient sera collected during the convalescent phase of dengue 3 infection, as determined by PCR. The results showed that the human sera reacted with eleven of the 15-mer peptides, distributed in 5 regions at amino acid positions 51-65, 71-90, 131-170, 196-210 and 246-260 and all of these, except the peptides 196-210 and 246-260 are hydrophilic according to Kyte and Doolittle hidrophilicity plots suggesting that these regions are exposed at the surface of the E protein. In conclusion, our study identified several immunodominant IgG-specific epitopes on the envelope of DENV-3. The peptides described here in conjunction with other well documented epitopes are potentially relevant for the development of diagnostic reagents and vaccine for the dengue virus.

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IDENTIFICATION OF DENGUE VIRUS IN PATIENTS WITH INESPECIFIC FEBRILE ILLNESS

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In endemic areas for dengue infection, the unnoticed transmission is a common situation that could provoke an underestimation of the actual incidence, especially in the absence of epidemic outbreaks. The purpose of this descriptive study was to explore the association between dengue infection, their serotype and clinical diagnosis in a hyperendemic area for dengue transmission. A total of 137 consecutive patients attended at the public health services in the state of Colima, México, with the clinical diagnosis of inespecific fever were included in the study. Clinical and demographic data were recorded. A venous blood sample was obtained in each patient in order to look for the presence of dengue virus and its serotype by means of the transcriptase reverse- polymerase chain reaction (RT- PCR) with specific primers for the four dengue serotypes. A total of 18 sera resulted positive to dengue infection (13.1%), 16 of them corresponding to DEN- 3 type and 2 of DEN- 1 serotype. The analysis with Poisson regression did not show association between dengue infection with sex, age group, type of community nor with clinical picture (only 2 patients had the clinical diagnosis of dengue). The result confirms the recent re- introduction of DEN- 3 serotype to Mexico. On the other hand, the findings support our previous findings that dengue transmission occurs in endemic communities in a continuous fashion, even in the absence of epidemics and that clinical diagnosis usually is not enough specific or sensitive to be considered as a reliable tool for epidemiologic surveillance.

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GENETIC CHARACTERIZATION OF DENGUE VIRUS SEROTYPES CIRCULATING IN OAXACA, MEXICO

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Dengue fever (DF) and dengue hemorrhagic fever/shock síndrome (DHF/DSS) are mosquito-borne infectious diseases that have become major international public health concerns. DF and DHF/DSS occur in tropical and sub-tropical regions around the world, predominantly in urban and sub-urban areas. There are four dengue serotypes, which are transmitted to humans principally through the bites of *Aedes aegypti*. Sequential infection with differents serotypes could be increases the risk of DHF, and this may be associated with the potentian virulent of the straints of DEN virus. A number of genetic markers have been proposed to condition these increased virulence, and recombination event could result in the generation of new, more virulent dengue viral genotypes. In this work, we studied the circulation of serotypes of *DEN virus* for serotipification of 6 isolates obtained of patients serum of the state of Oaxaca, Mexico. The maximun cytopatic effect (CPE) produced by virus DEN infection was obtained after three passage about 30 days in the mosquito cellular lines C6/36. The results of RT-PCR indicates that there are three DEN virus serotype 2 (DENV-2) and three DENV-3. This study indicates that several serotypes are circulating in Oaxaca increasing the risk of DHF. We made the secuence of protein E, and a portion of the C and prM genes. Phylogenetic analysis suggested that the isolate of DEN serotipe 2 was American/Asian genotype, which has DHF potential. The sequence analysis of NS5 compare with the genotipe elucidated by the aminoacid sequence of protein E, suggested some genetic markers in NS5 to genotype DEN virus serotype 2.

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CHLOROQUINE REDUCES DENGUE VIRUS REPLICATION IN VERO CELLS BUT NOT IN C6/36 CELLS, AND PARTIALLY PROTECTS MICE AGAINST VIRAL CHALLENGE

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Dengue represents the most important arboviral disease of humans. The only available way to control dengue is vector control since there is neither a vaccine to protect against this infection nor an antiviral that interferes with virus replication. To investigate whether chloroquine would interfere with dengue virus replication, Vero and C6/36 confluent cell monolayers were infected with dengue-2 virus (DEN2V) at a multiplicity of infection of 0.1. Viral replication inhibition assays were performed on Vero and C6/36-infected cells treated with chloroquine (50µg/mL) added either at 12- or 24-hour intervals after adsorption. Infected cell supernatants were collected after 0, 6, 12, 24, 48, 72, 96, 120, 144, and 168 hours after viral infection. Total RNA was extracted from these supernatants, and viral replication was assessed by real-time PCR. Reverse-transcription real-time PCR results showed that, compared to control cells, there was a statistically significant decrease in viral replication in chloroquine-treated Vero cells. The inhibition of viral replication was more striking on those cells treated with chloroquine at shorter time intervals. However, in C6/36 cells chloroquine induced a statistically significant increase in viral replication after 12 hours of infection when compared to control cells, probably because DEN2V uses a different strategy of penetration or uncoating in these cells. In order to investigate the *in vivo* influence of chloroquine on DEN2V infection, groups of 4 week-old Swiss mice were challenged with an intracerebral injection of a wild strain of the DEN2V, and treated with chloroquine by the intraperitoneal route. The chloroquine was administrated at 24-hour intervals during 7 days, and the animals observed during 21 days. The survival rates of dengue-2-infected mice treated with chloroquine were higher than in the untreated mouse group

(33% and 0%, respectively). This work shows that chloroquine interferes with dengue-2 replication *in vitro* and *in vivo*, and might represent an alternative to treatment of dengue infections in the near future.

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CONFIRMATION OF AN OUTBREAK OF SELVATIC YELLOW FEVER IN A NATIVE COMMUNITY IN THE PERUVIAN AMAZONIAN JUNGLE

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Yellow fever (YF) is still a worldwide important disease for public health, even tough an effective vaccine is available. In South America, Peru is one of the countries where YF is most prevalent. Endemic areas are northern and central parts of Peruvian Amazonian jungle. In December 2005, there was an outbreak of a condition characterized by fever, jaundice, and hemorrhage in a native community (the Awajun tribe) comprising 435 inhabitants in the Amazonian region, in a place very close to the Ecuadorian border, 700 meters above sea level. 106 febrile patients required medical attention, 33 presented with jaundice, 20 had hemorrhage, and 12 patients died. This study was undertaken to determine the causal agent of the condition characterized by fever, jaundice, and hemorrhage in the Awajun native community. We obtained serum and liver tissue samples from the affected patients. ELISA tests were performed for detecting IgM antibodies against yellow fever. Serum aliquots were also inoculated in cells for culture and in suckling mice. RT-PCR, as well as nucleotide sequencing was also performed in the serum samples and isolation, respectively. According to serology tests, viral isolation and RT-PCR yellow fever virus was the causative agent for the outbreak. Gene sequencing showed that this virus had 99% and 88% coincidence with other yellow fever viral sequences reported in Gene Bank from South America and Caribbean region, respectively. This outbreak had some particular features; its elevated attack rate; the occurrence of affected children less than 5 years old; the native population has never been immunized against yellow fever; there were no reports of dead monkeys; and no epizootics were reported in the affected area; and it was suspected that transmission occurred inside the village or in its peripheral area. *Haemagogus, Sabethes* and other mosquito vectors were found.

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EXPERIMENTAL EVIDENCE THAT RNA RECOMBINATION OCCURS IN JAPANESE ENCEPHALITIS VIRUS

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Japanese encephalitis (JE) virus, a major cause of acute viral encephalitis in human, is a member of the genus *Flavivirus* belonging to the family *Flaviviridae*. Due to the instability of genonic RNA, mutations accumulated during virus replication is known to be a force contributing to the viral evolution. In past years, RNA recombination has also been postulated to be another factor to cause genomic variation of JE virus. The first evidence, based on phylogenetic analysis on the E protein, demonstrating RNA recombination of JE virus was reported in 2003; showing at least two strains of JE virus isolated from Korea may be a recombinant form of strains originated from Japan and Korea, respectively. In the meantime, one strain from Thailand was shown possibly formed by strains originated from China and Thailand, respectively. Recently, we have experimentally demonstrated the occurrence of RNA recombination in JE virus by using two local strains isolated from Taiwan. The recombinant progeny virus has actually formed, based on the results of restriction fragment length polymorphism (RFLP), in BHK-21 cells that has been co-infected by two strains of the parent virus. In addition, two types of subgenomic viral RNA, one contains the 5′-UTR with a short RNA fragment at the 3′-end and the other has the 3′-UTR and the same RNA fragment at the 5′-end, were obtained via *in vitro* RNA-dependent RNA polymerase (RdRp) assay; from which a newly formed RNA containing both 5′-UTR and 3′-UTR has been identified. It further demonstrated that RNA recombination really occurs during the replication of two co-existing strains of JE virus.

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CD4+ T CELLS MEDIATE WEST NILE VIRUS CLEARANCE FROM THE CENTRAL NERVOUS SYSTEM DURING PRIMARY INFECTION

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West Nile virus (WNV) is a single-stranded positive sense RNA virus that is an important human and veterinary pathogen. Although studies have shown that innate and adaptive immune responses are important in controlling WNV infection, to date, the role of $CD4^+$ T lymphocytes and T-dependent helper responses in modulating infection is poorly understood. In this study, using a mouse model, we examined the function of $CD4^+$ T cells in coordinating a protective immune response against WNV. A genetic or acquired deficiency of $CD4^+$ T cells resulted in a protracted WNV infection in the central nervous system (CNS) that culminated in uniform lethality by 50 days after infection. Mice surviving past day ten after infection had high WNV titers in the CNS compared to wild type mice, even 40 days following infection. Immunohistochemistry of brain tissue samples showed persistent WNV antigen staining in the brains of mice lacking functional $CD4^+$ T cells at twenty days post-infection. Interestingly, the absence of $CD4^+$ T cell help did not affect clearance of WNV in the spleen, suggesting a role for CD4-independent responses in clearing virus in the periphery. WNV-specific IgM levels were similar to wild type mice in CD4-deficient mice early during infection, but dropped up to 20 fold at day 15 post-infection whereas IgG levels in CD4-deficient mice were 2-3 $\log_{10}$ lower than in wild-type mice throughout the course of infection. Despite this, T-independent antibody responses were sufficient to neutralize WNV in the blood. WNV-specific $CD8^+$ T cell activation and trafficking to the CNS were unaffected by the absence of $CD4^+$ T cells at day 9, but were markedly compromised at day 15. Based on these results, we suggest that $CD4^+$ T cells protect against WNV infection primarily by sustaining WNV-specific CD8+ T cell responses in the CNS.

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CONSTRUCTION AND SELECTION OF HUMAN MONOCLONAL FAB ANTIBODIES TO WEST NILE VIRUS USING A PHAGE DISPLAY COMBINATORIAL LIBRARY

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Passive immunity using immunoglobulin has shown efficacy in treating some patients with West Nile virus infection. This makes the development of humanized anti-WNV antibodies significant. The goal of this study was to construct a Fab antibody phage display library of WNV, and to identify and select clones with neutralizing activities. Total RNA was extracted from PBLC of two immune individuals. PT-PCR was used to amplify the heavy chain Fd and light chains. The amplified genes were sequentially cloned into the recombinant antibody expression vector pComb3-H. After transfecting E.coli XL1-blue, a Fab phage library was packaged with helper phage VCS-M13. Five rounds of panning were carried out with WNV E protein domain III and ELISA was used to select binding antibodies. Antigen binding specificity, CDR sequence of VH and VL, and neutralizing activity against WNV were analyzed *in vitro*. Fab antibody library was constructed with a capability of 7×10^7 clones/ml. Eight Fab monoclonal antibodies were obtained, which recognized linear E protein domain III. One of these, Fab1, exhibited significant neutralizing activity, and completely blocked 100 pfu WNV from infecting Vero cells, at a concentration 160 µg/ml. The other 2, Fab13 and Fab25, showed weaker neutralizing activity, and incompletely blocked 100 pfu WNV infection at

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concentrations of 320 µg /ml and 160 µg /ml, respectively. In conclusion, Fab antibodies may be valuable for immunoprophylaxis or treatment of WNV infection.

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MOSQUITO SALIVARY GLAND GENE EXPRESSION DURING LONG-TERM CYTOPATHOLOGICAL WEST NILE VIRUS INFECTION

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Persistent infection with West Nile virus (WNV) in the vector *Culex pipiens quinquefasciatus* is associated with salivary gland cell death and a reduction in virus transmission over time. Ultrastructural analyses of mosquito salivary glands infected with WNV suggest that apoptosis and extreme cellular degeneration and vacuolization occur during late stages of WNV infection. The aim of the present study was to explore the molecular basis of cytopathology in mosquito salivary glands during WNV infection. We designed oligonucleotide microarrays to test the hypothesis that genes involved in the physiology of the salivary glands, immunity, cell death, and stress response would be differentially transcribed in WNV-infected mosquito salivary gland cells as compared to uninfected, blood fed control mosquitoes. Expressed sequence tags from cDNA libraries of bacteria-inoculated mosquitoes and blood fed midguts were used to generate 60-mer oligonucleotides for spotted microarray slides. Three mosquito infections were performed, from which 100 mosquito salivary glands were dissected on days 14 and 21 post-infection for microarray analysis. Salivary gland genes were differentially expressed in WNV-infected mosquitoes compared to uninfected controls. These differences are discussed in the context of WNV-induced cytopathology observed by transmission electron microscopy. This study represents the first tissue-specific examination of gene expression during long-term flavivirus replication in a *Culex* mosquito vector and provides insight into transcriptional changes that accompany long-term mosquito infection.

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EPIZOOTIOLOGY OF WEST NILE VIRUS IN THE CENTRAL RED RIVER VALLEY OF NORTH DAKOTA AND MINNESOTA, USA 2002 - 2006

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The establishment and early history of West Nile virus (WNV) within the central Red River Valley of eastern North Dakota and northwestern Minnesota was chronicled from 2002 to 2006. Host-seeking mosquitoes were collected using Mosquito Magnet® traps, sorted by species and tested for WNV using reverse transcriptase polymerase chain reaction assays. *Culex tarsalis* was identified as the main WNV vector in the region. Passerine birds were collected and tested for anti-WNV antibodies using epitope-blocking enzyme linked immunosorbent assays. Environmental conditions from 2002 to 2005 produced a natural "field experiment" which demonstrated the differing magnitudes by which environmental temperature and host immunity affected local WNV activity. Despite warm temperatures and high vector abundance, WNV activity was low during its introductory year (=2002). The next year (2003) was an "epidemic year" for WNV, as indicated by the high number of human cases statewide and high infection rates in the local vector population. Passerine immunity was low, which probably contributed to the epidemic. In 2004, unusually cool weather prolonged vector larval development, adult emergence and arboviral extrinsic incubation period. As a result, WNV activity during 2004 was low and WNV had insufficient time to undergo extensive amplification cycles _ similar to the situation that occurred during the introductory year of 2002. However, the epidemic conditions of 2003 had produced a high level of immunity in the local bird population in 2004. This immunity carried over into 2005. In 2005, environmental temperature, length of transmission season, and vector abundance were all nearly identical to those of the epidemic year of 2003. Yet the intensity of WNV activity during 2005 was considerably less than that of 2003. The big difference between 2003 and 2005 was the level of passerine immunity. The high prevalence of immunity within passerines during 2005 may have contributed to preventing another epidemic, but it did not totally eliminate WNV activity. Results for the 2006 transmission season will be presented.

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SEARCHING FOR A SMALL MOLECULE INDICATOR OF *O. VOLVULUS* INFECTION

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An essential element currently lacking in river blindness eradication programs is the means of determining the presence of patent infection. The ability to evaluate this is essential for defining areas of need, monitoring progress of drug administration, verifying absence of infection/transmission within an area and assessing reoccurrence within a cleared area. A field test kit allowing aid workers to determine which persons in a population are capable of transmitting *Oncocerca volvulus* and thus require further ivermectin treatment, and which persons do not will greatly aid river blindness eradication programs. As an approach to this goal, we believe a unique metabolomic marker exists in the blood of persons patently infected with *O. volvulus* that could be used to monitor their infection status. An examination of the behavior and lifecycle of *O. volvulus* strongly suggests communication via pheromones for a number of behaviors. A key aspect of a pheromone is that it is inherently required to be species specific. Closely related species will use chemically distinct pheromones to avoid attracting inappropriate mates. The core idea is to detect the presence of adult parasites by utilizing the very method they use to attract each other. Rather than limiting ourselves to just a putative pheromone, we are conducting a metabolomic profile of plasma samples from infected and non-infected individuals as well as from adult worm extracts obtained from nodules. The aim is to identify and isolate any molecule unique to river blindness. We will present the results of our LC-MS metabolomic profiles of adult parasite extracts and methanol extracted plasma from infected and non-infected individuals of the Northwest Province of Cameroon.

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FIELD OBSERVATIONS THAT CHALLENGE THE CURRENT DOGMA CONCERNING THE PATHOGENESIS OF LYMPHATIC FILARIASIS

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A mass drug administration program against lymphatic filariasis has now been in place in Tanzanian for the past six years. The experience gained from the field-based observations made in this public health initiative has revealed a number of new questions about the biology of filariasis and also encourages a reassessment of some of the basic beliefs that such mass drug programs are based upon. The Tanzanian National LF Program has treated more than 12 million people along the coast, and area long known for a high prevalence of the disease. The Program has

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studied the acceptance of the MDA by the population, the effects of the two drugs (annual ivermectin and albendazole) on the ICT antigenaemia and circulating parasite loads, and on the clinical presentation by those affected with elephantiasis and associated disease. The need to show a positive effect on individual's already affected by the disease has been an important element in the Tanzanian Program and has driven the form of advocacy needed for a successful program; this has not been a major directive in Global Program before. It has been seen that many individuals that have taken the drug annually, as is proscribed by the Global Program, remain infected; the possible reasons for this will be discussed. Likewise the correlation between ICT positivity and circulating microfilaraemia has been found to be variable in hyper-endemic areas of Tanzania, suggesting that there may be a need for different antigen based tests. An unexpected finding has been the very significant improvement patients suffering from lymphatic filariasis have enjoyed and this is believed to be due an improvement in their ability to resist and combat secondary infections. The field remains a vital "laboratory" for gaining a basic understanding of the disease and its parasitology, and the observations made in the Tanzanian Program lay a strong basis for new research efforts that are likely to enhance the overall efforts to eradicate this disease. This presentation will discuss the type of research that is needed to address these new findings.

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EFFECTS OF ANTI-RICKETTSIA DRUGS ON THE MICROFILARIA SURVIVAL OF *DIROFILARIA IMMITIS*

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Canine and feline heartworm diseases (Dirofilariasis), caused by a filarial nematode, *Dirofilaria immitis*, are transmitted by mosquitoes. Human accidentally infected with *D. immitis* have been reported. Human pulmonary dirofilariasis develops when the parasites die, embolize, travel to the lung, and develop nodule in small branches of the pulmonary arteries. Anti-rickettsia drugs have bactericidal activity against the endosymbiont *Wolbachia*, required for fertility and survival of the filarial nematodes. Our study showed that doxycycline was the most effective compound. After 24 hours in culture, only doxycycline affected microfilariae motility. All microfilariae died at 48 hours, with the minimum effective concentrations (MEC) of 256 µg/ml. The effects of rifampicin (MEC = 256 µg/ml) and ciprofloxacin (MEC = 128 µg/ml) appeared later, (at day 4 and 10, respectively). The outcome of this study will be useful for treatment, and control of *D. immitis* infection and could be applied to control other human filarial parasites.

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ULTRASOUND ASSESSMENT OF SUBCLINICAL HYDROCELES IN A COMMUNITY COENDEMIC FOR WUCHERERIA BANCROFTI AND MANSONELLA PERSTANS

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Lymphatic filariasis (LF) due to *Wuchereria bancrofti* (Wb) infection is endemic throughout Mali with prevalences, based on circulating antigen assays, as high as 65%. Despite this, clinical manifestations of LF occur less frequently than expected. In Sabougou, where the prevalence of infection was 48%, lymphedema, elephantiasis and hydrocele were detected in 1.6%, 0.8% and 1.6%, respectively, of the 129 circulating-antigen positive subjects enrolled in a study of doxycycline treatment of *Mansonella perstans* (Mp) WB coinfection. The purpose of the present study was to determine the rate of subclinical hydrocele in male subjects coinfected with Wb and Mp in Sabougou. Only 4 of 62 subjects (6%) who underwent ultrasound examination had evidence of hydrocele on clinical examination. In contrast, 42 of 62 subjects (67%) had hydroceles (estimated volumes 10-800 ml) detectable on ultrasound exam. The "filaria dance sign" indicating the presence of live adult worms of Wb was observed in 24 of 62 subjects (38%), all of whom had hydroceles and evidence of lymphatic dilatation on ultrasound examination. Subcapsular calcifications were also common, occurring in 14 subjects. These results confirm the results of studies in other endemic areas, that have demonstrated increased sensitivity of ultrasound in detecting subclinical pathology in Wb-infected subjects. Although the effects of concomitant Mp infection on the clinical presentation of Wb infection remain uncertain, the degree of discordance between the clinical and ultrasound examinations in Sabougou is markedly greater than that reported from areas non-endemic for Mp infection. Clinical and ultrasound examination of Wb-infected male subjects from Sabougou without concomitant Mp infection should help clarify these issues.

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ONCHOCERCIASIS AND EPILEPSY IN PARTS OF THE IMO RIVER BASIN, NIGERIA: A PRELIMINARY REPORT

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The relationship between Onchocerciasis and epilepsy prevalence was investigated in an Onchocerciasis endemic area in the Imo River Basin, Nigeria. Individuals complaining of seizures were identified by means of a population census in 13 villages. A total of 72 individuals were identified as possible epilepsy patients during the survey. Active epilepsy was confirmed in 58 giving a crude prevalence of 1.2%. Epilepsy was prevalent in six out of the 13 villages investigated, with highest rates in Umulolo (2.8%), Amuro (2.2%) and Aku (1.8%) and lowest in Ajabo (0.5%) and Okanachi (0.4%). The age of epilepsy patients ranged from 4 years to 59 years with majority (64%) in the 20-29 age group. The sex difference in epilepsy prevalence was not significant ($p > 0.05$). The prevalence of Onchocerciasis in the villages ranged from 8.3% to 36.0%. The highest Onchocerciasis rates were correspondingly found in the villages where epilepsy was most prevalent. This finding suggests a geographical association between epilepsy and Onchocerciasis. If successful control of Onchocerciasis in the area were to be followed by a fall in the prevalence of epilepsy, this may lend credibility to a causal connection between epilepsy and Onchocerciasis prevalence.

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RAPID ASSESSMENT METHOD FOR PREVALENCE OF LOIASIS IN PARTS OF THE NIGER DELTA, IMO STATE, NIGERIA: A PRELIMINARY REPORT

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The prevalence of loiasis was investigated in 24 rural communities in parts of the Niger Delta Imo State, Nigeria using rapid assessment methods based on a history of eye worm (lasting 1-7 days together with confirmation by the guided recognition of a photograph of adult Loa loa in the eye) and Calabar swelling. A standardized questionnaire was administered to 1,921 individuals from randomly selected households, aged > 15 years and who had resided in the communities for at least 5 years. The results showed that the prevalence of loiasis was generally low for both diagnostic indices, history of eye worm (3.85%) and Calabar swelling (4.90%). There were insignificant differences between communities in the prevalence of eye worm and Calabar swelling ($p > 0.05$). Furthermore, the sex-related prevalence of history of eye worm and Calabar swelling were insignificant in males (3.02%; 3.38%) and females (5.09%, 7.03%) ($p > 0.05$) respectively. While the prevalence of history of eye worm was similar in all age categories, the prevalence of Calabar swelling increased with age to a peak of 15.11% in subjects 74 years and above. Both manifestations were more prevalent in farmers and traders than other occupational groups. The present findings show that rapid assessment of the prevalence of loiasis at the community level can be reliably achieved using a method based on the history of eye worm lasting 1-7 days together with confirmation by the guided recognition of a photograph of an adult Loa loa in the eye.

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REAL-TIME PCR FOR THE DETECTION OF *BRUGIA* DNA IN BLOOD AND MOSQUITO SAMPLES

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Improved diagnostic tests for brugian filariasis are needed to support filariasis elimination efforts. We have developed two real-time PCR assays for detecting *Brugia* DNA in blood and mosquitoes. The highly repeated AT-rich 320 bp "*Hha*I" DNA sequence was used as a target for Taqman (TaqM, amplicon size 320 bp) or Eclipse MGB (EMGB, amplicon size 120 bp) real-time PCR assays. The EMGB assay is more sensitive than the TaqM assay and detects as few as 22 copies of the target. The EMGB assay was as sensitive as membrane filtration and microscopy for detecting *B. malayi* in 36 night blood samples from infected individuals in Sulawesi, Indonesia. The EMGB assay also detected parasite DNA in 17 of 31 (55%) of microfilaria-negative day blood samples from these subjects. This test was more sensitive than conventional or TaqM PCR (and almost as sensitive as night blood filtration) for detecting parasites in night blood samples from a *B. timori*-endemic area on Alor Island, Indonesia, where infected people had low microfilaria densities after mass drug administration (MDA). To evaluate the assays for xenomonitoring, host-seeking mosquitoes were collected on Alor Island, after 1, 2 and 3 rounds of MDA. About 25 % of the collected mosquitoes were *Anopheles barbirostris*, the vector of *B. timori*, and ~75% were *Culex*. DNA was extracted from pools of 10 to 20 individuals and infection rates were estimated by the Poolscreen2 algorithm. Overall TaqM detected *B. timori* DNA in 36 of 88 *Anopheles* pools (infection rate 5.1%, 95% CI, 3.4-7.2%); EMGB detected 38 positive pools (infection rate 5.5%, CI 3.8-7.7%). Surprisingly, 10 and 12 of 87 *Culex* mosquito pools were positive by TaqM and EMGB assays, yielding estimated infection rates of 0.6 and 0.7%, respectively. *Culex* mosquitoes are not known to be vectors of *Brugia*, but microfilariae were presumably taken up with blood meals and their DNA appears to persist long enough to be detected by PCR. Our data show that real-time PCR is a sensitive means of detecting *Brugia* DNA in human blood and man-biting mosquitoes.

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ENHANCED EXPRESSIONS OF TGF-Β1 IN INFLAMMATORY CELLS, Α-SMA IN STELLATE CELLS, AND COLLAGEN ACCUMULATION IN EXPERIMENTAL GRANULOMATOUS HEPATITIS CAUSED BY *TOXOCARA CANIS* IN MICE

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Although toxocaral granulomatous hepatitis (TGH) characterized with a dominant-Th2 type immune response is a self-limiting disease, little is known concerning the role of fibrosis- related cytokine transforming growth factor β 1 (TGF-β 1) in pathogenesis of TGH. A detailed histological and quantitatively immunohistochemical analysis of TGF-β 1, α-smooth muscle actins (α-SMA), and collagen was performed on the liver tissues from mice infected with *T. canis* as assessed between day 1 and 42 weeks post infection (DPI or WPI). TGF-β1 was detected mainly in infiltrating cells in lesions with a peak at 12 WPI. Larvae *per se* also exhibited strong TGF-β1 expression in the trial. Alpha-SMA was detected predominantly in hepatic Stellate cells (Hospital for Sick Children) which surrounded the lesions, reaching a peak at 28 WPI. Collagen was observed to accumulate in inflammatory lesions and biliary basement with peak content at 24 and 12 WPI, respectively. In conclusion, although enhanced TGF- 1 in infiltrating cells and active Hospital for Sick Children with α-SMA expressions may contribute to healing of injured sites through up-stimulation of collagen deposition, abnormally persistent collagen accumulation may cause irreversible fibrotic injury in the TGH.

(ACMCIP Abstract)

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IMPACT OF MASS DRUG ADMINISTRATION ON THE DEVELOPMENT OF RESISTANCE IN HOOKWORM

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Hookworms infect an estimated 1.3 billion people worldwide. These parasites feed on blood, producing an iron-deficiency anemia that leads to malnutrition, stunting of growth, intellectual and cognitive retardation in children, and adversely affects intrauterine growth resulting in premature births and low birth weight. Treatment campaigns targeting hookworms and/or lymphatic filariasis using mass drug administration (MDA) have produced significant reductions in the prevalence and intensity of hookworm infections. However, mass treatment also brings with it the risk of drug resistance. Studies on several parasites of veterinary importance have demonstrated that levels of resistance are extremely high before resistance is recognized by phenotypic measures of drug efficacy. Therefore it is of critical importance to detect evolving resistance while the frequency of resistance alleles is still low and the drugs are still effective. We are investigating the potential impact of the MDA on the development of resistance in hookworms in Haiti, where benzimidazoles have been used in the WHO-sponsored program to eliminate lymphatic filariasis. In several nematode parasites of livestock, resistance to benzimidazoles is associated with point mutations (TTT to TAT or TTC to TAC) in positions 167 and 200 of β-tubulin gene, which replaces a phenylalanine (Phe) with a tyrosine (Tyr). However, mutations in other positions of the protein also may

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correspond to resistant phenotypes. We therefore cloned and sequenced the β-tubulin genes of the human hookworms *Ancylostoma duodenale* and *Necator americanus* and the dog hookworm *A. caninum*. Mutations are being investigated using a broad range of techniques including allele specific PCR, pyrosequencing and Real Time PCR. We are also interested in investigating the population genetics of drug resistance development and spread, using microsatellites as genomic markers. Genomic libraries enriched for microsatellite sequences were prepared for all three species, and we now have set of 34 usable microsatellite markers for *A. caninum*.

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A RARE CASE OF TRICHURIS TRICHURA AND HOOKWORM INFESTATION

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Heavy ***Trichuris trichura*** and hookworm infestation and surgical complications are rare in developed countries. Cases of intestinal complications including colitis, rectal prolapse, intussusceptions and perforated appendicitis with trichuriasis has been reported. We described a case of appendicular obstruction leading to necrotizing appendicitis related to heavy ***T. trichura*** and Hookworm infection in a healthy young male who had emigrated to United States from Honduras six months earlier. He presented to our hospital with lower abdominal pain, fever, jaundice and vomiting for four days. He was unable to pass flatus and had no bowel movements. Abdominal examination showed rebound tenderness with guarding at right lower quadrant with sluggish bowel sounds and rectum was empty. Laboratory investigations revealed peripheral leukocytosis and neutrophilia with no eosinophilia or anemia. The liver enzymes were elevated with hyperbiliburinemia. Hepatitis Profile was negative. CT Abdomen and Pelvis revealed appendicitis with abscess, inflammatory changes of the right colon, mild intrahepatic biliary ductal dilatation, and parasitic colonization of small/large bowel. Patient had appendectomy with abscess drainage and pathology revealed features of acute necrotizing appendicitis with periappendicitis.

Blood culture grew *Streptococcus milleri* and stool sent for ova and parasite showed many *T. trichura* and hookworms. Patient was treated with antibiotics and Mebendazole. Patient improved and was discharged home after nine days of hospitalization. Our case reveals complicated appendicitis and cholangitis from parasitic infestation with superimposed bacteremia. Intense local irritation, spasm of intestinal wall and increased plasma concentration of tumor necrosis factor in systemic circulation may have played a role in the pathogenesis of above manifestations.

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MOLECULAR CLONING, CHARACTERIZATION AND EXPRESSION OF CDNA DERIVED PHOSPHAGEN KINASE (PK) OF *T. CANIS*, *A. SUUM*, *F. HEPATICA* AND *S. JAPONICUM*

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Phosphagen kinases are the enzymes that catalyse the reversible transfer of the high-energy phosphoryl group of ATP to naturally occurring guanidino compounds such as creatine, glycocyamine, taurocyamine, lombricine and arginine, and have a key role in the interconnection of energy production and utilization in animals. In vertebrates the only phosphagen is phosphocreatine, and the corresponding phosphagen kinase is creatine kinase (CK). In invertebrates, at least six unique phosphagens (phosphoarginine, phosphoglycocyamine,phosphot aurocyamine,phospholombricine, phosphohypotaurocyamine and phospho-opheline) are present in addition to phosphocreatine, and the corresponding kinases for the first four such as, arginine kinase (AK), glycocyamine kinase (GK), taurocyamine kinase (TK) and lombricine kinase (LK), have also been identified. Here we report the isolation and characterization of cDNA derived amino acid sequences of phosphogen kinases of very important zoonotic parasites such as *Toxocara canis*, *Ascaris suum, Fasciola hepatica* and *Schistosoma japonicum*. The phosphagen kinase can be a very good drug target site and a new approach in the control of these parasites. In this study, cDNA was synthesized from these parasites and the PK gene was successfully amplified by PCR and completely sequenced. The PK gene of *S. japonicum* and *F. hepatica* contain two domains. In *S. japonicum* domain I comprises 1080bp of ORF coding for a 360-amino acid residue protein and domain II comprises 1021bp of ORF coding for a 357-amino acid residue protein. In *F. hepatica* domain II comprises 1071bp of ORF coding a 357-amino acid residue protein. The cDNA of *T. canis* and *A. suum* PK comprises 1300bp of ORF coding for a 400-amino acid residue protein and 1194bp of ORF coding for a 398-amino acid residue protein respectively. The cDNA derived neucleotide and amino acid sequences of *T. canis* and *A. suum* showed high neucleotide and aminoacid similarity with nematode AKs. The phylogenetic analysis indicated that both neucleotide and aminoacid sequences of *T. canis* and *A. suum* PK placed within the nematode AK cluster while *S. japonicum* and *F. hepatica* formed a separate cluster (trematode). Further, we cloned *T. canis* and *A. suum* PK in pMAL plasmid vector and expressed it in *E. coli* as a fusion protein with maltose-binding protein.

(ACMCIP Abstract)

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EPIDEMIOLOGY OF HEPATITIS C VIRUS INFECTION AND ASSOCIATION WITH HUMAN IMMUNODEFICIENCY VIRUS AMONG MEN WHO HAVE SEX WITH MEN IN LIMA, PERU

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To evaluate any potential association between hepatitis C virus (HCV) and human immunodeficiency virus (HIV), we studied 162 HIV-positive case subjects at screening and 324 age- and location-matched HIV-negative control subjects which were part of a cross-sectional HIV sentinel surveillance survey of 3,280 men who have sex with men (MSM) conducted in 6 major urban centers in Peru, during November 2002 and February 2003. Sexual behavior was assessed with a structured computer assisted self-interview (CASI); serum-based screening for HIV and HCV antibodies was performed by enzyme-linked immunosorbent assay (ELISA). The overall HCV prevalence in the case-control study group was 9.5%. Age-related increases and decreases were noted in HIV and HCV infection rates, respectively. HCV infection was found to be associated with HIV infection (odds ratio [OR] = 2.99), prior symptoms of sexually transmitted infections (STIs), such as urethritis (OR = 2.60) or proctitis (OR = 1.84), and a homosexual self-definition (OR = 1.80), but not with a history of illegal drug use. HCV infection among MSM in Peru is strongly associated with HIV seropositivity in a setting where injecting drug use is uncommon. STI

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prevention strategies may assist in the reduction of HCV infection among MSM in Peru.

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COMMUNITY BASED HIV/AIDS PROGRAM IN RURAL HAITI: WHAT TO BUILD, WHAT TO BORROW AND WHERE TO BEGIN

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Haiti is the poorest and most HIV/AIDS burdened country in the Western Hemisphere (Adult prevalence rate of 5.6%). Decades of political instability, international embargoes and economic sanctions have resulted in a public health and general healthcare infrastructure inadequate to meet the most basic needs of the majority of the population. Several non-government organizations have implemented HIV/AIDS programs with notable success in select Haitian communities. However, similar to most of Haiti's population, the peasants in the rural community of Fondwa had no consistent access to HIV/AIDS education, testing, treatment or care. The formal (clinic staff) and informal (community health promoters) healthcare workers in Fondwa requested assistance in designing and implementing a community HIV/AIDS program. A current literature review on HIV/AIDS in Haiti and relevant studies on HIV/AIDS in the developing world was performed. In addition, based on principles of community based participatory research, information was obtained from: (1) A focus group consisting of representatives from the formal and informal healthcare sectors and two US based nonprofit organizations providing long-term professional, educational, financial and material support to the primary care clinic. (2) In-depth interviews with long-term Fondwa Community volunteers and the Fondwa Clinic Director (3) Direct participant observation by the author who has been involved with a medical laboratory development project in Fondwa since 2002. Fondwa Clinic records were reviewed for general patient demographic data and results of a rapid community assessment survey were also considered. The community specific resources and needs are outlined and assessed in the current country specific context of Haiti. Recommendations for designing a model comprehensive HIV Prevention Education, Voluntary Testing and Counseling, Treatment and Care Program in rural Haiti are discussed as well as the challenges faced during the initial implementation and evaluation of the program.

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GENOTYPE DISTRIBUTION OF HIV-1 STRAINS AMONG CHILDREN IN LIMA, PERU, 2002-05

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The Acquired Immune Deficiency Syndrome (AIDS), represents the seventh leading cause of death in young adults and the ninth in children of 1 to 4 years of age worldwide. The number of HIV infected pregnant women in Peru has increased in the past 2-3 years and vertical transmission now accounts for approximately 4% of all HIV cases reported. There is no published data regarding the distribution of HIV genotypes among pediatric populations in Peru. We examined the genotypic distribution of circulating HIV strains among children born in Lima, Peru, during the years of 2002-05. The study population consisted of HIV-infected children who were suspected or confirmed of suffering from pulmonary tuberculosis (Tb) patients who were evaluated at the Instituto de Salud del Niño and/or at the Hospital Nacional Cayetano Heredia. The children's ages ranged between 6 months to 8 years old. All blood samples (n=86) that were confirmed by ELISA (EIA) and Western Blot (WB) were subjected to PCR amplification of the gag and env portions of the genome. Heteroduplex mobility assay (HMA) was performed with env PCR products followed by sequencing of the C2-V5 region of the envelope gene for undetermined samples. To gain better insight into the dynamic of the epidemics, we additionally sequenced the gag p24 and protease/RT (ProRT) region to monitor the prevalence of recombinant viruses. PCR Protease/RT amplified regions were sequenced using an ABI 3100 automated sequencer. A total of 84 (97.6%) were genotyped as subtype B by HMA and 2 (2.3%) of the specimens were found to be recombinant forms (CRF12_BF) representing B and F mixed genotypes by sequencing.

Co-circulation of a number of subtypes in a given population and super infection of a patient with a different virus may result in the emergence of recombinant viruses.

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HIGH PREVALENCE OF ENTEROAGGREGATIVE ESCHERICHIA COLI (EAEC) IN AIDS PATIENTS WITH DIARRHEA IN HAITI

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Enteroaggregative *Escherichia coli* (EAEC) infection is a cause of persistent diarrhea in AIDS patients. The prevalence of EAEC in Haitian patients with AIDS has not been examined previously. A matched-pair cohort study (25 patients in each category) to examine antiretroviral absorption in HIV-infected patients with and without diarrhea is currently enrolling patients at the GHESKIO Centers in Port au Prince, Haiti. The patients are matched by age, sex, and CD4 count. This abstract reports preliminary results from an investigation of the prevalence of EAEC in these Haitian AIDS patients initiating antiretroviral therapy. Polymerase chain reaction (PCR) targeting the plasmid-borne *aggR* gene of EAEC was performed on DNA from stool samples obtained at the time of anti-retroviral treatment (ART) initiation. DNA was extracted from frozen, unpreserved stool using the Qiagen DNA Stool Mini Kit according to the manufacturer's instructions. DNA was then amplified with AmpliTaq Gold (Applied Biosystems) using 40 cycles of amplification (55°C annealing temperature). We were able to detect down to 10,000 cfu bacteria per gram of stool using stool spiked with positive control EAEC bacteria (strain 17-2). The presence of the *aggR* gene was determined by a 457 bp band visualized after electrophoresis of PCR product on a 2% agarose gel. 23 stool samples from AIDS patients with diarrhea and 9 stool samples from AIDS patients without diarrhea have been tested with this protocol. 17/23 (74%) of the patients with diarrhea and 3/9 (33%) of those without diarrhea were positive for *aggR* by PCR. (p =.020) Patients with EAEC by PCR in their stools had significantly higher quantitative lactoferrin (42.1 µg/ml vs 7.6 µg/ml; p =.045), and they had significantly less weight gain in the first two weeks of ART (-0.48lb vs +3.3lb; p =.039) than patients without EAEC in their stools. In conclusion, performing PCR for EAEC directly from stool samples is feasible. EAEC is significantly more prevalent in AIDS patients with diarrhea relative to AIDS patients without diarrhea in this study population. Patients with positive stool PCR for EAEC have higher levels of lactoferrin, an indicator of intestinal inflammation, and poor weight gain after the initiation of ART.

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ONE YEAR FOLLOW UP OF MOTHERS FROM THE PMTCT PROGRAM IN ZIMBABWE: COMPLIANCE AND CHALLENGES

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This stiudy was undertaken to describe the outcome of a one year follow up of mothers from a national PMTCT program regarding defaulters, drop outs and compliance. Nested case control study was conducted. Three peri-urban primary health care clinics the city of Harare:Epworth, St Mary`s, Seke North. Pregnant women enrolled at 36 weeks of gestation were recruited for a follow up of mother and child from delivery, 6 weeks,

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4 and 9 months post partum. Follow up trend of HIV positive and negative mothers was compared regarding defaulting, drop outs, partial and full compliance. Statistical significance was computed using the chi-square test. Of the enrolled 1050 pregnant women with a known HIV status 851(81%) showed up at one or more visits scheduled up to 9 months. The denominator dropped at each point and time. The overall drop out was 19% without any significant difference between the HIV positive and negative women at delivery. The difference appeared at 6 weeks 7.7% versus12.9% (p=0.010) and at 4 months: 2.9% versus 7.7% (p=0.002) respectively. At 9 months the drop out rate was not different (p=0.747). The defaulter rate was significantly different at every stage between the HIV positive and negative mothers from delivery to 6 weeks becoming more significant at 4 and 9 months visits (p=<0.001). Overally full compliance at 9 months was 46.1% with a significant difference between the HIV positive (55.6%) versus (37.9%) for the HIV negative (p=<0.001). In conclusion, drop out is highest among the HIV negative as opposed to the HIV positive with the peak period being at 6 months. There is high defaulting among the HIV negative compared to the HIV positives with the peak period at 4 months. Full compliance is observed for the HIV positive whilst more HIV negatives comply partially. The challenge is in defining the threshold for keeping people in the cohort and for them to fully comply with the study. There is need to assess the predictors and characteristics of the mothers that dropped out, defaulted and those that remained in the study.

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THE CHARACTERISTICS, RISK BEHAVIORS AND STI PREVALENCES AMONG SOCIALLY MARGINALIZED WOMEN IN LOW-INCOME URBAN, COASTAL PERU

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This study was undertaken to describe the characteristics, risk behaviors, and STI prevalence among marginalized women in low-income, urban neighborhoods in three coastal Peruvian cities. The socially marginalized women are referred to as "movidas" (loose women) in these communities and were included in the National Institute of Mental Health Collaborative HIV/STD Prevention Trial as an ethnographic analysis of these communities suggested that they were at higher risk than women in the general population. Movidas were administered an epidemiologic survey to evaluate risk behavior and serologic tests to determine STI/HIV infection prevalence in 2001-2002. In the sample of 108 women, their mean age was 25.5 (range: 18-40), 38.9% had graduated from high school, and 56.5% had stable or occasional work while 38% were supported by their families. Their mean number of partners (standard deviation) in the past 6 months was 2.3 (7.8). Most (89.5%) had unprotected sex in the past six months with at least one of their past five partners and 10.5% had unprotected sex with a non-primary partner. Additionally, 27.7% of these women reported having been forced to have sex with one of their past five partners in the past six months. No HIV cases were found in the sample. The prevalence of HSV-2 infection was 43.0% (95% CI, 33.5% - 52.9%). The prevalence of gonorrhea was 2.8% (95% CI, 0.6% - 8.0%), Chlamydia prevalence was 18.7% (95% CI, 11.2% - 26.2%), and the prevalence of trichomonas was 6.5% (95% CI, 1.8 - 11.3%). In conclusion, movidas are a group with sexual risk behavior and STI prevalence higher than what has been found in the female general population in Peru, however they have not been included in STI/HIV prevention or control efforts. This is a highly vulnerable population with whom STI prevention interventions are warranted.

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IMMUNOPATHOLOGY AND *IN SITU* HYBRIDIZATION IN THE DETECTION OF CUTANEOUS LEISHMANIASIS IN AN ENDEMIC REGION OF WESTERN VENEZUELA

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A parasitological, immunological and molecular diagnosis of localized cutaneous leishmaniasis (CL) was conduced from 1966 to 2004, in 27 settments in western Merida state, an endemic Venezuelan Andes region for CL. The transmission is active, at a height of between 800 and 1800 metres in the humid mountainous zones where the anthropophilic *Lutzomyia* species are naturally infected. Patients were attended at the town of Tovar in Merida state site of the headquarters of the local Dermatology Service. Clinical histories revealed 5,545 infected human with 1 to 20 lesions over the forearms (10%), legs (35%), face (45%), and trunk (10%).The infection was equally common in children, women and men and the Montenegro intradermal reaction (MIDR) carried out each patient, with a dose of 0.1 ml of antigen injected intradermally on the inner surface of the left forearm was up to 29 mm in diameter. No significative differences between MIDR and sex, age, number and evolution of the lesions. The relationship between the number of lesions with age and sex was significative. The patients received treatment quimioterapic. Anti-*Leishmania* antibodies for indirect immunofluorescence antibody test and ELISA was 1:400 to 1:16400. Giemsa-stained imprints of the cut of the punch biopsies pressed on a glass slide, and skin biopsies sections of seven microns stained with Hematoxilin and Eosin and unlabelled peroxidase-anti-peroxidase and IFAT technique, showed inflammation site, amastigotes and *Leishmania* antigen. The disease was also seen in dogs and lesions rich in parasites were found in the nose and ulcerated scrotum and vagina of canids lesion by biopsy. The clinical presentation of the cutaneous lesions, the geographic origin of the infection and the caracterization from CL lesions of the humans and domestic animals by *in situ* hybridization was compatible with pattern strain of *L.* (*Viannia*) *braziliensis.*

(ACMCIP Abstract)

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INFECTION OF THE FETAL TISSUE IN CONGENITAL CHAGAS´ DISEASE IN THE WISTAR RAT

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This work was carried out on female Wistar rats intraperitoneally injected before mating with $5x10^4$ bloodstream l/Pas/Ve/00/planalto *Trypanosoma cruzi* strain, and pregnated 10 days later (GI) to study the effects of acute Chagas on the fetuses infection. Rats uninpregnated and infected with *T. cruzi* (GII), and pregnancy rats (GIII) were used as controls. Development of patent parasitemia in GI showed levels highest at the 18, 24 and 34 days post-infection (pi), with 6, 12 and 20 days of gestation respectively and with significant differences at the 1% level between GI and GII. The pregnant in infected rats with *T. cruzi* (GI) induces specific stimulation anti-*T. cruzi* antibodies (Ab). Serologic test in serum samples obtained during acute phase gave positive results, with titers Ab between 1:512 and 1:2048 at the 19 and 20 days pi, and significant difference at the 5%, when groups GI and GII were compared. *T. cruzi* was observed in amniotic fluid (AF) on a glass slides Giemsa stained. The 33% the AF samples from rats of GI with 20 days of gestation developed trypomastigotes in hemoculture NNN. Histopathological studies in sections of 6 micron Hematoxilinin and Eosin staining of fetal heart of 2 fetuses from rats GI with 34 days of gestation, showed parasitism in the miocardic tissue. Placentas showed moderate placentitis, inflammatory infiltration of mononuclear and polymorphonuclear cells with abundant neutrophils. The immunotintion with Fluorescein isiotiocianato-Propidium Iodide

and Peroxidase anti Peroxidase of placental and umbilical cord tissue of 3 rats from GI, showed intense fluorescente on *T. cruzi* antigen. These results confirmed that acute infection in gestanting rats produced fetal intrauterine infection. It is possible that the massive parasite invasion in the placenta, amniotic fluid and fetal cardiac tissue is related with high maternal patent parasitemia, and a congenital *T. cruzi* transplacental transmission occurred early in pregnancy rats.

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IDENTIFICATION OF THE *L. MEXICANA, L. AMAZONENSIS* AND SUBGENUS *VIANNIA* BASED ON ANALYSIS OF THE RRNA INTERNAL TRANSCRIBED SPACER 2

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Human cases of leishmaniasis are caused by approximately 20 *Leishmania* spp., some of which are found in the same geographic region. Species identification often has clinical relevance (e.g., influences decisions about whether/which treatment is indicated). However, the gold standard method for species identification, isoenzyme analysis, requires a positive culture that remains viable long enough to yield a large quantity of parasites. Molecular approaches for species identification, using various genetic markers, are being explored. In this study, we focused on the ITS 2. We designed *g*enus-specific primers to amplify a DNA fragment from the ITS 2 of virtually all pertinent *Leishmania* spp. and looked for regions in the fragment with potential utility for differentiating among species, with the ultimate goal of developing a multiplex approach. We extracted DNA from 50 isoenzyme-characterized specimens (21 skin/blood specimens and 29 cultured isolates); the species included *L. (V.) braziliensi*s, *L. (V.) panamensis*, *L (V.) guyanensis*, *L. tropica, L. major*, and species in the *L. donovani* and *L. mexicana* complexes. We conducted PCR and DNA sequence analysis of the amplified fragments (385 to 450 bp in length). Of note, no amplification was obtained when the primers were used on DNA extracted from *Trypanosoma cruzi* specimens (i.e., 3 blood and 3 culture specimens). For the *Leishmania* specimens, DNA sequence analysis of the amplified fragments revealed complex patterns of insertions/deletions and substitutions for the various species studied. However, we found 3 regions (3 to 21 bp in length) within the fragment that, when analyzed together, allowed robust differentiation among the species. These regions may be used to design probes for a multiplex approach for species identification.

(ACMCIP Abstract)

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DEVELOPMENT OF A NEW REAL-TIME PCR ASSAY TO IDENTIFY THE CAUSAL AGENTS OF LEISHMANIASIS IN PERU

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Five different species belonging to the genus *Leishmania* have been identified as a cause of american tegumentary leishmaniasis (ATL) in Peru, namely *L. braziliensis, L. peruviana, L. guyanensis, L. lainsoni* and *L. amazonensis.* These species present clinical outcomes that range from benign, self healing cutaneous lesions to diffuse cutaneous and mucosal ulcers. Of particular interest is the case of *L. (V.)* braziliensis, responsible for ~80% of the cases of ATL in Peru, which is more aggressive and causes mucosal metastases in approximately 10% of the patients. The identification of the agents to the species level is of critical importance for assessing the clinical outcome, providing adequate treatment and evaluating epidemiological risks. The current gold standard for the identification of the species, Multilocus Enzyme Electrophoresis (MLEE), is time consuming and requires parasite isolation and technical expertise. We cultured 45 isolates previously typed by MLEE and sequenced the complete coding region of 4 of the main isoenzymes showing differing migration patterns between species: malate dehydrogenase (MDH), glucose-6-phosphate dehydrogenase (G6PD), glucose phosphate isomerase (MPI) and mannose phosphate isomerase (MPI). Sequence analysis revealed polymorphisms that produced nonsynonymous mutations that resulted in a shift in the MLEE patterns. As expected from phylogenetic analyses previously published, several single nucleotide polymorphisms (SNPs) were found to be unique in the four genes from *L. lainsoni, L. guyanensis* and *L. amazonensis*, whose number were directly correlated to their evolutionary divergence. Accordingly, only one SNP in MPI and two in MDH allowed the differentiation between *L. braziliensis* and *L. peruviana*. We found a subpopulation of *L. braziliensis* strains that showed several unique SNPs and presented different migration patterns for G6PD, GPI and MDH and that will require further investigation. Based on the SNPs identified in the MDH and MPI genes we designed a FRET based real-time PCR assay that allows a rapid, sensitive and specific identification of these *Leishmania* species directly from clinical samples. Because of its swiftness and simplicity it is particularly useful for use in the field and routine laboratories in endemic regions and will allow an early diagnosis and better treatment of the patients affected with ACL.

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IMPACT OF CLIMATE VARIABILITY IN THE OCCURRENCE OF LEISHMANIASIS IN BOLIVIA

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Leishmaniasis transmission occurs in many countries in the Americas. Recent reports from Venezuela and Colombia have evidenced changes in cutaneous and visceral leishmaniasis epidemiology in relation to climate variability phenomena. We evaluated the potential impact of climatic events during 1991-2000 in leishmaniasis incidence in Bolivia. Satellite climatic and epidemiological data were obtained, the last from Bolivia's Ministry of Health. NOAA climatic classification and SOI/ONI indexes were determined as global climatic variability indicators. Yearly variations comparisons and median trends deviations for disease incidence and climatic variability were obtained. Statistical analysis was performed using SPSS. Considerable climatic variability was identified during the study period, (El Niño during 5 years and La Niña for the other 5). In this same period, 16,207 leishmaniasis cases were reported, mean 1620 cases/year. During La Niña years disease incidence increased 67%, while during El Niño years there was a decrease of 40%. We found significant differences in the mean annual number of cases between La Niña and El Niño years (2029 cases/year vs. 1212 cases/year, respectively $p<0.01$). Linear regression demonstrated that with lower values of ONI, a higher number of leishmaniasis cases were seen ($r^2=0.5257$; $p=0.018$). Higher values of SOI were associated with higher number of leishmaniasis cases ($r^2=0.7008$; $p=0.003$). In conclusion, our results support the growing body of evidence that demonstrate the potential impact of climate variability in the incidence of vector-borne diseases and suggest that prevention strategies of leishmaniasis need to take into account climate variability phenomena.

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ACUTE CHAGAS DISEASE IN COLIMA, MEXICO. REPORT OF A CASE AND REVIEW OF ITS EPIDEMIOLOGY

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Chagas disease still represents a relevant burden for health in many countries of Latin America. Its situation in Mexico is a matter of debate because the difficulties to document recent infection in humans in spite of extensive surveys. Here we present the case of a 17 years old boy who developed fever, lymphatic enlargement and a periorbital swelling 2 weeks before his consultation. The physical findings included Romaña´s sign and inoculation Chagoma in the frontoparietal area of the right side of the face, hepatomegaly and tender lymphatic enlargement in the neck. Lab examination showed moderate leucocytosis with neutrophylia and AST elevation, an echocardiogram was normal and chest X ray examination revealed moderate lung reticular infiltrates with right pleural effusion. A fresh smear from peripheric blood sample showed mobile trypomastigotes of T cruzi confirmed by Wright stain. Inoculation of newborn mice resulted in infection with significative parasitemia after 2 weeks. The patient lives in Cuahtemoc city, Colima, México, a place previously reported infested with domestic triatomine bugs. This case is the first acute documented infection in more than 20 years in Mexico and confirms our previous assumption that Chagas disease is currently an active trouble in this part of the country that deserves attention by the health authorities.

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BURDEN OF DISEASE AND DISABILITY-ADJUSTED LIFE YEARS (DALYS) ATTRIBUTED TO *TRYPANOSOMA BRUCEI GAMBIENSE* - DEMOCRATIC REPUBLIC OF CONGO, 2002

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Major advances in understanding the global burden of disease have been made by incorporating the years lost due to disability (YLD) into calculations of disability-adjusted life years (DALYs). Nevertheless, these morbidity estimates have not previously been included in DALY calculations for Human African Trypanosomiasis (HAT). In addition, due to the focal nature of HAT, country or continent-wide estimates do not reflect the severity of the health burden in local areas where the infection is endemic. The Democratic Republic of Congo, a country endemic for *Trypanosoma brucei gambiense*, is estimated to bear more than half of the global burden of HAT, and East Kasai province is the third most affected province in the country. We developed a disability weight for infection with *T. b. gambiense*, utilized province wide incidence data to recalculate the DALYs attributable to *T. b. gambiense* and performed a comparative analysis of the burden of disease attributable to HAT relative to tuberculosis in this province. Estimates of symptom duration and frequency are based on surveillance data from HAT treatment centers in Angola, Central African Republic, Cote d'Ivoire, Democratic Republic of Congo, Equatorial Guinea, Republic of Congo and Southern Sudan. The composite untreated disability weight (D_U) for *T. b. gambiense* is calculated to be 0.77, which is substantially higher than previous estimations of disability weights for treated and untreated forms of trypanosomiasis (0.35) (Murray and Lopez, 1996a p.413). Using standard expected years of life lost, age weight (0.04), discount rate (0.03), age of onset (30 years), and duration of disability (2 years), the DALYs lost per premature death due to *T.b. gambiense* is 29.6 years. In 2002, the total number of HAT cases approximated the total number of confirmed and suspected tuberculosis cases in East Kasai province (3,173 vs. 3,577 respectively). Disregarding undiagnosed HAT, the number of DALYs lost due to HAT in 2002 in East Kasai exceeded those lost to tuberculosis (13,544 vs 12,507). This is a first attempt to develop a disease-specific disability weight for *T. b. gambiense* and apply it to a recalculation of DALYs attributable to HAT. These data suggest that the relative health burden of HAT in sleeping sickness endemic areas of sub-Saharan Africa can approximate or surpass that of tuberculosis and support the need to revise the continent-wide estimates of the disease burden attributable to HAT.

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LYMPHOCYTE SUBSETS BEFORE AND AFTER SODIUM STIBOGLUCONATE TREATMENT

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Sodium stibogluconate (Pentostam®, Glaxo-Smith-Kline) is a mainstay of treatment for severe cutaneous leishmaniasis in U.S. military personnel returning from Iraq and Afghanistan. However, an increased incidence of reactivation of Varicella zoster virus (VZV) has been noted after treatment with this medication. To evaluate the effects of sodium stibogluconate on the immune system that might predispose to this adverse event, different subpopulations of immune cells were evaluated utilizing flow cytometry-based immunophenotyping in 10 patients with old world cutaneous leishmaniasis before and after 10 days of sodium stibogluconate treatment. Total white blood cells (WBCs) and total lymphocytes were analyzed along with lymphocyte subsets including helper T cells (CD3+CD4+), cytotoxic T cells (CD3+CD8+), memory T cells (CD3+CD45RO+), regulatory T cells (CD4+CD25+) and natural killer cells (CD16+CD56+). The absolute number of total WBCs decreased after sodium stibogluconate treatment by a median of 2400/mm^3 (p=0.004), total lymphocytes by 800/mm^3 (p=0.002), helper T cells by 265/mm^3 (p=0.002), cytotoxic T cells by 159/mm^3 (p=0.002), memory T cells by 321/mm^3 (p=0.002), regulatory T cells by 29/mm^3 (p=0.006) and natural killer cells by 54/mm^3 (p=0.004). The percentage of the total lymphocyte population for each lymphocyte subset did not change significantly except for a marginal increase in percentage of cytotoxic T cells by a median of 0.77% (p=0.049). Therefore, lymphocyte subset numbers decreased overall without predilection for any particular subset. Anti-VZV antibodies were measured in 7 patients before and after treatment, and did not change (p=0.69). The general decrease in lymphocytes and especially T cell subsets may account for the increased rate of Varicella zoster reactivation in patients treated with sodium stibogluconate. Further comparison of VZV-specific T cells before and after sodium stibogluconate treatment is ongoing using an intracellular cytokine secretion assay.

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PARASITE STRAIN-DEPENDENT VARIATION IN *TRANS*-SIALIDASE-SPECIFIC CD8+ T CELL RESPONSES IS A GENERAL CHARACTERISTIC OF EXPERIMENTAL *TRYPANOSOMA CRUZI* INFECTION

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The genome of *Trypanosoma cruzi*, the causative agent of Chagas' disease, contains a large number of *trans*-sialidase (ts) genes that encode peptides recognized by CD8+ T cells in mice and humans. Experimental infection of C57BL/6 mice yields dominant CD8+ T cell responses against the ts peptides TSKB20 (ANYKFTLV) and TSKB18 (ANYDFTLV). However, the kinetics and magnitudes of ts-specific CD8+ T cell responses vary

depending on the infecting parasite strain. Peak ts-specific CD8+ T cell responses are generated earliest following CL infection (approximately D15 post-infection), and later in Brazil or Y-infected mice (D19-D24 post-infection). In contrast, the highest frequencies of ts-specific CD8+ T cells are observed in Brazil infected mice (35% of all CD8+ T cells), followed by CL infected mice (20% of CD8+ T cells), and Y infected mice (less than 10% of CD8+ T cells). To determine whether strain-dependent differences in ts-specific CD8+ T cell responses are a general phenomenon of experimental infection, Balb/c mice were infected with Brazil, CL, or Y strain *T. cruzi* and responses to the ts peptide IYNVGQVSI (TSKD14) were examined. CD8+ T cells from Brazil-infected mice produced IFNγ at higher frequencies following TSKD14 stimulation than did CD8+ T cells from mice infected with CL strain. However, TSKD14-specific CD8+ T cell responses were observed earlier following CL infection (D14) than following Brazil infection (D17-24). TSKD14-specific recall responses were barely detectable from SC of Y strain-infected mice. These results document parasite strain-specific immunodominance patterns in T cell responses during *T. cruzi* infection. Such strain-specific patterns of responses in naturally infected hosts could allow for superinfection and could help account for differences in patterns of immune responses and disease outcomes in hosts.

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TOLERIZATION OF ANTIGEN-SPECIFIC CD8⁺ T CELL RESPONSES DURING EXPERIMENTAL *TRYPANOSMA CRUZI* INFECTION REVEALS REQUIREMENTS FOR IMMUNODOMINANT CD8⁺ T CELL SUBSETS

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CD8⁺ T cells are critical for host immune control of *Trypanosoma cruzi*, the causative agent of Chagas disease. C57BL6/J mice develop a highly focused CD8⁺ T cell response against two epitopes derived from the *trans*-sialidase (ts) gene family of surface proteins, TSKb20 (ANYKFTLV) and TSKb18 (ANYDFTLV). It is unknown if immunodominant CD8⁺ T cell responses are required for efficient control of *T. cruzi* infection and pathology. To assess the requirement of these immunodominant CD8⁺ T cell responses, we blocked the generation of antigen-specific populations by tolerization of TSKb20-specific CD8⁺ T cells using intravenous injection of the TSKb20 peptide. The efficacy of treatment was monitored by assaying for the presence of TSKb20-specific CD8⁺ T cells in peripheral blood by staining with MHC I-TSKb20 peptide tetramers. Tolerized mice generated significantly lower frequencies of TSKb20-specific CD8⁺ T cells (<1% TSKb20⁺ CD8⁺ peak) compared to untreated mice (~25% TSKb20⁺ CD8⁺ peak). Mice failing to generate a strong TSKb20-specific CD8⁺ T cell response due to tolerance induction succumbed to infection approximately 57 days after infection. Furthermore, TSKb20 treated mice contained massive numbers of parasites in their muscle tissue as measured by histopathology and quantitative PCR. The results of this study demonstrate that an immunodominant CD8⁺ T cell response focused on ts-derived epitopes is a critical element in control of *T. cruzi* infection. Ongoing studies are aimed at addressing the relative contribution that sub-dominant CD8⁺ responses make to the immune control of *T. cruzi*.

(ACMCIP Abstract)

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VACCINATING AGAINST *TRYPANOSOMA BRUCEI* USING RECOMBINANT *VIBRIO CHOLERAE* GHOSTS EXPRESSING TRYPANOSOMAL CA^{2+} PUMP PROTEIN

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Trypanosoma brucei spp. causes human African Trypanosomiasis (HAT, sleeping sickness) disease with a reported estimate of 50,000 new cases and equal number of deaths in Sub-Saharan Africa. Early symptoms of African Trypanosomiasis include fever, headaches, and joint pains. If treatment is not sought after several weeks, the parasite can then cross the blood-brain barrier to invade the nervous system which can result in coma and death. This severe epidemic calls for the development of an efficacious vaccine that is capable of protecting against infection. Past studies have shown the development of vaccines against HAT to be unsuccessful because of the parasites ability to evade the host immune system by antigenic variation due to its expression of distinct variable surface glycoproteins. We have designed a novel vaccine delivery system that is capable of eliciting antibodies against their epitope. Calcium ATPases have been identified and are localized in the less dense flagellar pocket where they function to maintain cytosolic calcium ion concentration for the survival and proliferation of the parasite. Therefore, we hypothesized that using recombinant *Vibrio cholerae* ghosts (rVCG) as a vaccine delivery vehicle expressing trypanosomal TBCA2, a calcium ATPase, will inhibit the function of these cation pumps preventing parasite survival. To test our hypothesis naïve mice were vaccinated, thrice at two-week intervals, with rVCG expressing TBCA2 or phosphate buffered saline. Two weeks following the last vaccination mice were challenged with *T. brucei* and observed for survival and parasitemia. Also splenic T-lymphocytes were harvested from mice to assess the induction of TNF-α, INF-γ, and IL-10. T-lymphocytes were cultured for five days in the presence or absence of the TBCA2 peptide. Supernatants were then collected and analyzed for TNF-α and IFN-γ; pro-inflammatory cytokines, and IL-10; anti-inflammatory cytokine. Although vaccinated mice tolerated higher parasitemia they were able to survive longer and expressed higher levels of TNF-α than the control group. Analysis of IFN-γ and IL-10 are in progress. Administering rVCG-TBCA2 as a vaccine increased mouse survival and can therefore, be used as a novel approach in the development of a vaccine against human African Trypanosomiasis.

(ACMCIP Abstract)

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RUNT DISEASE LIKE SYNDROME CAUSED BY *LEISHMANIA* PARASITES IN THE MURINE MODEL

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Runt disease in mice is a syndrome characterized by several clinical features agreed upon by most researchers. All the reports point to the same symptoms in fact the animals become wasted loosing their fur with cutaneous atrophy, loss of subcutaneous fat, muscle volume, and even bone mass. These subjects start shrinking very gradually to become significantly smaller in size compared to their non-injected littermates. Furthermore diarrhea if it develops would be an additional symptom to all of the above health problems. The pathophysiologic changes are identical with the features that characterize graft versus host disease. The pathologic findings on post mortum examination reveal invariably an enlarged spleen and liver. Abscesses in the liver are often observed. We report the changes that take place in animals (inbred Balb/c mice)

susceptible to *Leishmania* parasites injected with promastigote forms of this parasite grown in axenic cultures, collected and injected at the log phase of their growth. The course of illness starts a few weeks after the animals have received the inoculum. The gradual changes observed clinically and the time frame for the full blown picture of runting are described. The animals are sacrificed when their survival for even a few hours became doubtful. Our contention that these parasites are the cause of these changes is substantiated by several findings, beside the clinical picture. They comprise the gross findings at autopsy, in addition the histologic studies of the samples from the skin, the liver and the spleen support our suspicion..Finally the cultures showed no bacterial growth beside the expected parasitic growth. These results are discussed in view of what has been reported in the literature on runt disease in mice whether caused by a graft attacking the host or by particularly virulent microorganisms such as *Salmonella Typhi* or as in this report *Leishmania* in *Balb/c* mice.

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CIRCULATING CELL-DERIVED MICROPARTICLES IN MALARIA PATIENTS

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Activation of vascular endothelium and blood cells in a range of inflammatory and infectious diseases is associated with the formation of cell-derived microparticles (MPs), which are membrane vesicles with diameter <1.5 µm. Here we describe the quantity, the cellular origin and the possible pathogenesis of MPs in malaria. Using flow cytometry, we found that the median (range) of circulating MPs was increased in patients with falciparum malaria (N=29, 2051 (222-43280/µl), p<0.01), vivax malaria (N=5, 840 (376-1141/µl), p<0.01) and malariae malaria (N=2, 499 (499-500/µl, p<0.01) compared to healthy controls (N=11, 163 (81-375/µl). Patients with severe falciparum malaria showed higher numbers than uncomplicated falciparum malaria (N=19, 2567 (366-43280/µl) versus (N=10, 1947 (222-4107/µl), p<0.01). The number of MPs rapidly declined after start of antimalarial treatments and remarked below 800/ µl after 48 hours of admission. MPs were mainly released from red blood cells (RBCs), platelets (PLTs), and endothelial cells (ECs). In order to study the effect of oxidative malaria haem products on MP formation, we exposed RBCs, PLTs and human brain endothelial cells (HBECs) to haemin (12.5-100 µg/ml). Above 50 µg/ml haemin bleb formation on RBCs could be observed electron microscopically, and this correlated with an increase in the red cell derived microparticles (RMPs) numbers and a decrease in RBC surface diameter. This was not observed in PLTs and HBECs. Haemin induced RMP formation was inhibited by the anti-oxidant N-acetylcysteine (1 mg/ml). To establish RMP formation from *P. falciparum*-infected RBCs, we measured RMPs in the supernatant from synchronous culture. It was found that most RMPs were produced during schizogony. Altogether, this suggests that MP formation is increased during malaria infection and is associated with severity of disease. Formation of RMPs might result from haemin induced oxidative stress and schizogony.

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THE BURDEN OF MALARIA INFECTION ON PREGNANT WOMEN AND THE INFANTS

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Malaria infection is one of the major problems encountered by pregnant women in the tropical region. Its severity reduces with increase gravidity and age of the mother. This study investigated the effect of gravidity and age of the mother on the severity of malaria infection. It is also aimed to show the burden of infection on the haemoglobin level of the mother and the birth weight of the infants born to them. Peripheral blood was collected from 262 pregnant women who attended Ade-Oyo maternity hospital, Ibadan, Nigeria. Of the pregnant women studied, 128 and 134 were primigravidae and multigravidae respectively. Thick blood smears were prepared for parasite identification and quantification. Anaemia was detected by measuring Hb levels using Drabkin's solution. Age, gravidity, gestation, and history of treatment with antimalaria drugs were obtained from the pregnant women using questionnaire. The overall prevalence of infection was 41.8%. The prevalence of infection was higher in primigravidae (35%) than multigravidae (22%). Teenagers and primigravidae were more infected than the adults. Of the pregnant women studied, 76.4% were anaemic and this was associated with increase in parasitaemia. The severity of the anaemia was significantly higher ($p<0.05$) among malaria positive teenagers and primigravidae than adults and multigravidae. The mean birth weight of infants born to malaria positive was significantly lower ($p<0.05$) than those born to malaria negative mothers. Malaria positive teenagers and primigravidae had children with lowest birth weight as compared with adult and multigravidae. This study suggests that the prevalence of malaria infection and anaemia were higher among teenagers and primigravidae than adults and multigravidae. Malaria positive mothers had babies with very low birth weight than malaria negative mothers. Age and gravidity also affected the birth weight of the infants.

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PLASMODIUM FALCIPARUM CULTIVATION USING THE PETRI DISH: REVISITING THE EFFECT OF THE 'AGE' OF ERYTHROCYTES

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The Petri dish method is one of the most popular methods of cultivating the parasite *Plasmodium falciparum*. Differences in the characteristics of the culture conditions can influence the multiplication rate for this organism. In previous studies, ideal culture conditions for the growth of these organisms was achieved by using erythrocytes collected from blood that had been stored at least 2 weeks. In the present study, we studied the multiplication rate for *P. falciparum* in cultures containing erythrocytes of various "ages". *In vitro* multiplication rates for *P. falciparum* decreased as the duration of erythrocyte storage increased. This trend was consistent despite the interval of medium changes, which ranged from 12 to 48 hours. On the fourth day after inoculation (i.e., 2 life cycles), the parasitemia in the culture containing erythrocytes that had been stored for 28 days was approximately half that of the parasitemia in the culture containing fresh erythrocytes. When the medium was changed every 12

hours, the first 2 weeks of storage turned out to be critical, as indicated by the fact that the multiplication rate for *P. falciparum* decreased more steeply in cultures during the first 2 weeks of erythrocyte storage than the subsequent two weeks. However, when the medium was changed every 48 hours, the multiplication rate decreased more steeply in cultures during the latter period of erythrocyte storage. When the medium was changed every 24 hours, the multiplication rate decreased regularly along with the increase of erythrocyte storage duration. The results of this study strongly suggest that "younger" erythrocytes are better than aged ones for cultivating *P. falciparum*.

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CYTOADHERENCE OF PLASMODIUM FALCIPARUM STRAINS FROM SINGLE AND MULTIPLE GENOTYPE INFECTIONS FROM SYMPTOMATIC CHILDREN IN FRANCEVILLE, SOUTH-EASTERN GABON

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The virulence of *Plasmodium falciparum* is due to its ability to induce severe malaria by sequestration of parasitized red blood cells (PRBC) in the microvasculature of major organs such as the brain. The objective was to investigate whether the level of cytoadherence is influenced by *P. falciparum* multiple genotype infection (MGI) and to determine the relationship between cytoadherence and disease severity. The nested polymerase chain reaction was used to genotype *P. falciparum* isolates and identify SGI (single genotype infection) and MGI from symptomatic children. Cytoadherence of PRBC was determined using the *in vitro* model of human lung endothelial cells (HLEC). Genotype analysis of two highly polymorphic regions of the merozoite surface antigen (MSA)1 and (MSA) 2 and a dimorphic region of the erythrocyte binding antigen (EBA-175) revealed 9/42 (21.4%) SGI and 33/42 (78.6%) of MGI in symptomatic children. Cytoadherence varied from 58 to 1,811 PRBCs/mm^2 of HLEC for SGI and from 5 to 5,744 PRBCs/mm^2 of HLECs for MGI. There was no significant difference between mean cytoadherence in SGI (1021) and MGI (1028). No association was observed between cytoadherence levels and severe malaria (p=0.92). However, the K1 genotype of the MSA-1 locus was observed in 82% of isolates from individuals with severe malaria. These results showed that cytoadherence to HLECs was not influenced by the multiplicity of clones in *P. falciparum* infection and confirmed field evidence of an association between severe malaria and a specific genetic characteristic of the parasite.

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IN VITRO CULTURING OF PAPUA NEW GUINEAN *P. VIVAX* FIELD ISOLATES USING UMBILICAL CORD RETICULOCYTES

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Plasmodium vivax (Pv) has proven difficult to grow in culture and therefore thorough study of this parasite has been limited. Nine years ago, using the Pv Chesson strain, Golenda *et al.* successfully improved existing short term Pv culturing techniques by periodic supplementation with reticulocytes obtained from a hemochromatosis patient. Blood samples from this donor (3-5% reticulocytes) were processed by ultracentrifugation to enrich reticulocytes to a final working concentration of 10%. This technique, while useful, is labor intensive and relies upon frequent donation of blood from a patient with an uncommon blood disorder. In PNG Pv is endemic. To facilitate *in vitro* Pv culturing umbilical cord blood from post partum mothers (3-8 % reticulocytes) provides an ideal alternative source of reticulocytes to supplement *P. vivax* cultures. Furthermore, we have developed a technique that further enriches reticulocytes harvested from cord blood to 70%. In our studies we observed that a one-time addition of 20 uLof the reticulocyte solution at the initiation of cultures in 2 mLs McCoy's 5A Medium +20 % Human AB serum at a 5% hematocrit (Pv starting parasitemia.18%-.68%) enabled significantly longer (p=0.002) Pv viability (8 days, ± 0) compared to cultures initiated in media alone (4.25 days, ± 0.95). These results suggest that regular supplementation of Pv cultures will allow us to extend *in vitro* Pv cultures for multiple rounds of blood-stage replication. These studies will enable us to evaluate mechanisms of Pv erythrocyte invasion and drug susceptibility.

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INCREASED SEVERE ANEMIA IN HIV-1-EXPOSED AND HIV-1-POSITIVE INFANTS AND CHILDREN DURING ACUTE MALARIA

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Since the primary hematological complication in both pediatric HIV-1 and malaria is anemia, co-infection with these pathogens may promote life-threatening severe malarial anemia (SMA). The primary objective of the study was to determine if HIV-1 exposure [HIV-1(exp)] and/or HIV-1 infection [HIV-1(+)] increased the prevalence of SMA in children with acute malaria. The effect of HIV-1 exposure and HIV-1 infection on the prevalence of SMA (hemoglobin less than 6.0 g/dL), parasitemia (parasites/µL), high density parasitenia (HDP, 10,000 parasites/µL or greater) was investigated in children < 2 years of age presenting at hospital with acute *Plasmodium falciparum* malaria in a rural holoendemic malaria transmission area of western Kenya. Upon enrollment, a complete hematological and clinical evaluation was performed on all children. Malaria parasitemia was determined and children with acute *P. falciparum* malaria were evaluated for HIV-1 exposure and infection using two rapid serological antibody tests and HIV-1 DNA PCR, respectively. Relative to HIV-1(-) group (n=194), the HIV-1(exp) (n=100) and HIV-1(+) (n=23) groups had lower hemoglobin concentrations (P<0.001 and P<0.001, respectively), while parasitemia and HDP were comparable between the three groups. Multivariate analyses, controlling for age, gender, and sickle-cell trait demonstrated that the risk of SMA was elevated in HIV-1(exp) children (odds ratio, 2.17; 95% CI, 1.25-3.78; P<0.01) and HIV-1(+) children (odds ratio, 8.71; 95% CI, 3.37-22.51; P<0.0001). The multivariate model further revealed that HIV-1 exposure or infection were not significantly associated with HDP. Results presented here demonstrate that both HIV-1 exposure and HIV-1 infection are associated with increased prevalence of SMA during acute *P. falciparum* infection, independent of parasite density.

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MOLECULAR BASIS OF *PLASMODIUM FALCIPARUM* RECEPTOR BAEBL FOR BINDING TO ERYTHROCYTE LIGAND GLYCOPHORIN C

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Plasmodium falciparum invades human erythrocytes by redundant pathways. Unlike *P. vivax* that has one Duffy Binding-Like (DBL) receptor, *P. falciparum* has four members of the DBL receptor family. Furthermore, one of these DBL genes, BAEBL, has polymorphisms at four amino acids in region 2, the receptor region of the protein; each polymorphism binds to a different red blood cell (RBC) ligand. One BAEBL variant (VSTK) binds specifically to erythrocyte glycophorin C. BAEBL (VSTK) is the only one that had threonine at amino acid 121 (T121) in place of K or R. We modeled the structure of region 2 of BAEBL (VSTK) on the crystal structure of a related DBL receptor, EBA-175. Four charged amino acids, Arg 52, Arg 114, Glu 54 and Asp 125, are predicted to surround T121 on the model of BAEBL (VSTK). They were individually mutated to alanine and expressed on the surface of CHO cells. The wildtype binds poorly to Gerbich negative cells that have a deletion of exon 3 in glycophorin C. In contrast, the mutations in arginine 52 or 114 caused reduced binding to normal RBCs and had similar binding to Gerbich negative RBCs. Mutations of glutamic and aspartic acid had no effect on binding, that is, they still bound normal RBCs at the same efficiency and have markedly reduced binding to Gerbich negative RBCs. These findings suggest that the two arginine residues surrounding T121 are critical for RBC binding and may be critical for sialic acid binding

(ACMCIP Abstract)

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REDUCED STEM CELL GROWTH FACTOR PRODUCTION IS ASSOCIATED WITH THE DEVELOPMENT OF CHILDHOOD SEVERE MALARIAL ANEMIA

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In holoendemic *Plasmodium falciparum* transmission areas, such as sub-Saharan Africa, severe malarial anemia (SMA) is the leading cause of morbidity and mortality in malaria-infected individuals. The underlying causes of SMA are multifactorial and include direct and indirect destruction of parasitized and non-parasitized red blood cells (RBC), inefficient erythropoiesis, and dyserythropoiesis. Upon resolution of a malaria infection, new RBC production is essential for recovery from SMA. Although many soluble factors, such as erythropoietin, increase the erythropoietic response, it is currently unclear if decreased production of these factors contributes to the development of SMA. Stem cell growth factor (SCGF), a recently discovered hematopoietic growth factor, possesses burst-promoting activity for human bone marrow erythroid progenitors. Since no study to date has reported a role for SCGF in malaria, circulating SCGF levels were determined in children with varying severities of malarial anemia (n=128), and the relationship between SCGF, hemoglobin (Hb) concentrations, reticulocyte production index (RPI), and parasitemia were examined. Children with SMA (Hb <6.0 g/dL) had reduced circulating SCGF that was positively correlated with Hb levels (r=0.213 P=0.019) and the RPI (r=0.316 P=0.016). SCGF was not significantly associated with parasitemia. Peripheral blood mononuclear cells (PBMC) from children with malaria cultured under baseline and stimuli-induced conditions further revealed that children with SMA produced lower SCGF under baseline conditions (P<0.05). In vitro experiments with PBMC from healthy, malaria-naïve U.S. adults demonstrated that malarial pigment (hemozoin, pfHz) and synthetic Hz decreased SCGF transcript expression (P<0.05). Moreover, circulating SCGF in children with malaria was negatively correlated with the number of pigment-containing monocytes (r=-0.325 P=0.013). Taken together, these results illustrate that naturally acquired pfHz decreases mononuclear cell SCGF production and that reduced SCGF may be an important hematopoietic factor that contributes to the development of SMA.

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REAL-TIME BEDSIDE MEASUREMENT OF NITRIC OXIDE DEMONSTRATES IMPAIRED PRODUCTION IN ADULTS WITH SEVERE MALARIA IN PAPUA, INDONESIA

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Nitric oxide (NO) has been associated with protection from severe malaria in both children and adults. However, NO has a half-life of seconds and studies of the role of NO in malaria have been hampered by the difficulty in measuring production during disease. Most studies have measured concentrations of NO metabolites in body fluids. The results of many of these studies have been difficult to interpret because of the failure to control for dietary nitrate ingestion, nitrate retention in renal failure and perturbations in fluid volumes. Studies that have controlled for these variables have shown reduced levels of NO metabolites in severe disease. Other studies have shown impaired expression of NO synthase in circulating mononuclear cells in severe malaria. However, to date, there have been no real-time direct measurements of NO itself in malaria. In this longitudinal study conducted at Mitra Masyarakat Hospital in Timika, Papua province Indonesia, adult patients (age 18-60 years) with uncomplicated and severe malaria underwent serial bedside measurement of exhaled NO in parts per billion (ppb) using the NIOX apparatus and American Thoracic Society Guidelines. Measurement required the ability to sit and to cooperate with the exhalation technique and was not possible in subjects with cerebral malaria or prostration. Baseline measurements were possible in 60 patients with moderately severe malaria (patients requiring inpatient parenteral therapy but without WHO manifestations of severe malaria) and 12 with modified WHO criteria for severe malaria. Median exhaled NO was lower in severe malaria [10.5 ppb (IQR: 9.5-15.0)] than moderately severe malaria [18.5 ppb (IQR: 11.1-26.9)]; p=0.03. By 48 hours, exhaled NO in patients with severe disease had increased to levels comparable to those found in healthy controls (median 16.6 [IQR: 11.9-27.0]). Real time bedside measurement of exhaled NO allows direct measurement of NO production in malaria, and demonstrates impaired production in patients with severe malaria compared to those with moderately severe malaria. Results are consistent with a protective role for NO in malaria. Measurement of exhaled NO has potential utility in evaluating interventions targeting increased NO production in severe malaria.

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PYRUVATE KINASE DEFICIENCY PROTECTS AGAINST *PLASMODIUM FALCIPARUM* MALARIA

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In malaria-endemic regions ***Plasmodium falciparum*** infection constitutes a great challenge for the population. Epidemiological studies have demonstrated a correlation between malaria disease and genetic mutations, in particular, patients with hemoglobinopathies were shown to be protected against severe malaria. Two principal mechanisms have been postulated to explain this observation: first the inhibition of invasion and growth of parasites within mutated erythrocytes (E), and second the enhanced clearance of parasitized mutant E. After glucose-6-phosphate dehydrogenase deficiency, Pyruvate kinase deficiency (PKD) is the most frequent enzyme abnormality of the glycolytic pathway, and the most common cause of hereditary non-spherocytic hemolytic anemia. Recently PKD in mice was shown to be protective against malaria. Whether PKD protects against ***P. falciparum*** remains unknown. We show here using two strains of ***P. falciparum*** that the parasite invaded less PKD E than normal E. We also found that membrane-bound hemichromes, IgG and complement C3c fragments bound, and phagocytosis by human and mouse monocytes/macrophages were higher in ring-stage in PKD E than in normal E. Nevertheless, when ***P. falciparum*** parasites invade PKD E their growth is similar as in normal E. Reduced invasion and enhanced uptake of ring-parasitized PKD E may low the parasite load in the blood of PKD patient and protect them against severe malaria.

(ACMCIP Abstract)

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EFFECT OF SUPHADOXINE-PYRIMETHAMINE ON ANTIOXIDANT DEFENSE SYSTEM AND LIPID PEROXIDATION

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Due to the spread of resistance to chloroquine by ***Plasmodium falciparum***, Sulphadoxine-pyrimethamine (SP) became a cheap alternative drug of choice for the treatment of malaria in most endemic areas. Production of oxygen radicals forms part of the host defense and pathology of malaria. Exacerbation of intra-erythrocytic oxidative stress might contribute to the process of elimination of the parasites. The effect of treatment with SP on the antioxidant defense system was investigated using rabbit as a model.

Ten male rabbits were divided into two groups of 5 animals each. The first group was administered with normal saline and served as control. The second group received a single dose of SP (26.25mg/kg body weight). Blood samples were collected before and at 6, 12 and 24 hr after saline or drug administration. Activity of cellular enzymic antioxidants, superoxide dismutase (SOD) and catalase (CAT), and reduced glutathione(GSH) were assayed using standard photometric methods. Serum lipid peroxidation was assessed by the formation of malondialdehyde while protein content was assayed by the method of Lowry.

SOD activity was observed to increase progressively by 4.9, 63.4 and 120.8% at 6,12 and 24 hr respectively after drug administration. Similarly, CAT activity increased by 44.5, 82.6 and 116.3% at 6, 12 and 24hr respectively. Malondialdehyde levels also significantly increased by 45.5, 118.2 and 186.4%. However, the activity of GSH was observed to have decreased by 41.9% by 6hr and remained so till the 12th hour, but by 24 hours after drug administration, the activity has increased significantly up to 48.4% above the 0hr level. SP treatment altered the enzymatic antioxidant defense system and lipid peroxidation in blood and therefore could induce oxidative stress. The increase in SOD activity is an indication of generation of reactive oxygen species.

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DISRUPTION OF C5 CONFERS RESISTANCE TO CEREBRAL MALARIA

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Host genetic factors are important in determining susceptibility to cerebral malaria (CM) in both humans and in mouse models. Infection of known resistant and susceptible inbred mouse strains with *Plasmodium berghei* ANKA provides a good model system to identify host genetic determinants that regulate the development of CM. Complement component 5 (C5), which sits at the crossroads of the classical and alternate pathways of the complement system, has been implicated in susceptibility to other infectious diseases. Since activated C5 (C5a) participates in pro-inflammatory cascades, we hypothesized that it may contribute to the inflammatory cytokine-associated encephalopathy that characterizes human and murine CM and that C5 deficiency would confer protection. To examine this hypothesis, 5x10^5 *P. berghei* ANKA parasites were injected into a panel of mouse strains, mice were genotyped at the C5 locus and were monitored for survival and parasitemia. Infected mice were found to exhibit significantly different survival curves and could be divided into four groups: very susceptible (e.g. 129SV), susceptible (e.g. C57BL/6 or B6), resistant (e.g. A/J) and very resistant (e.g. AKRJ); however, all susceptible and very susceptible mice were wild type (WT) at the C5 locus. Transfer of the C5-defective allele from A/J onto a susceptible B6 genetic background conferred resistance to CM. Conversely, a congenic A/J strain containing the WT C5 allele from B6 mice were susceptible to CM. Additionally, in the closely related B10.D2/NsnJ and OsnJ, which differ only at their C5 locus, the C5 deficient mice had higher survival rates and lower parasitemias than WT mice. These data provide direct evidence that C5 contributes to the development of CM in the *P. berghei* ANKA model, suggesting a role for C5 and complement pathways in human CM.

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MICROFLUIDIC PLATFORMS FOR MALARIA PATHOGENESIS

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Severe malaria caused by the parasite *Plasmodium falciparum* is a potentially fatal disease, in part due to the failure of host organs brought about by the accumulation of parasitized red blood cells in the microvasculature. Severe disease symptoms are associated with capillary blockage due to the loss of deformability of infected erythrocytes, their sequestration to endothelial cells and their interaction with the host immune system. Despite much progress in understanding disease pathophysiology from postmortem autopsies and *in vitro* adhesion assays, there remains a need for experimental systems to study the pathogenesis of the disease in a controlled, multicellular environment that closely mimics capillaries. We show here that microfluidic devices with dimensions similar to those found in real capillaries can be engineered to grow and support both host cells and infected red blood cells under conditions of continuous fluid flow for time periods of up to 24 hours. Using these devices, we have been able to measure novel characteristics of sequestration of parasitized RBCs to cells and recombinant proteins such as CD36 and ICAM-1 in channels resembling narrow capillaries, and phagocytosis of infected red blood cells that are continuously exposed to fluid shear forces. The development of these microfluidic tools that mimic capillaries in the body can therefore shed light on how infected RBCs interact with different host cells *in vivo*. The devices also have the potential to be a valuable, portable research tool in malaria endemic areas.

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OPTIMIZATION OF A HEPATOCYTE CULTURE SYSTEM FOR *IN VITRO* SCREENING OF COMPOUNDS AGAINST LIVER STAGES OF *P. FALCIPARUM* AND *P. VIVAX*

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After mosquito injects malaria sporozoites into human body, the parasites firstly invade liver cells to proliferate and subsequently invade red blood cells. Advancement of knowledge regarding liver stage parasite development has been slower than in other developmental stages due to the lack of a simple *in vitro* or *in vivo* model. Development of effective antimalarial drugs against liver stages of human malaria will require an efficient *in vitro* system because this cannot be done in humans. We have established a new human hepatocyte line, HC-04, that supports the exo-erythrocytic (EE) development of both *Plasmodium falciparum* and *P. vivax*. To establish an *in vitro* system for screening of new compounds against liver stage malaria, this system is being optimized to increase sporozoite invasion and production of key proteins/enzymes in the hepatocyte cell line. Different culture conditions and parasite preparation methods have been compared. We have measured levels of different forms of CYP450 that are involved in drug metabolization and major protein production such as albumin, etc., using RT-PCR and quantitative PCR. *Plasmodium falciparum* or *P. vivax* sporozoites were inoculated into the HC-04 cell line and cultured under three different culture media, *i.e.* MEM/F-12, Hepatocyte Culture Medium (HCM, Cambrex), or DMEM/F-12 supplemented with Hepatocyte Growth Factor (HGF). Identification and quantification of the parasite development was accomplished using microscopic examination, quantitative PCR, and measurement of fluorescence of FITC tagged parasites using HIV-1 TAT protein-transduction domain. We also compared the gene expression profiles of major liver proteins and drug metabolizing enzymes among different cell passages and among parasite infected and non-infected cells using RT-PCR. This model will be further validated for *in vitro* screening of compounds against liver stages of *P. falciparum* and *P. vivax* once the system is optimized.

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EMERGENCE OF NEW GENOTYPES AND INCREASES IN DOMINANT GENOTYPE COPY NUMBER ARE ASSOCIATED WITH DEVELOPMENT OF SYMPTOMATIC MALARIA IN THE VILLAGE OF MISSIRA, MALI

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Previous PCR-based studies of polymporphic loci, such as Block 2 of merozoite surface protein 1 (*msp1*), have shown that *Plasmodium falciparum* infections have greater complexity in areas with high transmission intensity. In order to further study the role infection complexity plays in disease, we followed a cohort of children from 2 to 10 years of age with monthly filter paper blots using a combination of real-time PCR based on Block 2 of *msp1* (allotype-specific copy number) and capillary electrophoresis (genotype identification based on amplicon size) to define the genotypes present and the copy numbers for each genotype. To test the hypothesis that changes in parasite genotype and genotype copy number were associated with the development of disease, we identified 29 subjects who developed disease between September and November 2005 (symptomatic malaria = positive smear for asexual *P. falciparum* parasites plus fever, headache or other symptoms or signs of uncomplicated malaria) following an asymptomatic infection (positive smear, absence of clinical symptoms or signs), within a cohort of 401 children. In terms of genotype, 15 of the 29 children who developed disease had a new dominant genotype at the time of their illness, while 14 retained the same dominant genotype ($p > 0.05$). In terms of copy number, 16 of the 29 subjects who developed disease had a ≥ 50% increase in the copy number of the dominant genotype ($p > 0.05$). However, 24 of the 29 subjects either developed a new dominant genotype OR had a ($p = 0.001$). These results suggest that development of disease in areas with high transmission may be driven both by infection with new genotypes and by increases in the copy number of the dominant genotype.

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PYRUVATE KINASE DEFICIENCY PROTECTS AGAINST *PLASMODIUM FALCIPARUM* MALARIA

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A number of epidemiological studies have reported a correlation between malaria-exposure and selection for a variety of genetic erythrocyte disorders. In particular, several hemoglobinopathies have been reported to protect against severe and fatal malaria. Two principal mechanisms have been postulated to explain this observation: the inhibition of invasion and growth of parasites within altered RBCs and second, enhanced clearance of parasitized variant RBCs. After glucose-6-phosphate dehydrogenase deficiency (G6PDH), pyruvate kinase deficiency (PKD) is the most commonly recognized erythrocyte enzymopathy. Recent work by Min-Oo, et al. demonstrated that PKD is protective against severe and fatal malaria in a murine model (Min-Oo et al, Nature Genetics, 2003). However, whether PKD might be protective against *Plasmodium falciparum* malaria is unknown. In this study we investigated *Plasmodium falciparum* invasion, intracellular growth, and clearance of infected PKD RBCs versus normal erythrocytes. We demonstrate, using multiple *Plasmodium falciparum* clones, that there is an invasion defect for PKD RBCs compared to normal erythrocytes (invasion: 3.7±2.7 vs 10.8±9 %, $p = 0.0002$). We also show significantly increased membrane-bound hemichromes (0.162 vs 0.038 nmol/ml RBC membrane), IgG (0.669±0.011 vs 0.097±0.02 absorbance unit/min/10^7 RBCs, $p < 0.001$) and complement C3c fragments (1.66±0.017 vs 0.125±0.005 absorbance unit/min/10^7 RBCs, $p < 0.001$) on infected PKD RBCs as well as increased phagocytosis by human and mouse monocytes/macrophages of ring-stage PKD RBCs compared to infected normal RBCs (phagocytic index 29.9±8.3 vs 2.1±0.44 %, $p = 0.003$). Reduced invasion and enhanced uptake of ring-stage parasitized PKD RBCs may contribute to a lower parasite burden in PKD patients and confer protection against severe malaria.

(ACMCIP Abstract)

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RELATIONSHIP BETWEEN THE NEUROTOXICITY AND PHARMACOKINETIC PROFILES OF ARTEMISININ DERIVATIVES IN ANIMAL SPECIES

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Artemisinin has been identified as the active antimalarial component of qinghaosu, a plant used for centuries to treat malaria in China. Artemisinin, and its analogues dihydroartemisinin (DHA), artemether (AM), arteether (AE), artesunate (AS), and artelinate (AL), are highly effective against the erythrocytic stages of *Plasmodium falciparum in vivo* and *in vitro*. In the past decade, *in vivo* toxicity studies have showed a dose-dependent neurotoxic effect associated with movement disturbances, spasticity, brain tissue damage, and even death in animal species with the

oil-soluble artemisinin derivatives AM and AE. In contrast, few neurotoxic effects have been shown to be induced by the water-soluble artemisinins AS and AL0. Early studies have never induced any CNS side-effects in animal species after intravenous injection of AS or AL. To date, however, neurotoxicity has not been convincingly demonstrated in humans treated with any of the artemisinins. Pharmacokinetic profiles demonstrate that fatal neurotoxicity was caused by the oil-soluble artemisinins (sesame oil vehicle) as the drug depot in the intramuscular injection sites resulted in a long drug exposure time due to the slow and prolonged absorption in the muscle and accumulation in the blood. The mild and light toxicities induced by the water-soluble agents in contrast were found to be a result of their very short half-lives and lack of accumulation. In conclusion, drug exposure time has been demonstrated to play a more important role in producing neurotoxicity than drug exposure level, production of the more active metabolite (DHA), or having the drug located in the CNS system. Therefore, appropriate dose regiments with the correct formulations are necessary in avoiding neurotoxicity in humans and in animal species.

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POTENTIAL TOXICITY OBSERVED IN CHILDREN WITH ACUTE UNCOMPLICATED FALCIPARUM MALARIA WHILE ON TREATMENT WITH INCREASED DOSES OF CHLORPHENIRAMINE PLUS CHLOROQUINE COMBINATION

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This study was uncertaken to compare the safety of two regimens of chlorpheniramine plus chloroquine combination in the treatment of acute uncomplicated falciparum malaria in children. Ninety-nine children aged, 0.5 - 14 years, with acute uncomplicated malaria were randomised into two treatment groups. Forty-eight children were allotted to the high dose chlorpheniramine-chloroquine group and 51 children to the higher dose chlorpheniramine-chloroquine group. The children in the high dose group received 14 - 20 mg of chlorpheniramine daily for 7 days in combination with chloroquine 30mg/kg orally over 3 days while the higher dose group received, 20 - 28 mg of chlorpheniramine daily for 7 days in combination with chloroquine 30mg/kg orally over 3 days. Outcome measures were vital signs, clinical response and parasite clearance all of which were monitored on days 0-7 and day 14. The vital signs though showed no significant differences in both groups with respect to the pulse, systolic and diastolic blood pressure, the respiratory rate was significantly lower in the higher dose group relative to the high dose group on day 2 (28 ± 4 versus 33 ± 7 cycles/min, p=0.004). Drowsiness was also commoner in the higher dose group. The measures of therapeutic response showed no significant differences in the two treatment groups. The parasite clearance time was 2.8±0.7 days and 2.9±0.7 for the high dose and the higher dose group respectively (p=0.58), fever clearance time was 1.4±0.7 and 1.3±0.7 days respectively for the high dose and the higher dose group (p=0.68) and cure rate was 95.8% and 94.1% respectively (p=0.94). In conclusion, these data suggest that even though these combinations of chlorpheniramine with chloroquine may be generally safe and effective, the higher dose chlorpheniramine -chloroquine had no therapeutic advantage over the high dose. However, the lower respiratory rate observed in the higher dose chlorpheniramine plus chloroquine combination calls for caution in the clinical application of the combination.

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IN VITRO PHARMACODYNAMICS OF PYRIDONE DERIVATIVES

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4(1H)-pyridone derivatives are a family of antimalarial agents that act as potent selective inhibitors of *Plasmodium falciparum* mitochondrial function by blocking the electron transport chain. These derivatives show nanomolar IC_{50} against *Plasmodium falciparum* and have also been proven efficacious in mouse models of malaria. Although *in vitro* IC_{50s} and IC_{90s} are excellent predictors of the potency of an antimalarial, they provide essentiallly no information on the time course of antimalarial activity and on wether killing can be increased by higher drug concentrations. In order to determine the pharmacodynamic properties of pyridones, time-kill studies were performed with representative members of this family. For these experiments we used the 3D7A and FCR3 *P. falciparum* strains. Artemisinin, atovaquone, chloroquine and pyrimethamine were included as representative drugs with different rates of action. IC_{50s} were determined by the standard 48hrs *in vitro* isotopic method. Pyridone derivatives showed IC_{50} values from 1 to 10 ng/ml. Time-inhibition curves were performed at concentrations equivalent to 10, 30, 100, 300 and 1000 times the corresponding IC_{50} values. At predetermined time points, aliquots were taken, the corresponding drug was removed and the cultures were tested for [^{3}H]hypoxanthine incorporation. Experiments were performed in duplicate. In all the cases, pyridone derivatives demonstrated time-dependent antimalarial activity. Inhibition of parasite growth increased gradually with the time of exposure, reaching 90 % inhibition after 15-24hrs of treatment. Saturation of the inhbition rate occurred by ten times the IC_{50}, drug concentrations above these values do not inhibit parasites significantly faster or more extensively. According to this pattern of inhbition, the goal of a dosing regimen for pyridones would be to optimize the time that serum levels exceed some minimal value such as the IC_{90}. Further *in vitro* and *in vivo* PK/PD studies will be required to demonstrate this point.

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POPULATION PHARMACOKINETICS OF MEFLOQUINE FOR MALARIA PROPHYLAXIS IN AUSTRALIAN SOLDIERS

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The population pharmacokinetics (PK) of mefloquine (MQ) were determined in Australian soldiers on peace-keeping duties in East Timor following weekly prophylaxis with MQ (*Lariam*). Data was obtained from two clinical trials; a double-blinded randomised study (group A), and an open-label study (group B). Following a loading dose (250 mg MQ daily for 3 days for group A, and 250 mg MQ every second day on 3 occasions for group B), the subjects received an oral weekly maintenance dose of 250 mg MQ for 6 months. The PK study consisted of 1111 soldiers (group A: 162 subjects; group B: 949 subjects) who had a mean (range) age of 26 years (18-55) and weight of 82 kg (52-135). Blood samples were collected after the last loading dose, in weeks 4, 8, and 16 for group A, and in weeks 13 and 26 for group B. HPLC was used to measure plasma MQ concentrations. Population PK modelling was performed using NONMEM. A linear, two-compartment model with first-order absorption and interoccasion variability (IOV) on the clearance of MQ best described the data. The typical population PK parameter values were as follows: clearance (CL/F): 2.09 L h^{-1}; central volume of distribution (V/F): 528 L; absorption rate constant: 0.240 h^{-1}, inter-compartmental clearance (Q): 12.5 L h^{-1}; peripheral volume of distribution (Vp): 483 L; terminal half-life: 14.0 days. Body weight had a small proportional influence on V/F but was insufficient to warrant any alteration to dosing. The interindividual variability (coefficient of variation, CV%) for CL/F and V/F was 24.4% and 29.6%, respectively. The IOV for CL/F was 17.8%. The proportional residual unexplained variability component (CV%) for groups A and B was 11.5% and 19.5%, respectively, while the additive component (SD) was 57 µg L^{-1} and 149 µg L^{-1}, respectively. This is the first population pharmacokinetic model that describes the disposition of mefloquine when