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HOW LONG DO BEDNETS LAST? EVALUATION OF BEDNETS RETRIEVED FROM NORTHWEST GHANA AFTER 38 MONTHS OF HOUSEHOLD USE

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In December 2002, a district-wide bednet campaign distributed PermaNets®, DawaNets®, and conventionally-treated ITNs in the Lawra District of northwest Ghana. During a follow-up survey in February 2006, 250 of these nets were collected in order to evaluate their physical integrity, insecticide retention, and bioactivity. Holes having >1cm diameter were commonplace as were failures in the stitching between the top and sides of the bednet. Although repairs were common, smaller holes tended to be neglected. Complete analysis of physical condition, insecticide levels, and bioactivity will be reported in order to provide manufacturers and other investigators with guidance for future improvements in bednet textile design and treatment development.

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INSECTICIDE TREATED BEDNETS FOR THE CONTROL OF DENGUE VECTORS IN HAITI

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Dengue fever is currently the fastest spreading arboviral disease worldwide. In the absence of a vaccine, *Aedes aegypti* vector control remains the most effective strategy to prevent transmission. Although the protective efficacy of insecticide-treated bednets (ITNs) has clearly been shown with respect to malaria and other nocturnally-transmitted infections, effects on dengue vectors have not been evaluated. A cluster randomized trial in 18 clusters comprising a total of 1000 households in Leogane, Haiti in 2003-4 assessed the impact of insecticide treated bednets on the local dengue vector population. The effect was significant: not only did the bednets greatly reduce peridomestic dengue vector breeding (Breteau Index dropped from 15.9 to 6.4, $p<0.001$), but a serology survey showed that dengue IgM seroconversion rates dropped significantly (from 33.7% to 18.5%, $p<0.001$), 12 months after ITN deployment. A community-wide effect was also suggested, with dengue vector breeding also reduced in neighbouring control areas that had not received bednets. The results indicated that insecticide treated bednets might additionally prevent dengue transmission in areas where ITN use is promoted for control of other diseases, notably in those urban areas where both dengue transmission and ITN usage are high.

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THE USE OF PYRIPROXYFEN AS A CONTROL AGENT FOR *AEDES AEGYPTI* IN PERU

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Laboratory and field comparisons of larvicides in Peru (in the Amazon city of Iquitos and the coastal capital Lima) show that pyriproxyfen is an extremely effective choice for *Aedes aegypti* control. A series of trials using recommended doses (50 ppb a.i.) in large storage tanks with high turnover rates of water showed that it can prevent adult emergence for longer periods (>95% mortality for 6 months) than any of the other larvicides (temephos, methoprene, Bti) that we tested. We also present field data on its effect on other mosquitoes (Culex and Anopheles species), biting midges (Ceratopogonidae) and non-target aquatic arthropods in experimental conditions relevant to the tropics. Using results from laboratory and semi-field tests, we show that it is possible to use pyriproxyfen as an "egg sink" (oviposition traps treated with 50 ppb did not produce any viable adults for a six month period despite 1000s of eggs being laid in those containers), and as an agent for sterilizing female adult mosquitoes (mosquitoes resting on surfaces treated with 0.0125 g/m^2 exhibited 75% decreases in fecundity). Moreover, female adults that rested on those surfaces could transfer pyriproxyfen to other breeding sites and affect immature stages developing therein (80% pupal mortality was observed in some sites). We conclude that pyriproxyfen is a cost effective alternative to the larvicides currently in use in Peru, and that it can be used in the wider environment without undue risk. More speculatively, we suggest that it can be used in combination with ovitraps and resting traps to effect a more targeted means of mosquito control.

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SYSTEMATIC ANALYSIS OF PIGGYBAC STABILITY IN YELLOW FEVER MOSQUITOES, *AEDES AEGYPTI*

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PiggyBac based gene vector has been widely used for germline transformation in wide range of animals including insects of nearly 20 species. However, the post integration behavior of this gene vector is not elucidated in detail except in *Drosophila*. The post integration behavior will mainly determine the utility of this gene vector in future applications. Stability of integrated vectors is of major concern for biocontrol programs and medical applications. Applications like transposon mutagenesis, enhancer trapping and genetic drive systems required high rates of remobilization. Our experiments showed that the post integration behavior of *piggyBac* in *Aedes aegypti* is entirely different from its behavior in *Drosophila*. In the present study, we confirmed the long term stability of non-autonomous piggyBac element in soma (i) by direct injection of functional transposase containing plasmids into the embryos of non autonomous transgenic *Aedes piggyBac* single insert containing lines created in our lab and also used lines created in different labs and (ii) by creating heterozygous individuals by mating above mentioned non autonomous *piggyBac* lines with five different functional transposase containing transgenic lines. *Drosophila piggyBac* transgenic lines were used as control in both experiments for comparison. Germline stability was also confirmed in detail by using large scale screening of nearly 37000

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individuals and Transposable Element Display of about 350 individuals which showed eye color variation and possible transgene remobilization. Further analysis of stability showed no involvement of DNA methylation or silencing by interfering RNA. The causes of somatic and germline stabilization of integrated *piggyBac* elements remain unexplained.

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LINKAGE DISEQUILIBRIUM MAPPING OF INSECTICIDE RESISTANCE LOCI IN *ANOPHELES GAMBIAE*

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Linkage disequilibrium mapping involves testing for statistical independence between molecular markers and a defined phenotype. The principle underlying the approach is that genome regions statistically associated with the phenotype may contain a gene or genes that condition that phenotype. Our phenotype of interest is resistance to the major classes of insecticides that are used for the control of the malaria vector *Anopheles gambiae*. We have classified samples for susceptibility to pyrethroid and organochloride insecticides from sites in Ghana, Kenya and São Tome and Principé. We identified molecular markers (SNPs and microsatellites) that are clustered around known insecticide detoxification enzymes (e.g.Cytochrome P450s, GSTs and Esterases) or insecticide target sites (e.g. Sodium channel). Using this clustered approach we have identified loci that are significantly associated with insecticide resistance phenotypes. One of these loci is physically close to a cluster of cytochrome P450s on the right arm of chromosome 3. On the premise that metabolic resistance is usually a result of up regulation rather than allelic variation in detoxification enzymes we are investigating variation in *cis* acting regulatory factors.

(ACMCIP Abstract)

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ESTIMATES OF SELECTION PRESSURE ON AN INSECTICIDE RESISTANCE LOCUS: SNP ANALYSIS OF THE VOLTAGE-GATED SODIUM CHANNEL GENE IN *ANOPHELES GAMBIAE*

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Insecticide treated bednets have become the mainstay of malaria control programmes. To date, pyrethroids are the only class of insecticides approved for the treatment of nets. However, pyrethroid resistance in the form of the target site insensitivity mutation, *kdr*, is already widespread in *Anopheles gambiae* in Africa and could threaten the success of the future ITN based control programmes that remain solely reliant on this group of insecticides. Two separate *kdr* alleles have been described in *Anopheles gambiae:* one initially seen in West Africa, a leucine to phenylalanine amino acid substitution, and the other in East African populations, a leucine to serine change. Recently, all three alleles were detected in a Gabonese sample suggesting that the resistance mutations may be more widespread than previously thought. Whether this is a result of mutations becoming more widespread or a failure of earlier surveys to accurately genotype populations is unknown. To predict the spread of the *kdr* alleles, it is important to know the force of the selection pressure acting upon those mutations and the associated fitness costs. We identified and assayed exonic SNPs around the *kdr* locus in samples from populations with different *kdr* resistance allele frequecies that are exposed to differing insecticide selection pressure. By estimating the strengths of genomic hitch-hiking events associated with all three mutations, we may infer the relative age of the mutations and the strengths of the selection pressure to which they are subject. The relevance of these findings to predicting and controlling the spread insecticide resistance will be discussed.

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INVASIVE BACTERIAL INFECTIONS AMONG 0- TO 35-MONTH OLD CHILDREN TREATED AS OUTPATIENTS AT A PEDIATRIC REFERRAL CENTER IN BAMAKO, MALI

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Since 2002, we have been conducting surveillance for suspected invasive bacterial infections (IBI) among hospitalized children in Bamako, Mali. Only the most gravely ill children are hospitalized, as evidenced by the high mortality rates (14% of febrile hospital admissions die), suggesting that these data under-estimate the true incidence of IBI. Consequently, we extended surveillance to include ambulatory children evaluated in the Emergency Department (ED) and discharged home. We offer enrollment to children 0- to 35-months old with fever ($\geq$ 39°C) or suspected IBI who are evaluated in the ED of Hôpital Gabriel Touré (HGT), the major children's hospital in Bamako, and discharged home. A blood culture is performed on each child. Any other relevant body fluid collected by the treating physician is also cultured. If the culture is positive, the parent is notified and asked to bring the child for re-evaluation. From May 25, 2005 to September 30, 2005, 9283 children 0- to 35-months of age were evaluated in the HGT ED; 1300 of the 1301 study-eligibles were enrolled. Forty percent had fever alone and 60% had SIBI (+/- fever). A pathogen was isolated from 95 cases (7.3%) in cultures from blood (n=89), CSF (n=3), joint fluid (n=1), or cellulitis aspirate (n=1). The most common pathogens were *Streptococcus pneumoniae* (SP, 26%), non-typhoidal *Salmonella spp* (NTS, 23%), and *Haemophilus influenzae* type b (Hib, 21%). In comparison, the most common pathogens identified among inpatients are Hib (36%), SP (28%), and NTS (12%). At follow up, at least 6 bacteremic children who were sent home from the ED died (6.3%), including 4 infected with SP and 1 infected with Hib. In conclusion, these preliminary results indicate that ~1 in 15 Malian children 0- to 35-months old who is discharged from the ED after evaluation for high fever or suspected IBI is bacteremic and at considerable risk for death. Although the most common pathogens are similar to those found among hospitalized children in this age group, NTS is isolated more frequently (23% vs. 12%), and Hib less frequently (21% vs. 36%) in outpatients compared with inpatients.

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CHARACTERIZATION OF STREPTOCOCCUS PNEUMONIAE ISOLATED FROM DISSEMINATED DISEASE IN RURAL THAILAND

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Streptococcus pneumoniae commonly causes serious community-acquired pneumonia, often resulting in severe disseminated disease. The death rate of disseminated pneumococcal pneumonia is largely unchanged over the past 50 years despite considerable advances in diagnosis and treatment, with antibiotic resistance increasingly problematic. A heptavalent conjugate vaccine significantly reduces pneumococcal pneumonia in the population most at risk - children under the age of 5. In many countries, including Thailand, this vaccine is not widely used because of availability, cost, and lack of information on local disease burden. The goals of this

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study were to assess *in vitro* antibiotic susceptibility of recent isolates from patients with severe disseminated pneumococcal disease and determine the potential coverage of the 7-valent vaccine in this setting. *S. pneumoniae* were isolated from blood cultures collected from admitted patients at all 20 government hospitals in Sa Kaeo and Nakhon Phanom provinces from May 2005-May 2006. Identification was confirmed by conventional methods and isolates screened against chloramphenicol, clindamycin, cotrimoxazole, erythromycin, penicillin, and tetracycline by disk-diffusion with minimum inhibitory concentrations determined for resistant isolates. Serotyping was done by a previously validated PCR method. Of 36 unique isolates identified, 26 were from pneumonia cases, 6 from sepsis, and 4 from other severe disease. Eight were from children <5 years. Nine patients died. Among all isolates, 7% were resistant to chloramphenicol, 14% to clindamycin, 67% to cotrimoxazole, 25% to erythromycin, 39% to penicillin, and 56% to tetracycline. None were resistant to cefotaxime. Of the first 15 isolates serotyped, 11 (73%) were serotypes included in the existing 7-valent conjugate vaccine (4, 6B, 9V, 14, 19F, and 23F) and an additional 2 (13%) were vaccine-related serotypes (6A, 19A). In the isolates from children <5, 100% had serotypes in the 7-valent vaccine. In conclusion, *S. pneumoniae* isolates from severe disease are resistant to many antibiotics commonly used in this region. The current pediatric vaccine would provide significant protection in this setting.

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USE OF FRACTIONAL DOSE TETRAVALENT A, C, W135 AND Y MENINGOCOCCAL POLYSACCHARIDE VACCINE: A NON INFERIORITY TRIAL

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Since 2000, clusters of cases due to *Neisseria meningitidis* serogroup W135, or mixed outbreaks of serogroups A and W135, have posed serious challenges in vaccine choice for mass campaigns in Sub-Saharan Africa. The availability and affordability of vaccines protecting against W135 especially is a problem. Studies performed in the 1980's on healthy adult American volunteers have shown satisfactory immunological response with fractional doses of the polysaccharide vaccines. Thus, we explored the use of fractional doses of a licensed polysaccharide vaccine in an African population. A randomised, single-blind, non-inferiority trial was performed in Mbarara, Uganda, to compare the immunological response of the full dose (50µg) of the tetravalent Menomune® vaccine versus a fractional dose of 1/5 or 1/10 in healthy volunteers aged 2 to 19 years. Pre- and post-vaccination (4 weeks) sera were analyzed by serum bactericidal activity (SBA) using rabbit complement and IgG polysaccharide ELISA. In the non immune population prior vaccination, i.e. SBA titers<128, a responder was defined as showing 4-fold increase in SBA. Of 750 volunteers included, 291 received a full dose, 225 1/5 of the dose and 234 1/10 of the dose. For serogroup W135, 94% of the vaccinees in the 1/5 dose arm were responders and 97% in the 1/10 dose arm versus 94% in the full dose arm. For serogroup A, 92% of the vaccinees in the 1/5 dose arm were responders, 88% in the 1/10 dose arm versus 95% in the full dose arm. For both serogroup W135 (risk difference - 0.7% [-5.3%, 3.9 %]) and A (risk difference + 2.4% [-3.9%, 8.8%]), the non inferiority was demonstrated for 1/5 dose arm versus the full-dose group. For the 1/10 dose arm, the non inferiority was shown for serogroup W135 (risk difference -3.5% [-7.5%, 0.5%]but we could not conclude for serogroup A (risk difference + 6.3% [-0.01%, 13.3%]). The ELISA results were variable for the different polysaccharides and low correlation was seen between IgG titers and SBA responses. In conclusion, our study indicates that 1/5 dose of the licensed A/C/Y/W135 polysaccharide vaccine can confer a similar functional immune response as a full dose and be equally protective against serogroup A and W135 meningococcal disease. These results would enable an increase in the number of doses available and a lower

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A CASE OF BUBONIC PLAGUE IN URBAN LOS ANGELES

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No case of human plague has been reported in Los Angeles County (LAC) since 1984, but routine environmental surveillance in LAC foothills consistently detects animals with *Yersinia pestis* (YP). In April 2006 plague was diagnosed in a woman living in urban Los Angeles. LAC Public Health Laboratory tested the case YP isolate by DFA, PCR, and phage lysis. The case and family were interviewed repeatedly regarding potential exposures to animals and locations enzootic with YP; potential exposure sites were evaluated and animals were collected and tested for YP. PFGE analysis was done on human and animal YP isolates by Centers for Disease Control and Prevention. The isolate was confirmed as YP by DFA, PCR probes, and phage lysis test. Because the case denied any travel outside her urban area, sixteen persons who lived on the case premises were given antibiotic prophylaxis. Rodent traps inside and outside the home were empty. Two feral cats were trapped and tested negative for YP. Day trapping activities in a LAC park visited by the patient found 33 California ground squirrels; sera were negative for antibodies to YP. While initially denying outside activity, the husband later admitted he had hunted and skinned rabbits in the Mojave area of Kern County (KC). The patient handled the raw rabbit meat prior to cooking. Inspection of the KC hunting site revealed signs of a rabbit die-off. Surveillance yielded; 25 deer mice, two jack rabbits, and 5 rabbit carcasses. Five deer mice sera were positive by HA and one rabbit carcass was positive by FA and culture for YP. PFGE results showed that the rabbit and human isolates had indistinguishable patterns and were unique when compared with 363 unique patterns in our database representing over 1,100 PFGE entries. In conclusion, this case was likely caused by handling the carcass of an infected wild rabbit collected in the area of a recent plague epizootic. Repeated interviews may be needed to reveal risk factors when disease occurs in an unusual setting. Plague should be considered in persons who have visited or handled animals taken from enzootic areas. Public education regarding risk of plague in endemic areas is needed.

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TRENDS IN SHIGELLOSIS BURDEN OF DISEASE IN ASIA

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A review of the global burden of disease from shigellosis published in the Bulletin of the World Health Organization in 1999 estimated that over a million deaths were occurring annually from this infection. With encouragement from WHO, we repeated this analysis for Asia to update the estimate for this continent. The methods for this update were nearly identical to the original, but we limited data for this review to those epidemiological and clinical studies that were published since 1990. Our review suggests that the annual number of shigella infections in Asia is approximately 114 million episodes. Of these about 85,000 are fatal. This represents modest reduction in infections but about a 90% reduction in

fatalities since the earlier estimate which used data collected primarily during the 1980s. The large reduction in fatalities seems due primarily due to reduced case fatality rates. To explain this large reduction in shigellosis deaths, we cannot rule out improved therapy; however, this improvement seems more likely due to children being healthier at the time they become infected. Healthier children are less likely to develop fatal complications. Since the study was limited to data from Asia, this study is not able to assess the disease burden in Africa or Latin America, though unpublished data from Latin America suggests that a similar reduction in shigella deaths have occurred there as well. Speculation about changes in disease burden in Africa is complicated by concurrent infections with HIV-AIDS and the lack of diarrheal disease surveillance confirmed bacteriologically. We conclude that there has been a large reduction in shigella deaths during a period when there were no shigella specific interventions. We speculate that other non-specific interventions e.g. measles vaccination, vitamin A distribution, and improved nutrition have improved the general health of children which has lead to reduced case fatality rates and a marked reduction in shigellosis deaths in Asia.

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IMPROVING TB DIAGNOSIS IN HIGH BURDEN COUNTRIES BY USING FLUORESCENCE MICROSCOPES WITH LIGHT EMITTING DIODES (LEDS)

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During a cross-sectional study on 1398 TB suspects attending a specialized chest clinic in Nairobi, we showed that fluorescent microscopy on 2 auramine-stained sputum smears was superior to routine microscopy using 3 Ziehl-Neelsen (ZN) stained sputum smears with sensitivities of 78% and 60%, respectively, and also was more cost-effective. Moreover, the HIV positive status of suspects reduced the sensitivity of ZN microscopy, but had no effect on the sensitivity of fluorescence microscopy. Despite the relatively high purchase price of fluorescence microscopes, an argument that is often used against their use, fluorescence microscopy leads to savings from the viewpoints of both the health facility (less time needed for slide examination) and the patient (less visits to the clinic required). Unfortunately mercury-vapor short arc lamps, as used in fluorescence microscopes, are problematic and require not only high initial investment but also significant ongoing maintenance costs. Mercury-vapor lamps are expensive, inefficient requiring an expensive power supply, have a short life (typically 200 to 300 hours), and may fail, releasing toxic mercury. Critically repeated switching on and off, which may occur if the local power supply is unreliable, shortens the life of these lamps. We developed a simple adaptation of a standard fluorescence microscope for illumination using a high power LED and demonstrate this form of illumination is suitable for the detection of auramine O stained *Mycobacterium* spp. LEDs emitting at other wave lengths may be suitable for fluorescence microscopy in other applications, for example for malaria. The low cost, low power consumption, safety, and reliability of LEDs, makes LEDs attractive alternatives to mercury vapor lamps, making fluorescence microscopy even more cost-effective.

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LOCALIZATION OF GENDER-BIASED GENE EXPRESSION IN ADULT *BRUGIA MALAYI*

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We used *in situ* hybridization (ISH) to study gender-biased gene expression in the filarial parasite *Brugia malayi*. Digoxigenin-labeled RNA probes were synthesized from candidate genes identified by microarray and qRT-PCR studies and hybridized to frozen sections of adult worms. Genes assumed to be up-regulated in males (7 genes) and females (11 genes) were tested; a gene that is equally expressed in both sexes (BMC04302) served as a control. ISH confirmed gender-biased expression in all 18 genes and non-biased expression in the control. Hybridization signal intensities correlated with relative mRNA abundance (reflected by Ct values obtained by qRT-PCR). The expression of some gender-biased genes appeared to be strictly regulated during development of embryos and sperm. For example, expression of the female up-regulated caveolin homologue (MBC02383) was first detected in early morulae, peaked in late morulae, decreased in pretzel embryos, and was undetectable in stretched microfilariae. Major sperm protein (BMC02125) expression was detected in spermatocytes in the early spermatogenesis zone but not in the mitosis zone of the testis or in spermatides in the vas deferens. No signal was detected in inseminated females. A probe for the female up-regulated sheath protein *Shp-1* (BMC01695) labeled the uterine epithelium in areas with morulae or early pretzel embryos but not other tissues in females, more mature larvae, or males. This result suggests that at least some sheath proteins are derived from the uterus and not synthesized by intrauterine larvae. As expected, most of these genes are expressed in reproductive organs (gametes, developing larvae). ISH studies provide a means of independently confirming differential expression of genes identified by other methods. In addition, localization patterns can improve understanding of the biological role of known genes and help to further characterize the function of unknown or novel genes.

(ACMCIP Abstract)

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USE OF MICROARRAY AND REAL-TIME RT-PCR TO EVALUATE THE GENE EXPRESSION PATTERNS OF THE L3 AND L4 STAGES OF THE FILARIAL PARASITE *BRUGIA MALAYI*

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Microarrays and real-time RT-PCR were used to study the gene expression profiles of the L3 and L4 stages of *Brugia malayi* with the goal of identifying genes crucial for the parasite's ability to infect and perform the first molt in the human host. For this microarray study, the second version of the filarial microarray was used. The version 2 array includes over 18,000 oligonucleotide spots representing all of the known and predicted genes of *B. malayi*, *Onchocerca volvulus*, *Wuchereria bancrofti* and the *Wolbachia* endosymbiont of *B. malayi*. Arrays were hybridized with Cy3 and Cy5 dye labeled L3 and L4 cDNA. The experimental design included a technical (dye-flip) replicate. The data were normalized for background and probe intensity, and the relative abundance of hybridized cDNA for each spot was determined. Genes showing two-fold or greater differences with $P<0.05$ were considered stage-regulated candidates. The data demonstrated that 1,030 of the 18,153 oligonucleotides (6%) with signals above threshold were stage-regulated. These included 470 genes up-regulated in the L3 stage and 560 up-regulated in the L4 stage. Genes that had five-fold or greater differences with $P<0.05$ were classified into functional groups in order to determine stage-regulated trends in the L3 and L4; functional trends were confirmed with and analysis at $P<0.01$. Genes in the proteinase, immunological, and cellular metabolism groups were significantly up-regulated in the L3 stage while genes in the cuticle/collagen and oxidatitive stress functional groups were significantly up-regulated in the L4 stage. The data and trends generated by the microarray experiments were independently confirmed for eight genes using real-time RT-PCR; these data confirmed the stage-specific expression profiling of the microarray results for these genes. This approach for identifying stage-specific gene expression will lead to an increased understanding of the biology of filarial infection and the molt from L3 to L4 stage parasites. In addition, this strategy shows promise in identifying

putative targets for the development of new drugs or vaccines for the prevention of filarial infection.

(ACMCIP Abstract)

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EARLY EFFECTS OF DOXYCYCLINE ON *WOLBACHIA* AND PARASITE GENE EXPRESSION IN ADULT FEMALE *BRUGIA MALAYI*

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Most filarial nematodes contain *Wolbachia* symbionts. Prior studies have shown that these bacteria are essential for the normal development, reproduction and survival of *Brugia malayi* (Bm). Doxycycline (Doxy) clears *Wolbachia* (*w*Bm) from cultured Bm within a few days and inhibits the development and release of microfilariae by adult worms. Higher concentrations of Doxy kill adult Bm. The purpose of this study was to examine the early effects of Doxy on gene expression in Bm to better understand the molecular basis for the biological relationship between *w*Bm and filarial nematodes. Bm females were cultured for 24 hr in RPMI with or without Doxy (5 µg/ml). RNA recovered from treated and control worms was labeled by random priming and hybridized to the Version 2 Bm oligonucleotide microarray. This array contains 17,300 65-mers in duplicate that cover an estimated 89% of Bm genes and all known *w*Bm genes. Cy dye fluorescence intensity ratios were analyzed for two biological replicates (with technical dye-swap replicates) using RNA from treated and control worms (8 hybridizations per oligo on 4 slides). The data were normalized for background and probe intensity with internal controls. Genes with 2-fold differences in hybridization between treated worms and control worms with $P < 0.01$ were considered to be differentially expressed. 63 *w*Bm genes showed differential expression after Doxy treatment. Surprisingly, 61 of these were up-regulated after treatment. This suggests that treatment broadly induces transcription in *w*Bm; this may be a general response to Doxy-mediated inhibition of protein synthesis. 80 chromosomal Bm genes were differentially expressed after treatment. Of these, 31 were up-regulated including 14 that encode heat shock proteins. This suggests that Doxy induces a stress response in Bm, either directly or indirectly due to effects on *w*Bm. 49 Bm genes down-regulated after treatment are mostly novel genes or predicted genes with unknown functions. Ongoing KEGG analysis may provide clues regarding *w*Bm genes that are essential for Bm survival and reproduction.

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DISTINCT HOST EXPRESSION SIGNATURES INDUCED BY CLOSELY RELATED FILARIAL PARASITES

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Having previously demonstrated that the type of immune response induced by different pathogens is determined during their initial encounter with host antigen presenting cells we sought to utilize peripheral blood mononuclear cells (PBMCs) from normal individuals and patients with well-defined and -documented filarial infections to identify signature adaptive (but steady state) responses induced by phylogenetically similar but distinct parasites (*Loa loa* [Ll] and *Mansonella perstans* [(Mp]). Using banked PBMCs cryopreserved immediately *ex vivo* from patients with defined filarial infections (12 microfilarial (mf) positive Ll, 13 mf negative Ll, 5 mf positive Mp) or from normal donors (n=12), RNA was extracted, labelled and hybridized to Affymetrix U133 version 2.0+ microarrays, and the expression profiles compared using a cutoff of $p<0.001$ in ANOVA for determination of statistical significance. From a probe set of 54316, 5196 genes were differentially regulated in at least one group (versus the other 3). Further statistical analysis using t-tests to compare between groups means (one group to the next) at a $p<0.001$, we were able to define a 78 probe set signature that differentiated between mf+ and mf- Ll patients. Moreover, with a separate 223 probe set signature, mf+ Ll and mf+ Mp could easily be distinguished. Not surprisingly, normal blood bank donors could be distinguished from any of the filarial- infected groups simply by their ex vivo host response. These data not only provide insights into the identification of microbial determinants of the host response but suggests novel strategies for therapeutic intervention and possibly for prognostic markers of outcome.

(ACMCIP Abstract)

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IDENTIFICATION OF GENES THAT FUNCTION WITH CATHEPSIN L DURING EMBRYOGENESIS IN NEMATODES

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Cathepsin-like enzymes have been identified as potential targets for drug or vaccine development in many parasites, as their functions appear to be essential in a variety of important biological processes within the host, such as molting, cuticle remodeling, embryogenesis, feeding and immune evasion. Functional analysis of the *Caenorhabditis elegans* cathepsin L (*Ce-cpl-1*) has established that *cpl-1* is essential for early embryogenesis, potentially regulating the processing of yolk proteins. We have also shown using RNAi that the function of the homologous filarial genes in *Brugia malayi* (*Bm-cpl-1 and Bm-cpl-5*) is also essential during embryogenesis. To identify genes that are repressed or activated due to the deletion of *Ce-cpl-1* activity, we performed a microarray experiment using RNA derived from N2 young adult worms containing 4-6 embryos and from *Ce-cpl-1* (vc322) mutant worms at a similar stage of development. The *C. elegans* cDNA was tagged to allow pair-wise competitive hybridizations of mutant vs. wild type. Each experiment consisted of 3 pair-wise competitive hybridizations, 2 of which had the mutant labeled with Cy5 capture sequence and the third with the Cy3 sequence. 719 genes showing altered expressions were identified; 190 up-regulated and 529 down-regulated. Of the 529 down-regulated genes, 24 have been already shown to be essential for embryogenesis as their suppression by RNAi or the deletion mutants cause embryonic lethality and./or sterile phenotype, and including genes like F56F3.2 (*ndg-4*) which also causes accumulation of yolk in pseudocoelom. Further studies of these genes will potentially identify those that mediate or regulate *cpl-1's* function and thus participate in the pathway that regulate the processing of yolk proteins.

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PARASITE-DERIVED LYMPHANGIOGENIC MOLECULES: PUTATIVE ROLE IN MEDIATING THE LYMPHATIC DYSFUNCTION SEEN IN FILARIAL LYMPHEDEMA

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Human lymphatic filariasis (LF), caused by the nematode parasites *Brugia malayi and Wuchereria bancrofti,* can manifest as secondary lymphedema that can progress to elephantiasis typically through damage to or dysfunction of the lymphatic vessels. This filarial parasite-induced lymphatic dysfunction is poorly understood in large part because of a paucity of suitable *in vitro* / *in vivo* models. Utilizing podoplanin positive primary human lymphatic micro-vascular endothelial cells (HLMVEC), we have examined the effect of *Brugia malayi* mixed adult (BmA), adult male antigen (BmMAg) and microfilarial antigens on these HLMVEC. Proliferative studies indicate that BmA and BmMAg at doses of 5-50µg/ml induce significant proliferation in HLMVEC as assessed by ^{3}H-thymidine incorporation. This response was similar to levels seen with recombinant human VEGF165 (12.5ng/ml). In contrast, MfAg at similar doses (5-50µg/ml) significantly inhibited the proliferation of the HLMVEC, while

inducing proliferation at lower doses. Further, we demonstrate that the microfilarial antigens or live microfilariae (with or without contact) induce the formation of vascular tube formation of the HLMVEC in *in vitro* conditions. To identify those filarial molecules that may induce lymphatic proliferation and tube formation, we have used both an *in silico* approach and a cell based screening technique of a phage display library to identify first a *Brugia/Wuchereria* encoded VEGF/PDGF-like molecule and a 770 bp molecule that binds specifically to HLMVEC (and not to blood vascular endothelial cells) homologous to the human translationally controlled tumor protein (TCTP). Recombinant proteins are being expressed to prove definitively that these parasite molecules are responsible for the disordered lymphatic function seen in LF.

(ACMCIP Abstract)

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IMMUNIZATION WITH EARLY L3 ES ALTERS *BRUGIA PAHANGI* MIGRATION IN GERBILS

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It is hypothesized that L3 excretory/secretory products (ES) are important to the initial migratory phase of *Brugia* infections. A model for these early migrations has been established by inoculating L3s into the dermis (ID) of the permissive gerbil host (Porthouse et al., 2006). In this model, L3s injected ID in the lower hind limb travel to the popliteal lymph node by 3 days post infection. Parasites continue to migrate from this site and most are established as adults in the spermatic cord lymphatics by 28 days post infection. The majority of L3s injected into the peritoneal cavity (IP) do not migrate, thus ES may play a different and potentially less essential role in the establishment of these infections. L3 ES was collected from 24 hr *in vitro* cultures and concentrated. Immunization of gerbils with this mixture in RIBI adjuvant produced antibodies of multiple isotypes to a wide range of proteins as demonstrated in Western blots. Reaction with L3 surface was minimal. Gerbils were subsequently challenged either ID or IP with 100 L3s and euthanized at 3 or 106 days post infection (DPI). At 3 DPI ES immunization significantly slowed the migration of ID inoculated L3 compared to controls. The location of adult worms within lymphatic vessels and tissues at 106 DPI was also significantly altered from the norm following ES immunization. Nonetheless, while migration patterns were altered, immunization did not significantly reduce the numbers of parasites recovered from immunized animals at either time period in gerbils challenged either ID or IP. At 106DPI, immunized animals had fewer circulating microfilariae and significantly fewer microfilariae per female worm suggesting the antigens in L3 ES are shared by substances important in microfilariae production or the microfilariae themselves. Further, the number of intralymphatic parasite induced granulomas was also significantly reduced in ES immunized animals. It is important to note that microfilariae serve as an important nidus of these lesions.

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CYSTIC AND ALVEOLAR ECHINOCOCCOSIS TRANSMISSION AND RISK FACTORS IN NINGXIA HUI AUTONOMOUS REGION OF CHINA: CURRENT SITUATION AND EVOLUSION

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Ningxia Hui Autonomous Region (NHAR) of China, with a third of Hui Islamic population, sheep-farming as majority economy, is a well-known hyper-endemic for cystic (CE) and alveolar (AE) echinococcosis. A comprehensive survey was conducted recently. The results showed that human CE occurred throughout NHAR where the species is adapted to cycle between dogs and sheep and/other domestic animals. A co-endemic focus for both AE and CE is in the south. One of the main risk factors for AE and CE there is related to dog-ownership. Domestic dogs may become infected with *E. multilocularis* when they eat infected wild rodents. However, transmission patterns of AE/CE are considerably modified by human behavioural factors in recent past of the region. As a certain cultural situation, uncontrolled dogs live closely with people, uncontrolled slaughter of livestock and unsanitary living conditions, leading for both diseases to higher risk infection, for bias female taking responsibility for feeding dogs favour to the exposure in both infections. In addition, small mammal communities changed along an ecological succession from forest to farmland led to more intense transmission to humans with high prevalence AE up to 8% in certain geographic locally since plateau deforestation in the 1970s. Since the 1980s and the early 1990s, people commonly use rodenticides for crop protection, which resulted in secondary poisoning dogs, thus decreasing transmission and to parasite extinction locally. However, reforestation programmes carried out on farmland over years. Rodenticides were forbidden using by local government from 2004, and socio-economic and local-custom that watching-dog for sheepherding has led to dog-population increasing recently. These factors may paradoxically recreate conditions for AE or/and CE transmissions in the future. From this point of view, all government policies, such as grassland management, and disease control programs should consider incorporating principles of biological conservation and multi-purpose management of such socio-religion and local ecosystems.

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CYSTIC ECHINOCOCCOSIS IN FAMILIES AND NEIGHBORS OF PATIENTS RECENTLY DIAGNOSED WITH CE

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Cystic echinococcosis (CE) is a disease caused by the larval stage of *Echinoccocus granulosus*, which is endemic in sheep-raising regions of Asian, African, Mediterranean and South American countries, principally. In community wide screenings, 9-12.5% of households with infected individuals harbor two or more cases. To evaluate if targeted search

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may be useful, this case-control study assessed the prevalence of CE in household members of patients recently diagnosed with CE. The study was conducted in Junin, a sheep-raising place in the Andes of Peru, using serology by enzyme-linked immunoelectrotransfer blot (EITB, immunoblot), ultrasound and chest X-rays. The case group was constituted by 27 families (n=150 individuals) of individuals with CE; the control group, neighbors of CE patients, was constituted by 13 families (n=49 individuals). There were no differences between cases and controls in age or sex. Seven individuals with pulmonary CE were found in seven families in the case group (positive chest x ray), one had also liver involvement; only two of them were seropositive on EITB. In the control group only one individual with liver CE was diagnosed by ultrasound and EITB, and another 3 individuals were seropositive but had negative ultrasound and negative chest X-rays. While there was no statistically significant difference between both groups OR: 5.25 (CI 95%: 0.50 - 129.42, p=0.212) there was a sizable proportion of households with another infected individual.

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IDENTIFICATION OF NEW TREATMENT OPTIONS WITH PARASITOSTATIC AND PARASITOCIDAL POTENTIAL AGAINST ECHINOCOCCUS MULTILOCULARIS LARVAE

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Alveolar echinococcosis (AE) is characterized by primary hepatic invasion of ***Echinococcus multilocularis***. In most cases the diagnosis is only established at advanced stages of disease with diffuse infiltration of non-resectable structures. As a consequence, chemotherapy is the only means to impede further disease progression. Albendazole (ABZ) and Mebendazole are the only drugs licensed for treatment of human AE. In order to augment the therapeutic armentarium, we tested various drugs for their efficacy against ***E. multilocularis*** larvae. ***E. multilocularis*** larvae grown intraperitoneally in Mongolian gerbils were transferred into tissue culture. Vesicles budded from the tissue blocks and after 6 weeks, drugs were added and the effect on the vesicles was observed. We tested the following drugs at varying concentrations: ABZ, Artemether, Caspofungin, Itraconazole (ITZ), Ivermectin, Methiazole (MTZ), Miltefosine, Nitazoxanide (NTZ), Rifampicin and Trimethoprim/Sulfamethoxazole. ABZ, ITZ, MTZ and NTZ effectively destroyed parasite vesicles. At high NTZ doses of 10µg/ml, complete destruction of vesicles was observed after 7 days and was significantly more rapid than with ABZ at equal concentrations (21 days). After drug discontinuation, regrowth of vesicles occurred between 7 and 14 days for all 4 drugs, indicating a parasitostatic effect. Combination treatment with NTZ+ABZ at concentrations between 1 and 10µg/ml for either 3 weeks, 3 months or 6 months respectively, yielded no vesicle regrowth during 8 months after drug discontinuation. The so treated larval tissue was injected intraperitoneally into Mongolian gerbils and no regrowth of larval tissue was observed, suggesting a parasitocidal effect after combined treatment.

In conclusion, ITZ, MTZ and NTZ constitute new promising treatment alternatives against *E. multilocularis*, although they proved to be parasitostatic only. Our results suggest a synergistic and parasitocidal effect of NTZ plus ABZ. The combined treatment may solve the persisting task to effectively kill this human parasite.

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PREVALENCE, INCIDENCE AND SERO-REVERSION OF CYSTIC ECHINOCOCCOSIS (CE) IN THE HIGHLAND PERUVIAN COMMUNITIES USING CHEST X-RAY, ULTRASOUND AND EITB TEST

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CE is a disease caused by the larval stage of *Echinococcus granulosus*. It is endemic in sheep-raising regions of America, Africa, and Asia. Previous studies have demonstrated a prevalence of 5.7% (ultrasound and X-ray) while seroprevalence has been estimated in 9% in Peru. Incidence has been rarely measured in endemic populations, while seroreversion, from positive to negative status, has been described not only for CE but also for other parasites. This study followed up nine Peruvian communities in Pasco (Andean region) twice with one-year interval, using serology on Enzyme-linked Immmunoelectrotransfer Blot (EITB) assay, ultrasound (US) and chest X-ray to quantify the prevalence, incidence and seroreversion. Children <5 and pregnant women were excluded. In the first round, 52% (949/1836) of the population were surveyed. The overall prevalence (positive in at least 1 of the 3 test) was 12.5% (119/949, 95% CI: 11%-15%). The percentages of liver, lung cysts, and EITB-positives were 4.7% (45/949), 1.1% (9/829), and 8.9% (83/929), respectively. The prevalence in the second survey was 4.4% (34/780), 2.2% (17/760), and 8.7% (66/757) for liver CE, lung CE and EITB, respectively. The annual cumulative seroincidence was estimated as much as 2% (6/254) while the seroreversion was calculated in 37.5% (12/32) in one year. We confirmed the high prevalence in the area as well as the relatively high seroincidence. This finding also suggests that a significant proportion of seropositive subjects in echinococcus-endemic regions may have only transient antibody response without evidence of hydatid disease. Alternatively some of these subjects may have developed cysts undetected by imaging because of their size or location, or that resolved spontaneously.

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LINKING LANDSCAPE ECOLOGY AND *ECHINOCOCCUS MULTILOCULARIS* TRANSMISSION IN CHINA

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In 1986 Roberts and Gemmell presented a suite of differential equations to describe the transmission dynamics of ***E.granulosus*** in closed systems where steady state assumptions might be reasonable. Models have since been proposed for ***E.multilocularis*** transmission in Japan, Germany, Holland, Kazahkstan and also Sichuan, China. Transmission of these two cestodes differ in the relative importance of sylvatic vs domestic hosts. Due to complex population dynamics of small-mammals steady state assumptions often appear unreasonable for ***Em***, however key ecological parameters giving rise to spatial heterogeneity have yet to be fully identified. In China patterns of transmission intensity and stability are complicated by an endemic region which crosses numerous biogeographical areas comprising of varied host communities. Within that endemic area the Qinghai-Tibet plateau has been proposed to be a meta-stable transmission focus and there is growing evidence that human

activity there is interacting with environmental factors in ways which can elevate transmission. For example large numbers of free-roaming dogs are suspected to play an important role in transmission in and around areas of human habitation. Moreover, landscape management and grazing practices are known to affect habitat suitability for small-mammals implying that anthroprogenic activity affects reservoir populations and therefore transmission intensity.

The Roberts and Gemmell transmission model has recently been used to investigate the infection status of dogs in the area under steady state assumptions. However the infection pressure parameter *ß,* representing the number of infected rodents eaten per year by a dog, can vary naturally in space especially across study areas sufficiently large so as to include heterogeneity in habitat availability and suitability for key reservoir species. The current work aims to quantify the influences of landscape on transmission intensity. This requires parameterisation of at least two additional variables, i) habitat quality and ii) spatial position, and that attention be paid to issues of spatial scale. The effects of spatial position are being investigated in relation to small-mammal community data, as well as infection status data from dogs and humans within the Qinghai-Tibet focus and across the wider endemic area extending to unstable peripheral foci such as those found in the fragmented landscapes of Gansu and Ningxia.

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ECHINOCOCCOSIS TRANSMISSION IN EASTERN KAZAKHSTAN AND NOMADIC TIBETAN COMMUNITIES

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Echinococcosis is an emerging or re-emerging disease in central Asia and China. In nomadic Tibetan communities in western Sichaun province, PR China there are high levels of both human cystic echinococcosis (CE) and human alveolar echinococcosis (AE), caused by the larval stage of ***Echinococcus granulosus*** and ***E. multilocularis*** respectively. Local prevalences reach 5% or more for both parasites. In Kazakhstan there has been an increasing incidence of CE, since the collapse of the Soviet Union. Dogs are the main definitive host of ***E. granulosus*** and are also believed to play an important role in human transmission of AE. An ultrasound and serological surveillance study of 3139 inhabitants of a community in eastern Kazakhstan was undertaken in 2005. This was in a district where there are high levels of both ***E. granulosus*** and ***E. multilocularis*** infections in the local dog populations. The results indicate that there were 23 subjects that were ultrasound positive for CE with a further 24 reporting recent treatment for CE, giving a total of 47 with recent clinical evidence of CE or a prevalence of 1.5%. There were no cases of human AE detected. Serological results suggest a sero-prevalence of about 2.2% for CE, but of 0.1% for AE. These prevalences were much lower than in a Tibetan population where ultrasound prevalence rates for CE and AE were recorded as 4.6% and 4.9% respectively in a previous study of 3135 subjects. Purgation studies of the dog populations demonstrated a mean abundance of infection of ***E. granulosus*** of 1339 parasites per dog in Kazakhstan, but only 80 parasites per dog in China. Likewise for ***E. multilocularis***, purgation studies indicated a mean abundance of 122 parasites per dog in Kazakhstan and 131 parasites per dog in China. Thus the parasite biomass in the definitive host was far higher for ***E. granulosus*** in Kazakhstan but of similar levels between the two sites for ***E. multilocularis***. These results indicate that there are different ecological or socioeconomic factors that produce high dog to human transmission rates in China but much lower transmission rates in Kazakhstan. Different contact rate between humans and dogs in the two communities may be one possible explanation of these different transmission rates.

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A RANDOMIZED LONGITUDINAL CLINICAL TRIAL OF COMBINATION ANTIMALARIAL THERAPY IN A COHORT OF UGANDAN CHILDREN

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Combination antimalarial therapy is widely advocated in Africa due to the increasing spread of drug resistance. We are conducting a randomized clinical trial comparing the efficacies of combination therapies recommended by the WHO in a cohort of Ugandan children using a novel longitudinal design. From November 2004 to April 2005 we recruited a cohort of 601 children aged 1-10 from an urban community using probability sampling. Children are being followed for 3 years for all of their health needs. Malaria is diagnosed each time a child presents with a fever and a positive thick blood smear. When children are diagnosed with their first episode of uncomplicated malaria they are randomized to: 1) amodiaquine + sulfadoxine-pyrimethamine, 2) amodiaquine + artesunate, or 3) artemether-lumefantrine. The same therapy is given for all subsequent episodes of uncomplicated malaria. All episodes of complicated malaria and treatment failures occurring within 14 days are treated with quinine. Routine blood smears to detect asymptomatic parasitemia are done every 30 days and hemoglobin levels are measured every 90 days. Preliminary blinded results are presented after 682 person years of follow-up and a total of 651 treatments with study drugs. There have been no deaths or episodes of severe malaria. For all treatment groups combined, the risks of recurrent malaria (ETF or LCF) and recurrent parasitemia (ETF, LCF, or LPF) after 28 days of follow-up are 10% and 15%, respectively. The prevalence of anemia (Hb < 10 gm/dL) in asymptomatic children decreased from 9% at enrollment to 1% after 1 year of follow-up. The prevalence of asymptomatic parasitemia decreased from 19% at enrollment to 2% after 1 year of follow-up. Aggregate preliminary data suggest that all studied combination therapies are highly effective and that effective case management markedly diminishes the prevalence of asymptomatic parasitemia and anemia.. Un-blinding of the assigned treatment groups and early analysis of the data are planned for July 2006, and these results will be presented.

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ANTIMALARIAL ARTEMISIN-BASED COMBINATION TREATMENTS (ACTS): CURRENT STATUS IN WEST AND CENTRAL AFRICA WITH RESPECT TO ACCESS AND MONITORING DRUG USE AND EFFECTS

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Artemisinin based Combination Treatments (ACTs) are now adopted as first line treatment for malaria in most malaria endemic countries, but the implementation of such policy change is taking time. Access to and rational use of antimalarial drugs remains major challenges in low income countries. While effective and safe in clinical trials, concerned countries do not know how to improve access and how ACTs will perform in real-life. Monitoring coverage, parasite susceptibility, clinical efficacy and tolerability should be integral part of large-scale implementation. During 2005-2006, we carried out an evaluation of access to drug and case management in Cameroun, Senegal, and Central Africa, 3 countries having chosen artesunate - amodiaquine (AS/AQ) as first-line treatment of uncomplicated malaria. We recorded: availability of AS/AQ in urban and rural settings (private and public sector); training, information and prescriber's

adherence (physicians, health worker) to treatment policy; reasons for deviations from policy; adverse events as reported within the community. Results of the analysis of the data collected will be reported and discussed in the light of the implications of discrepancies between policy/regulations and practice, weakness of delivery systems, availability of registered and unregistered substandard antimalarial drugs, role of marketing based information and community health education.

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AMODIAQUINE PLUS ARTESUNATE VERSUS DIHYDROARTEMISININ-PIPERAQUINE FOR DRUG RESISTANT *P.FALCIPARUM* AND *P.VIVAX* IN PAPUA, INDONESIA

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In Papua, Indonesia, high levels of multidrug resistance (mdr) precludes the use of chloroquine and chloroquine plus sulfadoxine-pyrimethamine for the treatment of *Plasmodium falciparum* (PF) or *P. vivax* (PV). Amodiaquine plus artesunate (AQS) has been proposed as new treatment strategy in Indonesia. We conducted a chemotherapeutic trial to compare AQS with dihydroartemisinin-piperaquine (DP) for uncomplicated malaria in southern Papua. Patients presenting to a rural clinic with uncomplicated PF or PV were enrolled into prospective efficacy studies and randomised to either AQS or DP. Treatment was supervised and patients followed for 42 days. Between July and December 2005 231 patients with PF and 109 patients with PV were enrolled and received AQS (n=171) and DP (n=169). All regimens were well tolerated with no early treatment failures or major adverse effects. By day 42 the PCR corrected recrudescent rate of PF was 13% [95%CI: 6-20] after AQS versus 2.8% [95%CI: 0-5.9] with DP (p=0.01). The corresponding failure rates for PV were 30% [95%CI: 22-38] and 6.7% [95%CI: 2.4-11]; p<0.001. In addition DP reduced significantly the rate PF reinfection during a 42 day follow up: 11% [95%CI: 5.6-17] after AQS vs 2.9% [95%CI: 1.6-5.6] after DP; p=0.007. In conclusion, DP's efficacy against multidrug resistant malaria combined with its post treatment prophylaxis reducing reinfection and vivax relapse, makes it the preferred antimalarial option in this region.

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ARTEMETHER-LUMEFANTRINE FOR THE TREATMENT OF MODERATE MALARIA IN CHILDREN

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Clinical malaria is commonly classified as uncomplicated or severe. Patients with severe malaria are hospitalized and managed as in-patients. However, most children hospitalized for malaria do not have any of the strictly defined features of severe disease. But in practice, are treated with parenteral quinine. Reasons for hospitalization for these patients include frequent convulsions, vomiting, or clinical pallor. Because of the rapid parasite-killing rate associated with artemisinin-based combination therapy, Artemether-lumefantrine may be of benefit. However, variable lumefantrine bioavailability may be of concern. We have examined Artemether-lumefantrine efficacy and lumefantrine bioavailability in these patients. An open-label randomized controlled trial of the six-dose regimen of artemether-lumefantrine versus single dose sulfadoxine-pyrimethamine (SP) involving children aged six months to 13 years. Patients were hospitalized for the three-day treatment period and active follow-up was on Days 7, 14 and 28. Plasma lumefantrine concentration was determined on Day 7. Kenya adopted Artemether-lumefantrine as the first line treatment for uncomplicated malaria before the end of the trial. At this point, randomization to SP was considered unethical. However, the study continued as a single arm trial to evaluate the efficacy of Artemther-lumefantrine. Adequate clinical and parasitological response (ACPR) on Day 14 was 34/45 (76 %) and 44/46 (96 %) for SP and Artemether-lumefantrine respectively whereas on Day 28, ACPR was 21/36 (58 %) and 36/44 (82 %) respectively. In per protocol population involving all patients treated with Artemether-lumefantrine, PCR-adjusted ACPR on Day 28 was 83/88 (94 %). The median (Interquatile range) Day 7 plasma lumefantrine was 237 (130 - 345) ng/ml and Day 7 plasma lumefantrine concentration was below 280ng/ml, the putative *in vivo* MIC for lumefantrine, in 57/99 (51 %) of the patients. Despite plasma lumefantrine concentrations below the expected for cure in most patients with moderate malaria, Artemether-lumefantrine efficacy appears to be comparable with that in uncomplicated malaria and the six-dose regimen can be used in hospitalized paediatric patients (without signs of severe disease) with good results.

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PHARMACOVIGILANCE OF ANTIMALARIAL TREATMENT IN UGANDA

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Many African countries have recently adopted artemisinin-based combination therapy (ACT) for first-line treatment of uncomplicated malaria. Although widespread use of ACTs in Africa is imminent, data on the safety of these medicines, particularly with repeated use, are limited. Treatment safety is monitored through pharmacovigilance, beginning during product development and continuing into the post-marketing phase. However, infrastructure for pharmacovigilance is generally lacking in Africa, and monitoring for safety of antimalarial treatment may be challenging. Identifying when an adverse event has occurred, distinguishing possible drug reactions from malaria-related symptoms, and establishing causal relationships can be complicated. Presumptive treatment of fever with antimalarials in Africa is also common, frequently with drugs obtained without a prescription, which may increase the risk of adverse events. Pharmacovigilance for antimalarial treatment in Africa is essential, but presents a significant challenge. We propose to develop a pilot system for monitoring the safety of antimalarial drugs at sentinel sites within a malaria surveillance network in Uganda. We are currently investigating local beliefs and experiences with antimalarial treatment, and evaluating existing systems and innovative approaches for reporting adverse events that could be adopted. This qualitative baseline information will be used to develop working models for adverse event monitoring. A pilot system for reporting adverse events related to antimalarial treatment will be implemented and evaluated at sentinel sites in Uganda. We plan to conduct surveillance at the level of health care facilities and within the communities surrounding the sentinel sites by integrating with existing systems and ensuring adequate education and feedback. The results of the first phase of the project, a description of the pilot pharmacovigilance system, discussion of the challenges and opportunities, and preliminary results of the pilot will be presented.

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COMPARATIVE EFFICACY AND SAFETY OF TWO ARTEMISININ CONTAINING COMBINATION THERAPIES FOR ACUTE UNCOMPLICATED MALARIA IN NIGERIAN CHILDREN

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In response to the worsening antimalarial drug resistance situation, many countries including Nigeria have changed their national antimalarial policies to artemisinin containing combination therapy in line with WHO recommendation. In an open labelled randomised controlled clinical trial, 132 children aged 6 months to 10 years were enrolled into the study. Patients were allocated into one of two treatment groups according to a pre-generated randomisation table. Group 1 received artesunate at a dose of 4mg/kg body weight once daily for 3 days plus amodiaquine at a dose of 10mg/kg body weight once daily for 3 days. Artemether-lumefantrine was administered to the other group as a 6-dose regimen depending on body weight range of the patients (5-14 kg, 1tablet: 15-24 kg, 2tablets; and 25-35kg 3tablets) 2ce daily for 3 days. Efficacy was assessed using 2003 WHO guideline. Safety profile was assessed by the incidence of adverse events to the study drugs as well as assessment of the haematology and blood chemistry profiles. One hundred and twenty three (AL=62, AQAS=61) of 132 (93.2%) enrolled children completed the study. Day 28 cure rates in the per protocol population were 95.2% and 93.4% for AL and AS/AQ respectively (*p=0.49*). Day-28 PCR corrected cure rates for both drugs were 100%. The 24hour parasite reduction was 93.4% and 96.5% for AL and ASAQ respectively while mean parasite clearance time (PCT) and fever clearance time (FCT) were 1.86 days± 0.61 and 1.22 days ± 0.45 versus 1.77days ± 0.61 and 1.16days ± 0.45 in AL and ASAQ treated groups respectively. Both drugs were well tolerated and no patient was withdrawn on account of adverse event. In conclusion, artemether-lumefantrine and artesunate-amodiaquine are equally effective and safe in the treatment of acute uncomplicated malaria in children.

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AUDIOMETRIC CHANGES DURING TREATMENT OF FALCIPARUM MALARIA WITH ARTEMISININ CONTAINING COMBINATIONS IN NIGERIAN CHILDREN: A PRELIMINARY REPORT

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Artemisinin derivatives are now being deployed very widely in malaria control strategies because of worsening drug resistance in the malaria parasite. This underscores the need for careful assessment of the safety of artemisinin derivatives. As part of a larger open label randomized study, children aged 5 years to 12 years who were being treated for acute uncomplicated malaria were evaluated for audiometric changes. The children were treated with artemether-lumefantrine [AL] as a 6-dose regimen based on body weight twice daily for 3 days or artesunate (4mg/kg) plus amodiaquine (10mg/kg) [ASAQ] daily for three days. At enrolment, all children underwent thorough physical examination of their ears as well as othoscopy to ensure that there were no foreign bodies, impacted wax or discharge in the ear. Audiometrists were blinded to patients' drug treatment. Response of malaria to therapy with both drugs was prompt with 100% cure rate at day 14. Hearing assessment was carried out running through eight frequencies (500Hz, 1000Hz, 1500Hz, 2000Hz, 3000Hz, 4000Hz, 6000Hz and 8000Hz) on days 0, 3, 7 and 14.The degree of hearing loss detected by audiometric tests was assessed using the National Acoustic Laboratory 4 Frequency Averaging Formula. Results were categorized into three: normal, mild hearing loss and moderate/severe hearing loss. The audiometric findings of days 3, 7 and 14 were compared to findings on day 0. A total of 16 children have so far been assessed audiometrically. Ten received ASAQ while 6 received AL. Hearing at day 0 was within normal limits in all patients except one treated with ASAQ who had mild hearing loss in the right ear. The mild hearing loss in this patient remained unchanged at day14 assessment. Six of the remaining 9 children treated with ASAQ recorded improved hearing at the end of the study while the hearing in two children which were normal pretreatment remained the same. Hearing in all six children treated with AL remained within normal limits at the last day of assessment. Two of the 6 children treated with AL recorded improved hearing while 4 had no improvement in hearing of either both ears (3) or one (1) of the ears. In conclusion, neither ASAQ nor AL appears to cause hearing impairment following treatment of acute uncomplicated malaria. The observed improved hearing though still within normal limits suggests that some degree of mild hearing impairment may accompany acute uncomplicated malaria.

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ANALYSIS OF REPRODUCTIVE BARRIERS BETWEEN THE MOLECULAR FORMS OF *ANOPHELES GAMBIAE*

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The degree and nature of reproductive isolation between the molecular forms (M and S) of *Anopheles gambiae* has been the focus of intense research. Hybrids between the forms are virtually absent in sympatric areas. Although evidence implicated the contribution of assortative mating to the isolation, indirect evidence suggested selection against hybrids. However the specific mechanisms of isolation are not known. We tested i) that the forms mate in separated swarms in sypatric areas, 2) that the fitness of the hybrids and their backcrosses is lower than that of the pure forms. We studied the swarms of the molecular forms in Burkina Faso where the forms are sympatric. Out of 26 swarms, only four contained males of M and S forms. The frequency of mixed swarms was lower than that expected by chance based on the form composition in indoor resting collection. This suggests partial segregation between the swarms of the molecular forms, which probably contributes to their isolation. However, because the frequency of mixed swarms is too high to explain the low frequency of cross mating, we infer that mate recognition in a swarm is more important than swarm segregation. Multiple families (total of 154) representing all 12 possible combinations of crosses between the molecular forms and their hybrids were set up using forced mating between offspring of wild collected females near Bobo Dioulasso, Burkina Faso. The results showed that the reproductive output of hybrids and their backcrosses was equal or higher than that of the pure forms as measured by egg batch size, hatching rate, and development success into adults. No sex ratio distorsion was found among the offsprings. We conclude that postmating developmental barriers do not contribute to the isolation between the molecular forms.

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UNDERSTANDING THE ROLE OF RNA INTERFERENCE IN ARBOVIRUS-VECTOR INTERACTIONS

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RNAi is an important innate defense that insects have to control RNA virus invasion. RNAi is triggered by double stranded RNA (dsRNA) and degrades any mRNA with significant stretches of sequence identity with the dsRNA trigger. RNAi can be induced by viruses which form dsRNA intermediates

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as they replicate in permissive cells. In drosophila, the *Dcr2* gene product, Dicer-2, recognizes long dsRNAs and digests the dsRNA into 21-23 bp segments called short interfering RNAs (siRNAs). Dicer-2, a second protein, R2D2, and siRNAs combine with holo-RISC (RNA induced silencing complex) to form the RISC loading complex. The siRNA is unwound and R2D2 and one of the siRNA strands are displaced to form active RISC. The remaining siRNA strand (or guide strand) associated with RISC anneals to a homologous target mRNA and the protein argonaute-2 in RISC mediates mRNA degradation. Many of the genes (*Dcr2, Ago2, R2D2*) associated with RNAi have now been found in the *Aedes aegypti* genome. Functional assays have verified a role for several of these component gene products in RNAi. In transgenic *A. aegypti*, we have induced RNAi in the midgut to dengue virus type 2 (DENV2; *Flaviviridae*) by transcribing a DENV2 derived, inverted-repeat RNA (dsRNA) from the midgut-specific *carboxypeptidase A* promoter following ingestion of a viremic blood meal. These transgenic mosquitoes were highly resistant to midgut infection and dissemination and transmission of the different DENV2 genotypes. The resistance phenotype could be reversed when the RNAi pathway was impaired. Therefore the anti-viral properties of the RNAi pathway are functional in vector species when RNAi is triggered prior to, or concomitant with, virus infection. The question remains, how do arboviruses evade the RNAi pathway so that they can be successfully transmitted and maintained in nature? If we can understand how arboviruses circumvent RNAi, we might be able to successfully intervene in virus transmission.

(ACMCIP Abstract)

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AGE-STRUCTURE OF *AEDES AEGYPTI* POPULATIONS AND INTRA-ANNUAL VARIATION IN DENGUE TRANSMISSION

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Intra-annual (seasonal) variation in the number of dengue infections is a predictable pattern in most endemic areas. Explanations include (1) increased mosquito vector density during the rainy season due to more abundant larval development sites, (2) increased biting rate and/or decreased extrinsic incubation (EI) during warmer times of the year, and (3) increased survival during the rainy, more humid season. Results from earlier studies indicate that *Aedes aegypti* density does not significantly increase during the rainy season and biting rate is positively associated with seasonal fluctuations in dengue at some but not all locations. We tested the survival hypothesis by comparing *Ae. aegypti* population age structures across different transmission seasons. Using a cuticular hydrocarbon age-grading technique we estimated with greater accuracy than was previously possible chronological age of 1,333 female *Ae. aegypti* collected by aspiration from houses in 4 villages in northwestern Thailand during the 2000-2001 high and low dengue transmission seasons (4 collection periods). High transmission typically occurs from June-Nov and low transmission from Nov-March. Maximum estimated lifespan was the same across the low vs high 2000 transmission seasons (12 days), but was higher during the 2001 high than low transmission season (14 vs 10.3 days). Mean estimated age ranged from 4-5 days during low transmission seasons and 2-4 days during high transmission seasons. Using the Fisher Exact test with monte carlo simulation in Proc FREQ in SAS we did not detect ($P > 0.05$) significant differences in age structure across villages within or among collection periods even though temperature varied less (i.e., maximum temperatures were lower and minimum temperatures higher) during the high than low transmission seasons (t-test, $P < 0.05$). *Ae. aegypti* population age-structure, therefore, did not account for seasonal fluctuations in dengue transmission. We propose that differential affects of temperature on life span and EI drive seasonal variation in virus transmission; i.e., elevated minimum temperatures during the high transmission season reduce EI relative to population age-structure, which remains essentially unchanged from one season to the next. Consequently, more mosquitoes complete EI and transmit dengue virus during the warm than cool season.

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ENHANCEMENT OF *AEDES AEGYPTI* VECTORIAL CAPACITY BY VIRULENT DENGUE VIRUSES

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Dengue viruses associated with severe or hemorrhagic disease have been displacing less virulent strains in the Western Hemisphere: dengue serotype 2 viruses of American (AM) origin have been supplanted by viruses of Southeast Asian (SEA) origin. To investigate viral genetic differences contributing to this epidemiologic/ecologic displacement, we infected *Aedes aegypti* mosquitoes with six dengue serotype 2 viruses, three from each of these two genotypes. We measured viral RNA in midguts by quantitative RT-PCR and the presence of dengue antigen in midguts and salivary glands by indirect immunofluorescent antibody test, up to 14 days postinfection. Midguts infected with SEA viruses produced more viral RNA than did AM-infected tissues; however, at early timepoints, an AM virus produced more negative-strand, replicative RNA than did a SEA virus. Similarly, dengue antigen was evident in a larger proportion of midguts infected with SEA viruses than with AM viruses. Dissemination to the salivary glands was significantly faster for SEA viruses than AM viruses: 50% of salivary glands were infected by day 7 postinfection with SEA viruses, compared to day 14 for AM viruses. This drastic reduction in the extrinsic incubation period for SEA viruses leads to the potential of these mosquitoes to produce many more secondary cases (i.e. increased vectorial capacity). Depending on the probability of daily mosquito survival, SEA viruses may produce up to 65 times more secondary cases than AM viruses. This may be one of the mechanisms through which more virulent flaviviruses spread and displace others globally.

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A FIRST RESOLVED PHYLOGENY OF THE CULEX PIPIENS COMPLEX: MAIN SPECIES, SUBSPECIES, AND FORMS

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Mosquitoes in the *Culex pipiens* complex are principal vectors of several important wildlife and human diseases, including West Nile virus, St. Louis encephalitis, Lymphatic filariasis (elephantiasis), avian malaria and avian pox. As a whole this group of mosquitoes is found in every continent except for Antarctica, are often very numerous, and are closely associated with humans. Classically the complex includes *Cx. quinquefasciatus*, *Cx. australicus*, *Cx. pipiens pallens*, and finally *Cx. p. pipiens*, with its two forms "pipiens" and "molestus". Populations of mosquitoes in this complex have been shown to exhibit a wide array of epidemiologically crucial behavioral and physiological characteristics especially those linked to blood-host preference. Recent analyses using population level markers as well as variable introns have revealed both predictable genetic discontinuities between taxa as well as evidence of localized hybridization and introgression. The near absence of mitochondrial variability across the entire complex putatively due to selection driven by *Wolbachia pipientis* has made the phylogenetic analysis of the complex difficult. Besides sequencing three mtDNA loci (AT-rich region, ND4, and COI/II), we cloned and sequenced introns in three genes (acetylcholinesterase2, triosephosphate isomerase, and glucose-6-phosphate dehydrogenase) using an asymmetric PCR protocol with a proofreading polymerase. As outgroups we used the closely related species *Cx. pervigilans*, *Cx.*

torrentium, and *Cx. vagans*, as well as the more distantly related *Cx. restuans* and *Aedes vexans*. We were able to find fixed differences between all recognized taxa and create a resolved phylogeny for the *Culex pipiens* complex. An analysis of the scale of divergence across taxa allowed an evaluation of the timing and possible selective forces underlying the evolution of this group of important disease vectors.

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VARIATION IN VECTOR COMPETENCE FOR DENGUE 2 VIRUS AMONG COLLECTIONS OF *AEDES AEGYPTI* FROM THE YUCATAN AND VERA CRUZ REGIONS OF MEXICO

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The Southeast Asian genotype of dengue 2 virus (DENV-2) appears to be displacing the American genotype in the Western Hemisphere. Southern Mexico has historically recorded many dengue cases due to transmission by *Aedes aegypti* mosquitoes. Vector competence (VC) is defined as the intrinsic permissiveness of an arthropod vector to infection, replication, and transmission of a virus. Mosquito populations collected throughout Mexico in 2001 showed significant variance in VC for DENV-2, correlated with geographic location of the mosquito collections. Nineteen collections of *Ae. aegypti* eggs from selected locations in the Yucatan and Vera Cruz regions of Mexico were obtained in the summer of 2005, and each population was raised to adults and challenged orally with an artificial bloodmeal containing Southeast Asian genotype DENV-2 (JAM1409). Head and midgut tissues from the mosquitoes that had fully engorged were dissected 14 days post bloodmeal, and immunofluorescence assays (IFA) were performed on these tissues to determine the presence of DENV-2 antigen. Proportions with midgut infection barriers (MIB) and midgut escape barriers (MEB) were determined for each population. VC was defined as the proportion of total IFA positive head tissues in each collection. The VC for DENV-2 (JAM1409) of the collections ranged from 38% (Lerdo de Tejada) to 83% (Chetumal). There was an inverse correlation between VC and MEB. Populations from two sites exhibiting the highest and lowest VC, Chetumal and Lerdo de Tejada, were then challenged orally with a low passage American genotype DENV-2 (QRoo94). The VC of the Chetumal population dropped significantly to 37%, whereas the VC of the Lerdo de Tejada population remained relatively unchanged at 37%. These findings suggest that *Ae. aegypti* mosquitoes are genetically more susceptible to infection and transmission of Southeast Asian DENV-2 genotypes. This variance in vector competence also suggests coevolution of Mexican *Ae. aegypti* and the American genotype DENV-2.

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DOES WEST NILE VIRUS INFECTION DECREASE *CULEX TARSALIS* FITNESS?

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Although it was once assumed that arboviruses do not harm their vectors, evidence from recent studies indicates that this assumption may not be valid. West Nile virus (WNV) persistently infects many mosquito tissues including the midgut, salivary glands, nervous system, and fat body and has been associated with cytopathological changes in midgut muscles and salivary glands. However, it is not known if infection with WNV decreases mosquito fitness. We conducted a life table study of individually housed female *Culex tarsalis* to determine if WNV infection decreases mosquito survival and/or reproduction. After an initial blood meal from a WNV infected or uninfected chicken, mosquitoes were provided 10% sucrose ad lib and offered weekly blood meals via a hanging drop of defibrinated goose blood. WNV transmission status was determined weekly by testing the remaining blood drop for virus by plaque assay on Vero cell culture. Dead mosquitoes and eggs were collected daily. Mosquito legs and bodies were tested for WNV by plaque assay and eggs were counted and allowed to hatch. Two replicates of this experiment were performed with a total of 62 WNV-exposed mosquitoes (of which 21 became infected) and 43 unexposed mosquitoes. Although there were no gross differences in survival between infected and uninfected mosquitoes, infected mosquitoes exhibited significantly lower survival when adjusted for feeding rate, WNV titer, and average number of eggs per raft. Additionally, infected mosquitoes had significantly smaller egg rafts (100 vs. 129 eggs per egg raft) and a lower hatch rate (42% vs 60%) than uninfected mosquitoes. The percentage of mosquitoes feeding at each feeding opportunity was ~10% higher in infected compared to uninfected mosquitoes. A small amount of virus (average: 400 range: 50-5,000 PFU) was transmitted to the blood drops fed upon by infected mosquitoes starting at 10 days post-infection and continuing, in some individuals, throughout the entire lifespan. Our results indicate that WNV infection decreases reproductive output and may decrease survival in an important enzootic vector.

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ROLE OF SPHINGOMYELIN IN PREVENTING ACCESS OF ANTIBODIES TO LUNG STAGE SCHISTOSOMULA SURFACE MEMBRANE ANTIGENS

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Schistosoma mansoni and Schistosoma haematobium are the most prevalent and widespread of the species of schistosomes that cause schistosomiasis, a disease affecting more than 200 million people in 74 developing countries. Cercariae infect humans and animals by skin penetration and transform to schistosomula. Schistosomula reside in the skin for a few days before entering the blood circulation of the host, and then migrate through the vasculature successively to the lungs, liver, and portal vessels where they mature into adults. It is established that the lung-stage schistosomulum is the major target of natural and immune elimination of schistosomes in mice. Paradoxically, lung schistosomula apical membrane antigens fail to readily bind specific antibodies. This phenomenon is considered a major immune evasion mechanism; it was ascribed to antigen shedding, antigen masking by host molecules, or confinement of surface membrane antigens in immobile, lipid-rich sites in the external leaflet of the outer lipid bilayer. Support was given to the latter hypothesis as lung stage schistosomula surface membrane antigens were shown to bind specific antibodies following extraction of cholesterol by the membrane-impermeable, cholesterol-binding drug, methyl-β-cyclodextrin, or hydrolysis of sphingomyelin via activation of parasite, tegument-bound, neutral sphingomyelinase (nSMase). We herein report progress in Schistosoma nSMase gene cloning and expression, and attempt to explain how sphingomyelin prevents antibodies from accessing surface membrane antigens of intact lung stage schistosomula.

(ACMCIP Abstract)

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EXPRESSION OF FUCOSYLATED AND NON-FUCOSYLATED GLYCAN EPITOPES IN MIRACIDIA AND SPOROCYSTS OF *SCHISTOSOMA MANSONI*

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We report the use of previously defined anti-carbohydrate monoclonal antibodies to investigate developmental expression of the following terminal glycan epitopes on miracidia and sporocysts of *Schistosoma mansoni*: GalNAcβ1-4GlcNAc (LDN), GalNAcβ1-4(Fucα1-3)GlcNAc (LDNF),

GalNAcβ1-4(Fucα1-2Fucα1-3)GlcNAc (LDN-DF), Fucα1-3GalNAcβ1-4GlcNAc (F-LDN), Fucα1-3GalNAcβ1-4(Fucα1-3)GlcNAc (F-LDN-F), Galβ1-4(Fucα1-3)GlcNAc (Lewis X, Le^X), the repeating trisaccharide (-3Galβ1-4[Fucα1-3]GlcNAc β1-)$_n$ (Poly Lewis X, pLe^X), $Man_3GlcNAc_2$ (the mannosyl core structure), and the Circulating Anionic Antigen repeating glycotope (referred to hereafter as CAAg), (-6[GlcAβ1-4]GlcNAcβ1-)$_n$. Western blot analysis demonstrated the expression of LDN, LDN-F, LDN-DF, F-LDN, F-LDN-F, and the mannosyl core on both miracidial and 3-day sporocyst total protein extracts; glycoptope expression was evident on multiple proteins, many of which exhibited immunoreactivity with multiple antibodies. Miracidia and sporocyst extracts probed for Le^X, pLe^X, and CAAg exhibited only faint banding relative to control blots, indicating negligible expression. Although the glycan expression profiles of miracidia and sporocysts were qualitatively similar, quantitative differences in the number of immunoreactive bands and relative band intensities were observed. An immunofluorescence assay (IFA) confirmed the tegumental localization of LDN, LDN-F, LDN-DF, F-LDN, and F-LDN-F on miracidia as well as 2-day, 7-day and 15-day sporocysts, while Le^X, CAAg, and the mannosyl core were undetectable. The inability to detect the mannosyl core in IFA experiments suggests that proteins expressing terminal mannosyl core epitopes are localized internally and not at the tegument. Although preliminary IFA demonstrated pLe^X on miracidia and 2-day sporocysts, repeated trials failed to confirm this finding. Future experiments will more precisely characterize the glycotope expression profiles of miracidial and sporocyst surface proteins, while identifying individual tegument proteins that exhibit developmentally regulated glycosylation. By extension, we are interested in the developmental regulation of the glycosylation machinery, which determines the stage-specific expression of these glycotopes.

(ACMCIP Abstract)

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SCHISTOSOMA MANSONI AND *SCHISTOSOMA RODHAINI* IN WESTERN KENYA: A STUDY OF SPECIES BOUNDARIES

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We investigated the species boundary between *Schistosoma mansoni* and *S. rodhaini* in western Kenya. A natural hybrid of these species has previously been discovered where their ranges overlap in the Rift Valley region of Africa. Prior studies indicate that hybrids are successful at least to the F2 generation in the laboratory, and have a greater intermediate host range. Natural hybridization of these species is not only of public health interest because of potential affects on the pathobiology and transmission of *S. mansoni*, but also of evolutionary interest since it presents an opportunity to study the process of speciation of recently diverged species. Specifically, we examined how distinct these species are, and if distinct, how they maintain their identity despite hybridization. First, we established a technique to distinguish species and hybrids using molecular markers including sequences of mitochondrial and nuclear DNA and genotypes from 22 microsatellite loci. Second, we examined the prevalence of each species and hybrids in western Kenya through collection of snails in the Lake Victoria watershed. Finally, we examined the timing of cercarial emergence from snails as a potential isolating mechanism for these species. Field collections yielded both species of schistosomes, and hybrids or worms with hybrid ancestors. Timing of the emergence of cercariae differs between species; however, peak emergence only differs by 3 hours. This timing could allow considerable overlap in potential transmission times, which indicates that susceptible hosts can be exposed to both species of schistosomes simultaneously, and could possibly be facilitating hybridization.

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SURROGATE ESTIMATES OF SCHISTOSOME INFECTION INTENSITY ARE WAY OFF THE MARK

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In studies of human Schistosomiasis it is necessary to use indirect estimates of infection intensity to determine worm burdens, chemotherapeutic cure rates and vaccine efficacy. The parameters measured are faecal egg output and/or the level of parasite-derived antigens circulating in the blood (CAA and CCA). Unfortunately, it is not possible to establish the relationship between the worm burden and each of the surrogates, nor the threshold of detection in human subjects. In the course of experiments with the attenuated schistosome vaccine in the olive baboon, a highly permissive host for *Schistosoma mansoni*, we have generated a unique dataset that would be impossible to collect from humans. This has permitted us to establish the relationship between worm burden and faecal egg output, CAA and CCA in a primate host close in body scale to human children. Using worm burden as the definitive gold standard criterion, we demonstrated that the surrogate measures, faecal eggs and circulating antigens, consistently overestimated protection. The principal reason for overestimation was the threshold sensitivity of the assays, as determined by regression analysis of the surrogate parameters on worm burden. The insensitivity of the surrogate techniques was revealed by estimation of the threshold of detection from the regression equations, which on average were 40 worms. These observations have immense implications for diagnosis, evaluation of cure rates, and provide a powerful argument for the development of more sensitive measures of *S. mansoni* infection intensity before human vaccine trials begin.

(ACMCIP Abstract)

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LIVER FIBROSIS ASSOCIATED WITH EXPERIMENTAL *FASCIOLA HEPATICA* INFECTION: *IN VIVO* AND *IN VITRO* STUDIES

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The molecular mechanisms of liver fibrosis associated to *Fasciola hepatica* (FH) infection are unknown. Fas2, a cysteine proteinase derived from FH and considered a major antigen in the infection, might be involved in this process since it degrades collagen type I and elastin. Hepatic stellate cells (HSCs) are a major fibrogenic cell type in liver fibrosis. The aim of this study was to evaluate the mechanisms of liver fibrosis caused by FH *in vivo* (rat model) and *in vitro* (Fas2 fibrogenic gene expression in Hospital for Sick Children cell line LX2). *In vivo* study: 18 rats were infected with metacercariae of FH by gavage. Group I: 10 metacercariae (mild; n=6), Group II: 20 (moderate; n=6); and Group III: 30 (severe; n=6). Control group, n=3. Liver biopsies were performed at weeks 8 (early chronic

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infection) and 16 (advanced infection). Fibrosis was classified as mild (METAVIR F1-F2) or severe (METAVIR F3-F4). *In vitro* study: Serum-starved LX2 cells were exposed to Fas2 (1,5,10,20,30,50 and 70ug) for 24 hours. A cysteine proteinase inhibitor, E-67, alone or in combination with Fas2 were used as controls. The expression of the fibrotic genes: collagen-I, α smooth muscle actin (ASMA), PDGF-β receptor, TGF–β1 receptor, metalloproteinase (MMP-2) and tissue inhibitor of metalloproteinases I and II (TIMP-I and II) was evaluated by qRT-PCR. *In vivo*: The stage of fibrosis was directly correlated with the intensity of infection. At 8 weeks, Group I (n=6) = F1-F2:50%; F3-F4:33%. Group II (n=6): F1-F2:0%; F3-F4:33%. Group III (n=5): F1-F2:0%; F3-F4:67%. At 16 weeks, Group I (n=5; mortality 1 rat) = F1-F2:0%; F3-F4:60%. Group II (n=3; mortality 3 rats): F1-F2:0%; F3-F4:100%. Group III (n=0; mortality 6 rats). Compared to control group, analysis of the qRT-PCR data demonstrated a significant increase in mRNA levels of Coll1, PDGF-βR, MMP-2 and TIMP-I. *In vitro*: Compared to controls, LX2 cells exposed to Fas2 at doses ≥ 5ug increased the expression of fibrotic genes: Coll1, ASMA, PDGF–βR and TIMP-II. Cells incubated with Fas2+E-67 showed expression levels as control cells, indicating that protease activity of Fas2 is required for its fibrogenic activity. We conclude that the up-regulation of mRNAs expression of Coll1, PDFG–βR, MMP-2 and TIMP-I (*in vivo*) and Coll1, ASMA, PDFG-βR and TIMP-II (*in vitro*) suggests that FH infection is closely associated with hepatic fibrosis and Fas2 antigen might be a factor associated to the activation of Hospital for Sick Children in this disease.

(ACMCIP Abstract)

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TOWARDS SCHISTOSOMIASIS TRANSMISSION CONTROL AND ELIMINATION: CAN SNAIL PREPATENT INFECTION RATE SERVE AS AN INDICATOR OF RESIDUAL TRANSMISSION POTENTIAL?

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Large-scale drug treatment programs are a first approach to reducing schistosomiasis morbidity, yet such programs may not significantly affect parasite transmission in highly endemic areas in sub-Saharan Africa. As a result, the sustainability of their impact remains unsure. Residual infection and reinfection are likely to be associated with persistent 'subtle' morbidities such as anemia and undernutrition, and from a public health perspective, the projected indirect benefits of transmission control and the ultimate need for parasite elimination need to be reexamined. To determine whether extended long-term control projects can also achieve long-term reduction of transmission, a practical tool is required for rapid and sensitive transmission monitoring. We propose that newly available DNA-based monitoring of prepatent schistosome infection in snails, which is detectable from the earliest stages of snail infection, can be simplified and used as a practical indicator of transmission potential, representing, as it does, a summation of factors mediating human-to-snail transmission. Current results for this form of 'xenomonitoring' suggest a significant association between prevalence (and mean intensity) of human *Schistosoma haematobium* infection and the levels of prepatent infection among host bulinid snails in nearby water-bodies. Application of a new assays for species-specific identification of S. haematobium within snail extracts or blots, as well as emerging ambient-temperature molecular tools for field monitoring of prepatent snail infection, will enable effective assessment of transmission potential over extended regions and for expanded periods of time. Integration of transmission results will indicate the best means to optimize (or supplement) drug-based control in the design of the next generation of schistosomiasis control initiatives.

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LOCAL VARIATIONS IN *SCHISTOSOMA HAEMATOBIUM* TRANSMISSION IN MSAMBWENI, KENYA

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Urinary schistosomiasis is a significant cause of morbidity in Msambweni on the southern coast of Kenya, where transmission is associated with *Bulinus nasutus* snails in temporary ponds. Given that aquatic habitats in Msambweni vary with regard to their connectivity and suitability for sustaining *B. nasutus* populations, spatial patterns of human infection with *Schistosoma haematobium* need to be considered in the context of the entire water drainage system, rather than in association with an individual pond. Clustering of spatial and temporal patterns of children's infection densities were assessed as a function of household distance and direction to several water sources, including those infested with *B. nasutus* snails during two study periods, 2000 and 1984. The spatial extent and degree of infection clustering were quantified. Clusters of high and low levels of infection among school age children were detected. High infection levels were clustered around ponds containing snails shedding *S. haematobium* cercariae, while low infection levels were concentrated near a river, where *B. nasutus* is rarely found. The spatial distribution pattern of infections varied by age groups and by study periods. The predominant transmission focus around which high levels of infection were clustered varied between the two study periods. In 2000, high infection levels among younger children were clustered more closely than those of older children around the main transmission focus. In older children, high infection levels were clustered both near the 1984 focus and near the 2000 focus, suggesting some residual effects of the older focus on this group. Simultaneous consideration of the effects of infested and uninfested aquatic habitats on human infection patterns will allow for more targeted application of control measures aimed at interrupting *S. haematobium* transmission.

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GENETIC VARIABILITY, POPULATION STRUCTURE AND PHYLOGEOGRAPHY OF ARGENTINIAN AND OTHER SOUTHAMERICAN *TRIATOMA INFESTANS* POPULATIONS BASED ON *COI*

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Knowledge on the genetic variability, population structure and evolutionary history of *Triatoma infestans* may be useful for developing vector control strategies. A total of 222 bugs from nine Argentinian provinces, and individuals from Uruguay, Peru, and Bolivia (including domestic bugs, sylvatic bugs and one dark morph), were sequenced for a 661bp fragment of the mtDNA gene *COI*, and several population genetics and phylogeographic analyses were performed. High levels of nucleotide variation were found within Argentina, with 37 variable sites distributed among 30 haplotypes. Total heterozygosity ranged from 0.0097 to 0.0052 according to $_{w}$ and estimators, the highest value reported for *T. infestans* to date. The domestic Bolivian sample showed four haplotypes not found in Argentina, and had lower levels of nucleotide variation. Three of these haplotypes were shared with the sylvatic Andean bugs, indicating a possible domestic origin. The dark morph, in contrast, exhibited a private haplotype. Departures from neutrality expectations suggest a recent population expansion of *T. infestans* in Argentina, whereas AMOVA and

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K_{ST}^* analysis support a strong population structure, but not following an isolation by distance model as appeared to have operated in Bolivia. A haplotype network showed an Argentinian/Uruguayan clade and a Bolivian/Peruvian clade. The dark morph, however, was separated from these two clusters by seven mutational steps, suggesting that it is an ancient form, strongly isolated from the remaining populations. Interestingly, the Argentinian/Uruguayan clade had a general star-like shape but also five highly divergent haplotypes. This unusual haplotype structure may be explained by incomplete lineage sorting or population admixture. These results arise new questions about the genetic variability of *T. infestans* and its potential response to selective forces such as insecticide spraying, the gene flow between sylvatic and domestic bugs, and the historical patterns of dispersal of the species.

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EFFECTIVENESS OF COMMUNITY-BASED SELECTIVE INSECTICIDE SPRAYING ON REINFESTATION BY *TRIATOMA INFESTANS* IN NORTHWESTERN ARGENTINA

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The interruption of vector-borne transmission of Chagas disease in Central and South America relies mostly on reducing triatomine bugs infestation using residual insecticides and incorporating community participation. As a part of a larger project on the eco-epidemiology of Chagas disease in northwestern Argentina, we assessed the effects of selective residual insecticide spraying by residents on the reinfestation pattern of *Triatoma infestans* in a rural community. A community-wide insecticide spraying was applied in 1992, and in 1995-96 surveillance activities were transferred from the National Vector Control Program and research team to the communities. In 1997-2002, the capture of one *T. infestans* bug in any domestic or peridomestic site prompted selective insecticide spraying by residents of each compound. Spraying was evenly distributed between domestic and peridomestic sites. A higher proportion of sites was sprayed in November 1997-May 1998 (49%) than in 1998-2002 (10-34%) with an overall spraying coverage of infested sites 45% (63/169), decreasing from 79% in November 1997 to 15% in May 2000, and increasing in late 2002 (36%). Selective spraying by residents resulted in a sharp reduction of infestations by *T. infestans* - from 25% (56/226) in 1997 to 4-13% (11/298- 47/350) in 1998-2002. Of the sites that were found infested in 1997 and were immediately sprayed by residents, 8% (3/37) were found reinfested six months later, a 12.5 fold reduction in the prevalence of infestation. In contrast, for 131 non-infested sites in 1997 (either sprayed or not), reinfestation was only 2-3% six months later. Thus, following selective spraying by residents, 5-6% of the sites were infested one year after the peak of spraying activities in 1997, and 10-13% were infested following less intense spraying in 2000-2002. Selective spraying by residents proved to be an effective strategy to reduce the infestation by *T. infestans* only when a high spraying coverage of infested sites occurred within six months of *T. infestans* detection.

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TICK ACTIVITY DURING 2005-2006 ON THE TEXAS A&M INTERNATIONAL CAMPUS (WEBB COUNTY, TEXAS)

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We conducted a one year study of tick activity on the Texas A&M International University Campus beginning March 2005. During this time we collected and identified over 14,000 ticks. 98% of the ticks were *Amblyomma cajennense*, 2% were *A. inornatum*. We also detected low levels of *A. maculatum*(<0.1%), *Dermacentor variabalis* (0.4%), *D. albipictus* (0.2%), *D. halli* (<0.01%) and Haemaphysalis leporispalustris (0.2%). In spite of abundant deer activity on campus we did not detect any *Ixodes scapularis*. Tick activity peaked during the Spring and Fall, dropping to almost no activity in the summer and winter. Larval *A cajennense* was observed to mostly hatch during the Fall although larva were detected through winter into the Spring. Peak larval activity was from late September through early November. Nymph activity reached highest levels during the Spring. Nymph activity showed a negative correlation with higher temperature (3 week mean temperature of 75-84 - median of 7.9 ticks per trap, 3 week mean temperature of 85-94 - median of 1.75 ticks per trap). Adult ticks showed a positive correlation ($p < .05$) with increased temperature (3 week mean temperature of 75-84 - median of 0.2 ticks per trap, 3 week mean temperature of 85-94 - median of 0.34 ticks per trap). *A. cajennense* is known to be a vector of rickettsias, including RMSF, Thai tick typhus, and *Rickettsia amblyomii*. *D. variabalis* is known to be a vector of RMSF, ehrlichiosis, tularemia, and others. At this time *A. inornatum* and *A. maculatum* are not well characterized as vectors of disease. We are now determining the prevalence of tickborne diseases in these ticks. In conclusion, unlike in northern climates where tick populations increase throughout the summer, in the South Texas ecosystem there is a decrease in tick activity during the summer months. The dominant tick species on the TAMIU campus was *A cajennense*.

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A LONGITUDINAL STUDY OF THE SAND FLY POPULATION IN BARAOULI, MALI

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For several decades cutaneous leishmaniasis (CL) has been sporadically reported from Mali. *Phlebotomus duboscqi* was suspected to be the primary vector of the disease. However, its involvement in the transmission of CL remains unknown. The aim of this study was to determine the temporal composition and abundance of the sand fly population and identify the sand fly species responsible for transmission of CL in Sudan savanna areas of Mali. We carried out a monthly survey in two neighboring villages (Kemena and Sougoula), located 5 Km apart in Baraouli District in the Sudan savanna areas of Mali where CL cases have been reported.Collection of sand flies was performed from October 2004 to September 2005 using Centers for Disease Control and Prevention light traps and aspiration peridomestically, and oiled paper sheets in both peridomestic and sylvatic niches. The blood feeding preference of *P. duboscqi* was investigated using PCR aimed at distinguishing human, cattle, rabbit, etc blood. Of 7539 sand flies collected over 12 months from the village of Kemena, the genus *Phlebotomus* represented 24% (n=1810) and that of *Sergentomyia* 76% (n=5729). Of the *Phlebotomus* sand flies collected, 99% were *P. duboscqi* with *P. rodhaini* representing less than 1%. The genus *Sergentomyia* was mainly composed of 11 species dominated by S. *schwetzi (35%)*, followed by *S. dubia*, *S. africana*, *S. antennata* and, *S. clydei*. *P.duboscqi* represented up to 90% of the total monthly collection of October. The peak of the sand fly season (including *P. duboscqi* and *Sergentomyia sp*) was observed in April during the dry and hot season. *P. duboscqi* was present all year long and was mainly collected by Centers for Disease Control and Prevention light traps indoors from human dwellings. In contrast, *S. schwetzi* and other *Sergentomyia* flies were mostly collected using sticky traps placed outdoors and outside the villages. The proportion of blood fed *P. duboscqi* represented 22%. None of 9 groups of sandflies (8 sandflies/group) tested had evidence of blood from pig, dog, goat, sheep, or cow. All groups tested positive for human blood. indicating that *P. duboscqi* is anthropophagic. A similar pattern of the sand fly population distribution was found in the neighboring village of Sougoula. In conclusion, the high frequency of *P. duboscqi* combined

with its anthropophilic/anthropophagic behaviour suggests that this species may be the vector of CL in this region of Mali.

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CASE REPORT OF *VIBRIO CHOLERAE* ASSOCIATED WITH SEVERE GASTROENTERITIS IN A US TRAVELER

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Vibrio cholerae, a common cause of profuse watery diarrhea and epidemics, is well known to humanity as early as 1563. Better sanitary hygiene has contributed in literally extinction of this disease from developed countries. However, because of better and quick modes of transportation, sporadic cases have been known to occur in non-endemic areas of the world. Cases of cholera rarely occur in the United States, and cholera epidemics are unlikely. Five cases were reported last year in USA. Rare incidence of cholera can not only result in delayed but also missed diagnosis by our physicians since special instructions are required for the isolation of this pathogen by our laboratories. Routine culture media does not grow *Vibrio cholerae*. 25 to 50% of typical untreated cases can be fatal. Here we report a case in a traveler who presented with watery diarrhea and severe metabolic acidosis. Colonoscopy revealed copious amount of fluid and punched out lesions in colon. Biopsy of the lesions confirmed inflammation of crypts. Early diagnosis with aggressive hydration resulted in decreased morbidity and potential spread of the disease. Cholera remains a small but persistent risk in the United States and for travelers. With advances in traveling modalities, diseases like cholera should always be considered in any traveler coming from endemic area and presenting with rice water diarrhea and dehydration.

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THE CARRIAGE OF ENTERIC PATHOGENS BY DOMESTIC COCKROACHES IN AN URBAN AREA OF NORTH QUEENSLAND, AUSTRALIA

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Hind gut sterilely collected from 150 domestic cockroaches captured from a variety of locations in Townsville were macerated and inoculated on to selective enrichment culture media for the isolation of *Salmonella, Shigella* and *Campylobacter.* The carriage rate of Salmonella was 14.7%. The most common serotype was *Salmonella thompson,* followed by *Salmonella aberdeen* and *Salmonella welikade.* Neither *Shigella* nor *Campylobacter* were isolated. This study highlights the potential role of cockroaches in the spread of *Salmonella* in North Queensland and should prompt similar investigations elsewhere.

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MYCOBACTERIUM AVIUM COMPLEX PULMONARY NODULE: THE MIMICKER OF MALIGNANCY

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Mycobacterium avium complex(MAC) organisms are ubiquitous and have been found in soil, water, food and animals. *MAC* has now surpassed tuberculosis(TB) as a more common respiratory pathogen. The most important factor for acquisition appears to be underlying lung disease. *MAC* pulmonary infection was seen almost exclusively in men with lung disease in earlier studies. Now, more women seem to be afflicted. Patients often have a chronic cough. The chest radiograph can include infiltrates, cavitation or nodules. Isolated nodules have been reported but this may be an underestimation since cultures are not routinely done on surgical specimens. Nodules that show granuloma or acid-fast bacilli(AFB) are assumed to be due to TB. *MAC* nodules typically do not grow over time, are not >1cm and have not been studied with positron emission tomography(PET). We present a case of a woman with multiple nodules, one of which grew rapidly to >2.5cm, initially felt to be malignancy and ultimately determined to be infectious. Case: A 75-year-old woman prior smoker presented with a pulmonary nodule that doubled in size over a 6 month period on serial chest computerized tomography(CT) scans. Her PPD test was negative. She had a chronic cough without other symptoms. The CT scan of the chest showed a 2.5cm focal opacity in the upper lobe of left lung and a nodular opacity 1.7x1.3cm within the lingula; the latter demonstrated abnormal metabolic activity consistent with a malignant nodule on PET scan. A focus of abnormal metabolic activity was noted in the right lower lobe as well. A fine needle biopsy yielded only minimal interstitial fibrosis. A left upper lobectomy showed a necrotizing granuloma without AFB which grew *MAC*. In conclusion, there have been <100 case reports of pulmonary nodules due to *MAC*. The size has ranged from 15-50mm, much smaller than our patient's. Some feel surgery is curative whereas others feel that micronodules may exist and relapse. We suggest all biopsied pulmonary nodules be cultured, since all necrotizing granulomas are not TB and therapy is different.

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THE IMPACT OF TUBERCULOSIS AND CANCER IN THE ASIAN COMMUNITY

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Approximately one to five percent of tuberculosis (TB) patients have coexistent cancer. This is particularly important in the Asian community where TB is endemic and smoking is widely prevalent. Previously, the emphasis has been on the inappropriate delay of diagnosing lung cancer in the setting of TB; this can average between 6-9 months. Paradoxically, the diagnosis of TB may be overlooked in the pursuit of malignancy in this population. Acid-fast bacilli stains and mycobacterial cultures are not ordered routinely in the workup of lymph nodes and nodules. Patients may undergo immunosuppression with chemotherapy agents and any concurrent or latent infections must be addressed as well. Cases: Two patients presented in early 2006 with the presumptive diagnosis of malignancy based on CT findings. Both underwent a surgical procedure involving either partial lobectomy and/or lymph node biopsy. Both were Asian and were long time smokers. None had constitutional symptoms suggestive of an infectious disease. Because of the endemic population and findings of necrotizing granulomata on biopsies, mycobacterial cultures were also requested along with the pathologic specimens. To our surprise, both cancer and *M. tuberculosis* were diagnosed. The cancers found were Squamous cell and Small cell. In conclusion, the concurrent diagnosis of TB with lung cancer is often overlooked. When these diseases affect different locations of the lung, it may be easier to make the diagnosis of both diseases. The mechanism of how an individual can develop both diseases can be dual 1) scar tissue plays a role in lung cancer or 2) lung cancer may develop in a region where inactive TB resided, thereby causing reactivation. In endemic areas, TB must be aggressively ruled out even when not suspected. Radiologic findings that support both TB and cancer may include progression of one area while another is regressing, a > 3cm mass lesion with infiltrative disease, hilar lymph nodes in chronic pulmonary TB and post-obstructive atelectasis. Once lung cancer treatments are instituted, it may be crucial to identify underlying TB since these patients will undergo immunosuppressive regimens and make an infectious disease progress. We suggest that both mycobacterial culture and pathologic specimens be ordered when lung nodules and lymph nodes are excised especially in the Asian population.

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IN VITRO AND IN VIVO MODELS FOR THE INVESTIGATION OF PROTECTIVE IMMUNITY AGAINST LEPTOSPIRA INTERROGANS

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To investigate whether anti-leptospiral hyperimmunity in an area of endemicity confers protective immunity towards subsequent infections with a *Leptospira interrogans*, *in vitro* and *in vivo* models of infection were employed. Serum was collected from previously identified individuals with high anti-leptospiral titers from the hyperendemic area of Iquitos, Peru and intraperitoneally injected into guinea pigs which were later infected with a strain of *L. interrogans* isolated from a patient in Iquitos. Following infection all animals were observed clinically, including weighing animals daily, for 14 days or until death, then autopsied and kidney, liver and lung samples harvested for determination of leptospiral burden using RT-PCR targeting the 16S rRNA gene. For an *in vitro* correlate to infection, *L. interrogans* were incubated in 40% human serum for two hours at 37°C and then incubated in EMJH culture media for 4 days at 28°C. Following incubation, viable Leptospira were quantified using a Petroff-Hauser counting chamber. In the *in vivo* studies, the group of guinea pigs (n=5) injected with hyperimmune human serum exhibited a mean survival period of 10.6 days following challenge infection. In the cohort of animals receiving normal human serum (n=6) the mean survival following infection with *L. interrogans* was 10.2 days. Clinically there was a greater difference between groups as animals in the hyperimmune serum group had a mean of 11.0 days until a 10% decrease in body weight was observed, while the control group had a mean of 8.8 days. Upon autopsy, all animals exhibited characteristic lesions including pulmonary hemorrhage, retroperitoneal hemorrhage and jaundice. The *in vitro* experiments demonstrated a leptospiral survival rate of 33.4% in hyperimmune human serum versus 38.4% in the normal human serum. Results from RT-PCR quantification of leptospiral burden are pending. The data indicate that passive transfer of antibodies delays clinical symptoms but does not protect from lethal infection. These data raise the possibility that protective immunity in humans be not entirely be due to serovar-specific anti-leptospiral antibodies.

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EVALUATION OF *AOTUS NANCYMAE* AS AN ANIMAL MODEL FOR *BARTONELLA BACILLIFORMIS* INFECTION

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Bartonellosis, caused by the bacterium *Bartonella bacilliformis,* is a complicated, multi-stage infectious disease that is almost exclusively limited to the Andes mountain region of South America due to the limited habitat of its sand fly vector, *Lutzomia verrucarum*. The disease is characterized by an acute bacteremic phase usually beginning 2-3 weeks after exposure causing fever and red blood cell destruction (Oroya Fever or Carrion's Disease) and a more chronic phase that can manifest months after primary exposure that causes the growth of warty, skin nodules (Verruga Peruana). In order to properly study this disease, a reliable animal model must be developed that approximates the condition in humans. An experimental study was designed to evaluate the susceptibility of the New World monkey, *Aotus nancymae,* to *B. bacilliformis* infection. Two groups of three animals each received 1.13×10^8 cfu and 1.13×10^6 cfu *B. bacilliformis* by the intradermal (ID) and intravenous (IV) routes, respectively, and were observed for the development of clinical signs for 140 days. The animals were subsequently monitored daily over a 7 week period for acute clinical signs of bartonellosis and were evaluated weekly for evidence of infection, including physical exam, CBC, and hematocrit; confirmation of infection was determined by Giemsa-stained blood smear, PCR and blood culture. To date (Day 21 post-inoculation) the blood smears from two animals in each group are positive for erythrocyte infection (1-4% of erythrocytes) and one animal from each group developed dermal lesions. Lesion histopathology and culture results are pending. Culture and PCR analysis of blood samples have thus far been negative, which is not surprising with such a low bacterial burden. CBC and hematocrit results have been unremarkable and no difference is observed from baseline values. Serologic analysis of inoculated animals using stored sera from blood draws during the evaluation period will be performed at the conclusion of the study and results will be presented. This work demonstrates that *A. nancymae* are susceptible to infection with *B. bacilliformis* by both the IV and ID routes manifesting similarities to acute bartonellosis in humans (i.e. erythrocyte invasion), while other acute clinical signs (i.e. febrile anemia) have yet to be observed. Animals are currently being evaluated for signs of the chronic, verrucous stage of disease.

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LATENT RISK OF REEMERGENCE EPIDEMIC TYPHUS IN MEXICO

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Until the first half of the 20th century, like in other countries in the world, the epidemic typhus was a great public health problem. In 1951, Mexico began the National Campaign against epidemic typhus focused in 18 states. In 1964 the epidemic encompassed 8 states. In 1969 the last outbreak was registered. In 1983 in Chiapas there was an outbreak of 33 cases with lethality of 42% and in 1986, 51 cases with lethality of 18% and in 1983 there were an outbreak in the State of Mexico with 22 cases with lethality of 5%. Although in Mexico there have not been registered epidemic typhus outbreaks, the risk is present for the following reasons: 1) Isolated cases exist and are probably not diagnosed or misdiagnosed as other illness. 2) Persistence of marginalized groups with body lice. 3)There exists a high percentage of adult and elderly population that previously had typhus. They are potentials reservoirs or *Rickettsia prowazekii* and they can present recrudescence (Brill Zinsser illness). 4) The existence of flying squirrels Glaucomys volans in an area from Tamaulipas to Chiapas, México. These animals are potentials reservoirs of *R. prowazekii* through their ectoparasites could transmit the infection to humans. 5) The probability that other arthropods could be vectors of epidemic typhus illustrated by the discovery of *R. prowazekii* in ticks collected in the state of Nuevo Leon, Mexico. The ticks are reservoirs of rickettsiae and have transovarian transmission for many generations. 6) The ticks are highly abundant in tropical and subtropical areas, as is the greater part of Mexico. 7) Recent seroepidemiologicals studies in Nuevo Leon and Veracruz, Mexico, showed high seroprevalence to *R. prowazekii* 11% and 37% respectively. The population studied included children situation that suggest actually circulations or R. *prowazekii*. In summary, these facts suggest that in Mexico *R. prowazekii* had been transmitted or is transmitted epidemically in humans through body lice and endemically through ticks. The fact that ticks can be infected with rickettsiae facilitates the transmission to animals who could be reservoirs for *R. prowazekii.* These results raises important clinical epidemiological questions that could drive the study of this illness, theirs vectors, and reservoirs. These pathologies continue to be present and refuse to stay in the historical past.

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INDUCTION OF MUCOSAL IMMUNE RESPONSE TO *MYCOBACTERIUM TUBERCULOSIS* USING MICROSPARTICLE ENCAPSULATED WHOLE DEAD CELL ANTIGENS

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The *Mycobacterium bovis* Bacilli Calmette-Guerin (BCG) tuberculosis vaccine is not recommended for children and adults who are infected with HIV because of the potential adverse reactions associated with the use of live bacteria in these persons. Also, most vaccines including BCG are administered systemically and they, therefore, stimulate only poor mucosal immunity to prevent the establishment of infections. The objective of the project, therefore, was to develop biodegradable microspheres for oral delivery of *M. tuberculosis* dead cell antigens and to investigate the induction of mucosal immune response to the antigens. Encapsulated *M. tuberculosis* dead cell microspheres were produced by spray-drying using glutaraldehyde cross-linked BSA as the polymer matrix. Parameters for the spray drying were optimized for protein formulation. Particle size and Zeta potential were determined using Spectrex Laser Particle Counter PC-2000 and Malvern Zetasizer respectively. DSC and FTIR were used to measure changes in melting point and the major peaks of the protein. The bioactivity of the antigen was confirmed by probing with antigen specific antibodies. In vivo studies were done on six rats and boosters of the antigens were given on weeks 1, 10 and 12. Serum, saliva and faecal samples were collected on zero time, and weeks 1, 3, 7 and 18. Nasal wash was collected on week 18. Antigen specific IgG and IgA in the serum and IgA in the mucosal samples were determined by ELISA. The particle sizes and zeta-potential of the microspheres were ideal for uptake by immune cells. There were no significant changes in the physico-chemical characteristics and biological activity of the encapsulated antigens. There was significant production of antibodies to the *M. tuberculosis antigens* in both the serum and mucosal surfaces in test animals. Unlike BCG, the antibody titres increased with boosters of antigens. In conclusion, the results showed that micro-encapsulation with BSA by the spray drying method did not affect the bioactivity of the antigen. The oral administration was also successful in inducing both systemic and mucosal immune responses.

(ACMCIP Abstract)

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USE OF SYSTEMIC SCOLICIDALS [BEZIMEDAZOLE] IN HYDATD CYST SURGERY

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Hydatid Cyst (HC) constitutes a major health problem in Iraq and Yemen. Until recently, surgery was the only option for the treatment of echinococcal cysts. However, experience is growing with two other potential treatment modalities: chemotherapy and the PAIR procedure. As recurrence rate continue to be high and eradication or prevention is not optimal, a combination of approaches now is often instituted. This study was conducted to evaluate the effectiveness of combined use of surgery and Benzimidazole in reducing recurrence rate and preventing secondary hydatidosis. A prospective trial using combined conventional surgical treatment (using hydrogen peroxide as local scolicidal intra-operatively) with oral Benzimidazoles therapy (two weeks preoperatively and 2 - 4 weeks post operatively except for patients with acute presentation were given 4 weeks post operatively). The study was conducted in 3 teaching hospitals (two in Iraq between 1985 -1998 and 1 in Yemen between 2001 -2002). Reducing recurrence and preventing secondary hydatidosis were the primary end point. Small cysts situated in the liver were excluded. Seventy one patients were included in the study. Patients were followed every 3-6 month with routine clinical examination and imaging (Ultrasound and X-rays) for up to 5.7 years. Seventy percent were female. Mean age of patients was 31 years old (range 5-60). Liver was involved in 87% of cases (17% of which had other organ involved). Most common presentation was abdominal pain. Other organs infected were Lung, thyroid gland, spleen and bone. Only 4 cases presented acutely (two cases with anaphylaxis and other 2 with major intra-biliary rupture). By using the above protocol, we had recurrence rate of 8.4% and found no secondary HC on further follow up. In addition, we had no associated mortality. In conclusion, safe, recurrence free treatment of HC can be achieved using combined approach of surgery and short course of perioperative oral scolicidal agents.

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DISEASE MANAGEMENT IN ALVEOLAR ECHINOCOCCOSIS GUIDED BY PET-CT

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[^{18}F]Fluoro-Deoxyglucose-Positron-Emission-Tomography (PET) has been shown to demarcate areas of parasitic activity in alveolar echinococcosis (AE). Further progress was made when the integrated PET-CT became available, enabling the correlation between disease activity and anatomic structures. We evaluated the accuracy of PET-CT for monitoring the course of AE in different therapeutic settings. Between 2002 and 2005, PET-CT (Discovery LS) was performed in 91 AE-patients and we analyzed the number, location, extension, calcifications and metabolic activity of hepatic AE-lesions. 46 patients had unresectable AE-lesions and were receiving benzimidazole treatment at the time of PET-CT (group 1). 15 patients had newly diagnosed AE (group 2) and in 30 patients hepatic AE-lesions had been previously operated (group 3). Metabolic activity around AE-lesions was found in 36/46 cases in group 1. In group 2, lesions in 13/15 patients were metabolically active and only 4 patients had lesions that were classified as resectable in toto. In group 3, active lesions were detected in 11/30 patients with extrahepatic affection in 8/30. Metabolic activity was detectable in 5 lesions despite extensive calcifications. Extrahepatic disease was found in a total of 24 cases with affection of abdominal organs(10), thoracic organs(9), bones(3) and lymph nodes(2). Nine patients had discontinued benzimidazoles in 2002 due to lack of metabolic activity. In 2005, 3/9 showed newly detectable metabolic activity and drug treatment had to be restarted.

In conclusion, PET-CT is a valuable tool in the management of this chronic parasitic disease. PET-CT allows for accurate planning prior to hepatic surgery, because wider safety margins can be foreseen around metabolically active areas. Extrahepatic disease and small residual hepatic disease is often difficult to detect by CT alone. Integrated PET-CT has increased diagnostic accuracy during postoperative follow-up by detection of minor alterations of metabolic function and the precise correlation with underlying anatomic structures. In patients with inoperable disease and long-term medical treatment, interruption of benzimidazole treatment should only be attempted in patients with inactive lesions. However, disease activity may recur even after extended periods without drug treatment, warranting long-term follow up with PET-CT.

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RISK FACTORS FOR CYSTIC ECHINOCOCCOSIS IN PERUVIAN PATIENTS

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Human echinococcosis caused by *Echinococcus granulosus* is a serious public health problem in sheep raising areas of Peru. We conducted a case-control study to determine risk factors for infection with *E. granulosus* in Peru. Thirty one patients who underwent surgery for cystic echinococcosis (CE) at Hospital Nacional Hipolito Unanue in Lima city were selected as cases and were matched by age, gender and place of longest residence with 31 population controls. Initial matched univariate analysis found that cases were more likely than controls to have worked at home in the past (OR=8.0; 95% CI:1.0-63.9), slaughtered livestock in the field (OR=8.0; 95%CI=1.0-63.9), fed viscera to dogs (OR=4.0; 95% CI:1.1-14.2), played frequently with dogs before age 15 (OR=9.0; 95%CI=1.1-71.0), kept shepherd or guardian dogs at home (OR=12.0; 95%CI=1.6-92.3), and have observed dogs being fed with hydatid infected viscera (OR=5.5; 95%CI=1.2-24.8). Consumption of tap water without boiling (OR=0.1; 95%CI=0.01-0.9), and storage of water in covered containers (OR=0.3; 95%CI=0.9-1.2) were inversely related to CE. In multivariate analysis with conditional logistic regression, having played frequently with dogs before age 15 (OR=30.3; 95%CI=2.1-443.1) and storage of water in covered containers (OR=0.1; 95%CI=0.01-0.8) were statistically significant. In this study, close contact of humans and dogs through play was an important risk factor for CE. In addition, our findings suggest that covering containers used for water storage may limit their contamination with eggs of *E. granulosus*.

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GROUP A STREPTOCOCCAL DISEASE IN MALI: A PILOT STUDY

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Group A Streptococcus (GAS) is one of the most common and versatile human pathogens, causing superficial and invasive infections as well as rheumatic fever and other immunologic sequelae. Recently, multivalent M type-specific vaccines have shown promise in trials. To use such vaccines in developing countries, the GAS burden and distribution of *emm*-types must be characterized. Throat culture was performed on 157 5- to 15-year olds living in Bamako, Mali and seeking care for pharyngitis at the Otolaryngology Clinic of the major children's hospital (Hôpital Gabriel Touré [HGT]) over a rainy (June to Sept 2003) and dry season (January to April 2004) and on 733 asymptomatic controls. A lesion culture was obtained from 181 children with impetigo at a Dermatology Clinic at the Centre National d'Appui à la lutte contre la Maladie. Blood cultures were obtained from 0- to 15- year-olds admitted to HGT with fever ($\geq$ 39°C) and/or suspected invasive bacterial infection (n=5514, June 2002 to May 2005). GAS isolates are *emm*-typed according to the Centers for Disease Control and Prevention and Prevention protocol. GAS was cultured from the throat of 21 cases of pharyngitis (13%) and 41 controls (6%) and from the lesion of 61 cases of impetigo (33%). Seasonal and age variations were not apparent. Fifteen cases of invasive GAS disease were identified, including sepsis (7), bacteremic pneumonia (2), empyema (1), meningitis (3) and soft tissue infection (2). Two children with invasive infection died (case-fatality 13.3%). The annual incidence of invasive infection in Bamako was 1.3, 1.1, and 0.3 per 100,000 children ages 0-11 months, 1-4 years, and 5-15 years, respectively. *Emm*-typing (in progress) of 21 throat isolates identified type 77 (3), type 65 (2), and types 3, 4, 44, 55, 65, 71, 79, 81 and 82 (1 each). In conclusion, these preliminary results suggest that GAS causes invasive and noninvasive infections among Malian children. *Emm*-type distribution of pharyngitis cases appears to be broad and dissimilar to that targeted by current type-specific vaccines.

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GAMETOCYTAEMIA AFTER DRUG TREATMENT OF ASYMPTOMATIC *PLASMODIUM FALCIPARUM*

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Treatment of *Plasmodium falciparum* malaria with sulfadoxine-pyrimethamine (SP) is followed by a sharp rise in the prevalence and density of gametocytes. We did a randomized trial to determine the effect of treatment of asymptomatic infections with SP or SP plus artesunate (AS) on gametocyte carriage. We randomized asymptomatic carriers of *P. falciparum* to receive antimalarial treatment or placebo, and recorded the prevalence and density of gametocytes over the next 2 months. The trial was conducted during the dry (low malaria transmission) season in four rural villages in The Gambia. Study subjects were adults and children aged over 6 months with asexual *P. falciparum* infection and confirmed free of clinical symptoms of malaria over a 2-day screening period. Participants were randomized to receive a single dose of SP, or SP plus a single dose of artesunate (SP+AS), or placebo. Outcome measures were presence of gametocytes 7 and 56 days after treatment, and the duration and density of gametocytaemia over 2 months. 372 asymptomatic carriers were randomized. Gametocyte prevalence on day 7 was 10.5% in the placebo group and 11.2% in the SP group (risk difference 0.7%, 95% CI -7.4%,8.7%, P = 0.87) and 7.1% (risk difference to placebo 4.1% (-3.3%,12%) P=0.279) in the SP+AS group. By day 56, gametocyte prevalence was 13% in the placebo group and 2% in both drug-treated groups. Gametocyte carriage (the area under the curve of gametocyte density versus time), was reduced by 71% in the SP group, and by 74% in the SP+AS group, compared to placebo. Gametocyte carriage varied with age and was greater among children under 15 than among adults. Treatment of asymptomatic carriers of *P.falciparum* with SP does not increase gametocyte carriage or density. Effective treatment of asexual parasitaemia in the dry season reduces gametocyte carriage to very low levels after 4 weeks.

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A RANDOMIZED, INVESTIGATOR-BLINDED, MULTICENTER, PARALLEL-GROUP STUDY TO COMPARE EFFICACY, SAFETY AND TOLERABILITY OF ARTEMETHER - LUMEFANTRINE DISPERSIBLE TABLET FORMULATION VS. ARTEMETHER - LUMEFANTRINE 6-DOSE CRUSHED TABLETS IN THE TREATMENT OF ACUTE UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA IN INFANTS AND CHILDREN: AN INNOVATIVE DESIGN

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Malaria is primarily a disease of the very young, but specific pediatric formulations are scant. This randomized, investigator-blinded, multi-center, parallel-group study was designed to evaluate efficacy, safety, tolerability and pharmacokinetic data for a new artemether-lumefantrine pediatric dispersible tablet (DT) formulation for oral suspension, developed

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with MMV, in male and female infants and children (less than or equal to 12 years of age; greater than or equal to 5kg and less than 35kg) with microscopically confirmed acute uncomplicated *Plasmodium falciparum* malaria. It aims to demonstrate non-inferiority of the 6-dose regimen pediatric formulation vs. the current WHO-supported commercial 6-dose regimen of crushed conventional tablet at the standard dosage according to bodyweight. It compares 14-day and 28-day (primary objective) PCR-corrected parasitological cure rates between the two treatment groups on a total of 890 randomized patients, as well as time to parasite, fever and gametocyte clearance. It includes a futility interim analysis (IA) based on the uncorrected 7-day parasitological cure rate. AEs, general laboratory findings, vital signs, and ECG measurements are recorded. Eligible patients are randomized (1:1) to either treatment group (DT or crushed tablets) and receive 6 doses of artemether-lumefantrine. The 3-day treatment phase is followed by a 39-day observation period. Once the first 160 patients have completed the 7-day follow-up, data will be reviewed by an independent Data Monitoring Board (DMB). Assuming 75 evaluable patients in the DT arm, the DMB would probably recommend the study be stopped for futility if < or = 67 treatment successes are observed in the DT arm (using an assumed true success rate of 95%). The probability of stopping for futility when the true success rate is 95% is ~3.4%. If the IA outcome is positive, recruitment will proceed including additional centers. Artemether, DHA and lumefantrine plasma levels will be measured. Analysis of 28-day PCR-corrected cure rates will be performed on the PP population and analyses of secondary efficacy objectives on the ITT population.

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A HIGH PROPORTION OF SUSPECTED US CASES OF AVIAN INFLUENZA A (H5N1) HAVE HUMAN INFLUENZA A INFECTIONS

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As of May 2006, avian Influenza A(H5N1) has not been detected in the United States. Therefore, US residents are currently at risk only if they travel to H5N1-affected countries. We describe diagnostic and epidemiologic data among suspected human cases of H5N1 reported to Centers for Disease Control and Prevention. We retrospectively analyzed available data on suspected H5N1 cases in the United States that were reported to Centers for Disease Control and Prevention from February 2003 to May 2006. Clinical and epidemiologic data about suspected cases were communicated to Centers for Disease Control and Prevention by clinicians and public health departments. If warranted, diagnostic testing of suspected cases for H5N1 was performed at Centers for Disease Control and Prevention or state health department laboratories. No evidence of H5N1 infection was detected among the fifty-six suspected H5N1 cases that were reported to Centers for Disease Control and Prevention from February 2003 to May 2006. Fifty-three (95%) had traveled to at least one of 11 Asian countries with confirmed H5N1 infections in birds. None had touched domesticated birds or recently butchered poultry, and none had close contact with any confirmed or suspected human H5N1 cases. Thirty-five (63%) had diagnostic testing done at Centers for Disease Control and Prevention. Twenty-six (46%) cases met the Centers for Disease Control and Prevention suspect case definition, 18 (32%) were hospitalized for suspected respiratory infections, and 2 (4%) had fatal outcomes. Twenty-three (41%) cases tested positive for human influenza A (H3) virus by real-time RT-PCR. Other diagnoses occurring once included bronchiolitis obliterans and organizing pneumonia, community-acquired pneumonia, lymphoma, and rickettsial typhus. Twenty-nine (52%) cases had no laboratory diagnosis. Of the influenza A (H3) positive cases, 4 (17%) occurred outside of the US influenza season, with symptom onset in June, August (2), and September. In conclusion, our findings suggest that the current risk of acquiring H5N1 virus infection in US residents traveling overseas is extremely low. Clinicians should strongly consider human influenza A virus infection as a diagnosis in returned travelers with influenza-like illness year-round, including among persons who have traveled to countries with H5N1-infected poultry.

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MORTALITY AMONG CHILDREN ADMITTED TO A PEDIATRIC HOSPITAL IN BAMAKO, MALI

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Sub-Saharan Africa countries experience among the highest infant mortality and under-5 mortality rates in the world. While bacterial infections are thought to cause many of these deaths, little microbiological data have been available. In order to identify these bacterial pathogens and investigate the outcome of these cases, we have had ongoing hospital-based surveillance at the principal pediatric hospital in Bamako. Children age 0-15 years with fever $\geq 39^{o}$C or syndromes compatible with invasive bacterial disease (e.g. meningitis, pneumonia) and admitted to Hôpital Gabriel Touré were eligible. Blood and relevant body fluid (e.g. cerebrospinal fluid (CSF)) were cultured. Bacteria were identified by standard microbiologic techniques. Enrolled children were followed until the end of the hospitalization. Among the 11671 children admitted to HGT from June 2002 to May 2005, 6087 were eligible and 5514 (90.6%) were enrolled. During the course of the hospitalization 573 children died, representing at least 5% of those admitted and 10% of those enrolled. There was an increased risk of death among those < 1 year old ($p < 0.001$) and those from whom a pathogen was isolated (14.9% vs 9.0%, $p < 0.001$). This risk was decreased among children who had been treated with antibiotics prior to admission compared to those who had not, regardless of the presence of a pathogen ($p < 0.001$). The highest case fatality rates were observed among children infected with Gram negative bacilli (other than *Salmonella spp.*) (23.6%; 36/138), *Streptococcus pneumoniae* (SP, 19.4%; 73/377), non-typhoidal *Salmonella* spp (13.4%; 27/205, and *Haemphilus influenzae* b (Hib, 11.4%; 43/335). Nonetheless, SP and Hib contributed the highest number of deaths. In conclusion, invasive bacterial infections are associated with high case fatality among Malian children even after hospitalization. Vaccination and other strategies aimed at prevention and early detection and treatment are needed, particularly targeting young infants.

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SIMULTANEOUS DETECTION AND DIFFERENTIATION OF *CRYPTOSPORIDIUM PARVUM* AND *CRYPTOSPORIDIUM HOMINIS* BY USING TAQMAN® ASSAYS

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Identification of *Cryptosporidium parvum* and *C. hominis* at species level is an important initial step during outbreak investigations because it provides robust laboratory data to link outbreak cases of infection. These two species are morphologically identical, but they can be distinguished at the genetic level. Techniques currently available for differentiating *C. parvum* and *C. hominis* involve conventional PCR followed by DNA sequencing or RFLP; both being time consuming and labor intensive. We report a real-time PCR procedure that combines targeting of 18S

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rRNA with the chromosomal region to detect *Cryptosporidium* sp. and distinguish between *C. parvum* from *C. hominis* in clinical specimens. This assay uses a duplex TaqMan® assay to detect *Cryptosporidium* sp. by targeting the 18S rRNA gene and *C. parvum* by targeting a chromosomal sequence of unknown function. The assay also includes a separate TaqMan® assay to specifically detect *C. hominis* by targeting the same chromosomal region used for *C. parvum* detection. The duplex assay was validated by blindly testing DNA from 60 samples confirmed to be positive for *Cryptosporidium* sp. by microscopic techniques and characterized at species level (i.e., *C. parvum* or *C. hominis*) with DNA sequencing analysis after PCR amplification of at least one of the genetic markers known to discriminate these two species. The agreement between the TaqMan assay and the reference techniques was 100%. The detection limit of this technique was 1 oocyst per volume of sample processed (300 µl of stool) using stool samples spiked with different concentrations of *C. parvum* oocysts. No cross-amplification was obtained when testing DNA extracted from microscopy-negative samples (n=10) and samples positive for *Cyclospora caytanensis* (n=4), *Giardia intestinalis* (n= 2), microsporidia (n=7), *E. histolytica* (n=2), and *E. dispar* (n=2). After validation, this TaqMan PCR assay was employed as a diagnostic tool during one outbreak investigation in which two stool samples were found to contain both *C. parvum* and *C. hominis* (i.e., mixed infection) using these PCR assays. Further molecular analysis of these samples confirmed these results. This real-time PCR assay has been found to be a rapid, simple, and sensitive method for identification of *C. parvum* and *C. hominis* in stool specimens.

(ACMCIP Abstract)

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PANCREATIC CYSTADENOMA MIMICKING ECHINOCOCCAL CYST. A CASE REPORT

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A 25 year-old African woman from Benin presented with upper abdominal discomfort. Ultrasound (US) and Computed Tomography (CT) showed a cystic, septated mass measuring 6 x 6.5 cm. On US, the septae did not suggest a parasitic origin as they were slightly irregular and unlike the walls of daughter cysts. Serological tests for cystic echinococcosis (IHA, ELISA and Western Blotting) were negative. The patient was admitted to the Division of Infectious and Tropical Diseases where she underwent a US-guided aspiration of the cyst. Clear, thick fluid was aspirated from the main cavity, while yellowish, slightly turbid material was drawn from an adjacent cavity. Microscopic examination of the material showed no protoscolices or *E. granulosus* components. The patient was referred to a surgeon. A spleen-preserving distal pancreatectomy was performed and histopathological examination of the mass showed a pancreatic mucinous cystadenoma. The patient recovered and is now in good health. This case report shows that US appearance may suggest the non-parasitic nature of a septated cyst; US-guided aspiration can rule out an echinococcal cyst, but surgery is required for definitive diagnosis in such cases.

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CARDIAC ECHINOCOCCAL CYST: A CASE REPORT

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Cardiac involvement in cystic echinococcosis (CE) is very rare,with a prevalence of 0,5 to 2 % of all cases. Most cardiac cysts are located in the left ventricular wall and in the interventricular septum. We report a case of cardiac CE in a 47 year-old patient from Morocco. He was admitted to the Pulmonary Medicine Unit because of chest pain, fatigue and coughing. He had a history of acute myocardial infarction and pulmonary embolism due to deep vein thrombosis. ECG showed signs of previous infarction and Computed Tomography revealed complex masses in the mediastinum and a mass at the base of the heart. A CT-guided percutaneous aspiration of one of the masses located at the base of the heart yielded thick, yellowish material compatible with echinococcosis. IHA and ELISA tests were performed and showed a high titer of antibodies against *Echinococcus granulosus* (IHA: 1/8192; ELISA 4,930). The patient was transferred to the Division of Infectious and Tropical Diseases and underwent transthoracic echocardiography, which showed a grape-like, multivesiculated lesion in the left ventricular wall consistent with CE2 echinococcal cyst (WHO classification). Transesophageal echocardiography did not add any relevant findings. ECG-gated CT and MR showed another multilvesiculated cyst in the pericardium. The patient was started on albendazole and discharged. Interestingly, the heart was the only organ affected by CE, as shown by a whole body helical CT scan. A review of the literature revealed that this occurs in 32% of cases with cardiac involvement. CE should be included in the differential diagnosis of mediastinic and cardiac masses in patients coming from endemic areas; transthoracic echocardiography is the first-line diagnostic tool for confirmation. ECG-gated CT and MR are crucial for evaluation of the extent of disease.

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SENSITIVE LC-MS ASSAY FOR THE DETERMINATION OF THE ANTIMALARIAL PYRONARIDINE IN HUMAN BLOOD

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A sensitive method has been developed for the determination of pyronaridine in human whole blood using amodiaquine as an internal standard. Liquid-liquid extraction was used for sample preparation. Analysis was performed on a Shimadzu LC-MS-2010A in single ion monitoring positive mode using APCI as an interface. Positive ions are measured using extracted ion chromatogram mode. The extracted ion for pyronaridine was m/z 518.2 and for amodiaquine was m/z 356.1. Chromatography was carried out using a Gemini 5µ C_{18} 3*150 mm column using a 2mM perfluorooctanoic acid, acetonitrile mixture as the mobile phase in gradient mode delivered at a flow rate of 0.5 ml/min. The retention times of pyronaridine and amodiaquine are 9.1 and 8.1 minutes, respectively. The total run time is 14 minutes. The assay is linear over a range of 1.1-855 ng/ml for pyronaridine (r^2 0.991, weighted by 1/C). The analysis of quality control samples for pyronaridine at 10, 285 and 769.5 ng/ml demonstrated excellent precision with relative standard deviations of 5.9, 6.4 and 5.4 %, respectively (n = 5). Recoveries at concentrations of 10, 285 and 769.5 ng/ml are all greater than 85%. This LC-MS method for the determination of pyronaridine requires only 200 µl of whole blood and has excellent specifications for sensitivity, reproducibility and accuracy. This sensitive method can reliably quantitate pyronaridine blood concentrations as low as 1.1 ng/ml. The method will be used to quantify human blood for pharmacokinetic and drug safety studies.

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DETECTION OF *CRYPTOSPORIDIUM* SPP. ANTIGEN IN HUMAN FECAL SPECIMENS USING THE *CRYPTOSPORIDIUM II* ELISA TEST

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The *CRYPTOSPORIDIUM II* test (TECHLAB, Inc., Blacksburg, VA) is a 96-well ELISA assay that detects *Cryptosporidium* spp. antigen in human fecal specimens (fresh, frozen, stored in Cary Blair transport media, preserved

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in 10% formalin, or preserved in SAF). Positive samples are indicated by a yellow color in the well that can be analyzed spectrophotometrically or interpreted visually. A positive test result indicates the specimen contains *Cryptosporidium* spp. antigen. Assay steps include tube preparation (diluent, patient sample); addition of sample, conjugate and development reagents to the assay wells; two plate washing steps; and interpretation of results (Total incubation time = 100 minutes). Test performance was evaluated using 185 human fecal specimens, 44 positive for *Cryptosporidium* based on results from the Meridian Biosciences, Inc. Merifluor *Giardia/Cryptosporidium* combination reagent direct immunofluorescence assay (IFA). Specimens showing discrepant results were retested using the Merifluor combination reagent assay. Based on the reference methods, the *CRYPTOSPORIDIUM II* test had a sensitivity of 97.7%, a specificity of 100%, a positive predictive value of 100%, a negative predictive value of 99.3%, and a correlation of 99.5%. A false negative *Cryptosporidium* result was obtained from a specimen with low parasite numbers, fewer than 5 oocysts per IFA well. No cross reactivity was seen with 10 different protozoa (174 challenges), 8 different helminths (21 challenges), or human cells (3 challenges) found in fecal samples. Visual interpretation of the test correlated with spectrophotometric results. In conclusion, the *CRYPTOSPORIDIUM II* ELISA is a new reliable assay for the detection of *Cryptosporidium* in human fecal specimens.

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CELLULOSE ACETATE ELECTROPHORESIS (CAE) A VITAL TECHNIQUE IN LEISHMANIA DIAGNOSIS

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Leishmania parasites speciation is a very important tool to determine the course of treatment on infected individuals. Since the symptoms, characteristics and outcome of the disease vary from self healing to life threatening, it is often critically important to know which species is involved in the disease process. While there are many techniques for parasite identification (speciation), CAE is one of the most reliable, inexpensive, sensitive and accurate methods that identifies all known species of *Leishmania*. For world travelers and the United States Armed Forces exposed to endemic areas, it is necessary to identify the species to proceed with the appropriate treatment. Besides identifying all known species of *Leishmania*, this technique is very effective in detecting dual infections or more than one species of *Leishmania* infections. Dual infections can not be detected using any other diagnostic technique and examples of this will be provided in the presentation of our data along with other examples of unique diagnostic capabilities with this methodology.

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ENHANCEMENT OF ROUTINE HEALTH INFORMATION BY THE USE OF PERSONAL DIGITAL ASSISTANTS IN SOUTHERN TANZANIA

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The ability to plan and manage health services depends heavily on the availability of reliable health information. In tropical Africa the labour-intensive, time-consuming and error-prone manual process of routine data collection and collation rarely results in the availability of reliable data to assist in the planning and evaluation of health interventions. We are evaluating the use of Personal Digital Assistants (PDAs) for the collection of routine data in rural African health facilities. One person in each of 11 sentinel health centres in rural southern Tanzania was trained to use a Palm m130 PDA and solar charger. None of the recruits had prior experience of computers but all were able to collect data reliably after one week's training. The PDAs were programmed to ensure that data entry fields were not left blank, that data entry values were in acceptable pre-defined ranges and that responses were internally consistent with each other. Data has been entered directly into the PDAs on a daily basis from vaccination and children's outpatient clinics and from laboratories. The PDA operators are visited monthly by a supervisor who synchronises the PDA with an Access database on a laptop computer using Pendragon Forms v4.0. Programs are run at the health facility to produce summaries of routine data for forwarding to the district level and which are discussed on site with health workers. Data will be presented which show the benefits of such feedback in enhancing the completeness and quality of data. Furthermore, PDAs have facilitated the introduction and use individual identification codes which enable the evaluation of the effects of interventions delivered, for example, at the time of vaccination on outpatient attendances and laboratory results. Electronic handheld devices can help improve the quality, completeness and availability of routine health data even in rural, tropical, resource-poor settings.

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BLOOD AGAR SUBSTITUTE IN GROWING LEISHMANIA

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Leishmania diagnosis is essential to determine the best course of treatment and foresee its effectiveness in the infected patients. Until new and more effective techniques are found, culturing the parasites is a key step in treating this disease. Currently there are many ways to grow and expand *Leishmania* parasites, but the technique most commonly used is growing the parasites using Novy- MacNeal- Nicolle (NNN, blood agar). This blood agar medium for culture is used to grow and expand all Leishmania species and is more effective when parasites are very sensitive and difficult to expand in other culturing media. NNN is also very difficult to use as a standard media. NNN is very expensive and time consuming in its preparation. It is also easily contaminated because of the rich nutrients that compose the medium, which allow other unwanted microorganisms to grow and interfere with identification of leishmania parasites. A control temperature environment is also needed to keep this culture medium active and free of contamination. With the development of a blood agar substitute, I intend to demonstrate a potentially better way to grow *Leishmania* parasites considering cost of preparing media, preparation time, time of durability of the media, ease of contamination, parasite growth and environmental measures that affect the effectiveness of the media in various conditions.

(ACMCIP Abstract)

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CROSS REFERENCE BETWEEN CELLULOSE ACETATE ELECTROPHORESIS (CAE) AND POLYMERASE CHAIN REACTION (PCR) IN LEISHMANIA DIAGNOSIS

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Effective diagnostic techniques for Leishmania are very important and critical in starting treatment of an infected patient. Leishmaniasis is a potentially life threatening and mutilating disease and various species are lethal if not treated on time and appropriately. PCR is becoming the preferred tool in *Leishmania* diagnosis due to its rapidity in providing results, but is still lacking the full effectiveness of speciating all species of the *Leishmania* parasites. CAE is a technique that is been available for a very long time, and it requires live parasites and a large number of promastigotes to perform well, but it remains the most effective technique in performing speciation of *Leishmania* parasites. Both techniques

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accommodate the diagnostic needs if used together, but until PCR is fully developed, CAE remains the leading technique for *Leishmania* classification and speciation of all known's species of Leishmania parasites. In this presentation, my intention is to examine how well CAE and PCR perform side by side in speciation and where are the common grounds in *Leishmania* identification and speciation.

(ACMCIP Abstract)

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FIRST DENGUE HEMORRHAGIC FEVER (DHF) OUTBREAK IN IQUITOS, PERU, 2004

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The first documented dengue fever (DF) outbreak in Peru was described in Iquitos between March and July 1990. At that time dengue virus (DEN) serotypes 1, 2 and 4 were found and approximately 25% of the population was infected. During 2000 and 2001 until December, only DEN-1 was active. DEN-1 and DEN-2 were the predominant serotypes until June 2002 when DEN-3 completed replaced other serotypes. Surveillance for DEN has been conducted in health centers and hospitals in Iquitos for more than 5 years. We followed WHO criteria for dengue hemorrhagic fever (DHF), and confirmed DEN etiology by virus isolation, RT-PCR, and serology. We documented 7 DHF cases from 12/3-12/13. The DHF incidence rate was 4/100,000 population during December. Six of the 7 cases were adults. Clinical course was marked by fever, chills, malaise, poor appetite, arthralgia, myalgia, rash, abdominal pain and vomiting. A single fatality occurred in a 6-year old who had shock (grade IV). The other six patients were (grade I- III). All 7 patients were positive by RT-PCR, and had DEN IgM and 6 of the 7 had evidence of previous infection with another DEN serotype. Fourteen years after the first DF outbreak, DHF appeared in Iquitos. All cases were caused by DEN-3 and most were secondary infections.

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COLLEGE OF AMERICAN PATHOLOGY (CAP) AND ITS PROGRESS IN THE LEISHMANIA DIAGNOSIS

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The progress of any diagnostic laboratory is evident when they follow the rules and regulations established by the Good Laboratory Practices. As the first College of American Pathology (CAP) *Leishmania* Laboratory in the world we can provide the expertise, experiences and knowledge acquired while dealing with the college of accreditation to other institutions with similar interests. Pre and post accreditation progress has been measured to challenge our laboratory personnel with the ultimate purpose of finding and determining the best routes to maximize our Leishmania diagnostic capabilities. Our progress evaluations have served the purpose of exposing any advances or deficiencies during our development and evolution. During the conduct of this project, we intended to evaluate all the factors involved in handling the submitted samples (number of samples, contamination, patient travel history, positive or negative results, smears, PCR, rK39, and speciation). This year is our third full CAP inspection, therefore the data will go back six year before and six years after CAP accreditation. Our self impose proficiency test will be used as a final evaluation to measure sterility lab effectiveness and personnel qualifications.

(ACMCIP Abstract)

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CHARACTERISTIC FEATURES OF ANEMIA IN VISCERAL LEISHMANIASIS (KALA-AZAR)

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Visceral leishmaniasis (kala-azar) is one of the most important and neglected endemic diseases in Africa, yet there is a paucity of information identifying the type and/or pathogenesis of anemia apparent in this disease. This study was undertaken to investigate the hematological features of the Leishmaniasis epidemic in Eastern Sudan. To determine whether the anemia is corrected by anti-leishmanial therapy alone without haematinic administration and to determine the effect of nutritional deficiency on the pathogenesis of the anemia. Patients with a diagnosis of Kala-azar were admitted to the El Geraif Kala-azar health center in Khartoum by Medicine San Frontier (MSF) from 1990-1992. This was a case-control study with 40 patients and 40 controls. The mean hemoglobin value in patients was 7.4 gm/dl before treatment, and 8.5 gm/dl after treatment ($p= 0.003$). The mean value of platelet counts before treatment was 131,000 and after treatment was 153,840 ($p = 0.09$). The anemia was normocytic in the majority of cases (76%) and the peripheral blood picture showed an increase in rouleux formation. In terms of anemia, there was a significant difference between the controls (772) and patients after treatment (427). There was no significant difference in vitamin B12 or Folic Acid deficiency before and after treatment. In conclusion, the anemia that complicates Kala azar is likely due to multiple etiological factors. The anemia of Kala-azar showed features of anemia of chronic infections, nutritional deficiency and iron deficiency. Among our patients, all were displaced, undernourished and diseased. The anemia in this epidemic was shown to be mild and responded well to treatment. Absence of marked leucopenia and thrombocytopenia suggested that bone marrow depression was not a major factor in the pathology of anemia. It appears that nutritional factors may play an important role as well as levels of ferritin and its utilization in chronic diseases; therefore, more detailed studies are needed.

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THE UTILITY OF HRP-2/P-LDH MALARIA RAPID DIAGNOSTIC TESTS IN SEMI-IMMUNE POPULATIONS OF SUB-SAHARA AFRICA

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The utilization of malaria rapid diagnostic tests in Sub-Sahara Africa is rapidly growing. The utility of these tests in populations who live in areas of high endemicity for malaria has been highly debated. 431 rapid malaria (HRP2/p-LDH based) dipsticks were conducted in parallel with microscopy in conjunction with regulated clinical trials in Kenya. Total positive and negative malaria slides where compared with the total positive and negative rapid diagnostics results to compute the sensitivity and specificity of the rapid diagnostic's ability to accurately predict malaria or its absence in this population. Subset analysis was also conducted on species for mono and mixed infections. Mean parasite density in this predominately non-symptomatic population was 435 parasites/μl with a geometric mean of 272 parasites/microliter. Analysis to date shows the sensitivity and specificity for this test was 87.9% and 81% respectively. In conclusion, while further analysis should be conducted, preliminary results suggest that malaria rapid diagnostic tests could be utilized as an effective devise for the diagnosis of malaria in a highly endemic population.

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PNEUMONIA ADVERSELY AFFECTS GROWTH IN CHILDREN

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Pneumonia is the leading cause of death in children under 5 years but has not been identified as a major cause of growth faltering. We hypothesized that pneumonia leads to persistent stunting and weight loss, and that vitamin A and/or zinc supplements might ameliorate these adverse effects. We conducted a prospective study to test these hypotheses. The Vitamin A and Zinc: Prevention of Pneumonia (VAZPOP) study was a multi-year nutritionally-stratified, placebo-controlled, double-blind study of low-dose vitamin A and/or zinc in 2,582 normal and malnourished urban children aged 6 to 36 months in Quito, Ecuador. Four group of ~ 645 children were enrolled in 2000, 2001, 2002, and 2003 with each child participating for up to 50 weeks. Children were visited 4 days each week. Outcome Measures. Anthropometric data were obtained at baseline, and then every 10 weeks. Pneumonia was diagnosed using WHO screening criteria and by experienced clinicians. We detected 518 cases of pneumonia in 378 children, with 0.254 cases of pneumonia per child-year. Pneumonia episodes had a significant, persistent negative impact on height-for-age Z-score (HAZ), weight-for-age Z-score (WAZ), and mid-upper arm circumference (MUAC). The decrement in HAZ was -0.187 (0.036) (SE) ($p< 0.001$), in WAZ was -0.091 (0.032) ($p = 0.004$), and MUAC was -0.094 (0.036) cm ($p = 0.009$). These effects were greatest in children >18 months of age and in females. The micronutrient supplements did not ameliorate the nutritional consequences of pneumonia. The magnitude of these individual effects greatly outweighed those of diarrheal disease by a factor of 3.5 to 4. The overall, population-wide adverse nutritional impact of pneumonia may be comparable to that of diarrheal disease. Children with pneumonia at risk of stunting and weight loss may benefit from nutritional support to decrease subsequent adverse medical, cognitive, and social effects.

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EVALUATION OF ONE-YEAR SURVIVAL RATES OF CHILDREN WITH BURKITT'S LYMPHOMA IN TANZANIA AFTER CYCLOPHOSPHAMIDE MONOTHERAPY

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The objective of this study was to assess one-year survival rates in Tanzanian children with Burkitt's Lymphoma (BL) receiving cyclophosphamide (CPM) monotherapy treatment. In addition, to assess the pattern of presentation of Burkitt's Lymphoma in Tanzanian children - mandibular versus abdominal involvement. A retrospective study of 47 children with BL, confirmed by fine needle aspirates (FNA), was performed. Treatment for children was carried out using CPM monotherapy (IV CPM at 40 mg/kg/dose at 14-day intervals for 4 weeks). Monotherapy (vs multiple treatment therapy) was used due to limited funding at Shirati Hospital, Tanzania. All 47 cases studied had information regarding the type of BL (mandibular vs. abdominal); however, information on survival was only obtainable in 28 cases. Results: Out of 47 patients, abdominal BL was the most frequent site of involvement (76%), with 36 cases total (8 of which also concurrently displayed mandibular involvement). The M:F ratio was 3:2 and the median age of diagnosis was <7 y/o. Among the 28 cases with survival data, the overall survival rate was approximately 30 %, with at least 50% of these children dying within the 1st year after treatment. Only 2 of the 8 cases with mandibular lymphoma survived (25%) and 6 of 21 abdominal lymphoma cases survived. In conclusion, through this study we were able to determine that montherapy with CPM is not adequate to cure children with mandibular and/or abdominal BL in Tanzania. Other studies using multi-therapies have shown survival rates between 81-90%. Unfortunately, poor record-keeping to U.S. standards precluded us from receiving comprehensive clinical data and accurate survival estimates for all children. A prospective study with proper staging, assessment of FNA, marrow and cerebrospinal fluid collection using modern techniques, plus adequate follow-up would help us better evaluate the current therapeutic value of CPM monotherapy versus short, intensive polychemotherapy (shown effective in other clinical studies). Further investigation is needed to evaluate the most effective treatment for Tanzanian children suffering from BL so as to minimize their morbidity and ensure their survival.

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RAPID DIAGNOSTIC MALARIA TESTS FOR THE DIAGNOSIS OF MALARIA VERSES CLINICAL JUDGMENT

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Areas in Sub-Sahara Africa where malaria is holo-endemic are often poorly resourced to make accurate diagnosis of malaria. The lack of funding, proper equipment and effective training, coupled with huge patient to physician ratios place extreme pressure on medical personnel to make quick diagnostic and therapeutic decisions, often on clinical grounds alone. In patient and out-patient records at a local hospital showed a 3 year history of malaria diagnosis and treatment averaging approximately 18 cases per day. Over a 9 week period subjects at this local hospital presumed to have malaria were referred for HRP2 rapid diagnostic tests (RDTs). These WHO approved rapid diagnostic tests were conducted over approximately 4-5 hours a day for 51 days. Based on the prevalence of disease, historical records and an "over diagnosis rate" of 10%, the expected positive test rate was approximately 50%. 814 tests were conducted, 743 negative and 71 positive were observed resulting in an 8.7% positive test rate. In conclusion, malaria over diagnosis is a serious problem resulting in patients being exposed to the side effects of unneeded medication, the lack of proper treatment for the proper diagnosis, added expense, as well as decreasing the efficacy of life saving anti-malarial drugs through the development of resistance. Malaria RDTs could decrease the rate of over diagnosis, and thus over treatment as well as save time and vital resources. Further research is needed in this area.

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A LITTLE SUGAR GOES A LONG WAY: TRAVELING WITH DIABETES

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Diabetes is a global epidemic affecting over 21 million people. Control of diabetes is important on a daily basis but can be challenging as people travel for business and pleasure. Changes in time zones, meal times, types of food and activity are examples of the challenges that travelers with diabetes face. This session will focus on some strategies for pre trip preparation, medication adjustment across time zones and resources such as the National Diabetes Education Program, a joint Centers for Disease Control and Prevention and Prevention (Centers for Disease Control and Prevention), and National Institutes of Health (National Institutes of Health) program to help the person with diabetes travel successfully.

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A PHASE II, OPEN LABEL, STUDY OF THE SAFETY, TOLERABILITY, EFFICACY AND PHARMACOKINETICS OF INTRAVENOUS ARTESUNATE IN ADULTS WITH UNCOMPLICATED MALARIA

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New rapid and effective parenteral malaria treatments for severe and complicated malaria are urgently needed in Sub-Saharan Africa as well as for the traveler who acquires falciparum malaria. GMP Intravenous Artesunate (Walter Reed Army Institute of Research, Silver Spring, Maryland) was administered as part of a phase II pharmacokinetic (PK), efficacy and safety study in Kenya. After informed consent, 30 African adults who presented with symptomatic, uncomplicated falciparum malaria were treated intravenously with GMP Artesunate at 2.4mg/kg/day for 3 days, followed by a full-course of Malarone for cure. Samples for a 9 time point PK profile were collected along with blood smears and safety laboratories. Preliminary review of the data show marked clearing of parasites within 24 hours of administration and dramatic clinical improvement. The artesunate was generally well tolerated, and no attributable severe adverse events were noted to the artesunate. Additional safety and efficacy data will be presented. This is the first study of parenteral GMP artesunate in Sub-Saharan Africa. Further studies, including safety and PK assessments in African children, are needed.

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GAMETOCYTE CLEARANCE TIME AND ARTEMISININ COMBINATION THERAPY FOR UNCOMPLICATED MULTIDRUG RESISTANT FALCIPARUM MALARIA IN VENEZUELA: PRELIMINARY RESULTS

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Artemisinin based combination therapy has been widely used for treatment of uncomplicated falciparum malaria. In the Americas, the aim of the antimalarial treatment is to eliminate parasitemia and radical cure has been used in the last fifty years using primaquine as gametocidal drug in single dose in all countries. Many reports showed that artemisinin derivatives reduce gametocyte carriers but not enough data on gametocyte clearance has been published. Countries in the Americas started changing their national malaria treatment guidelines including ACT plus primaquine (Bolivia, Guyana). Before recommending the use primaquine with ACT, the Venezuelan Ministry of Health decided to evaluated prospectively the gametocyte clearance time and gametocyte profiles during follow up among patients with uncomplicated malaria that received supervised treatment with mefloquine+artesunate 3 days, mefloquine+artesunate 2 days, Coartem 3 days, artemether). Patients were selected from clinical trials and systematic cases´ follow up which involved follow up visits on days 1, 2, 3, 7, 14, 21 and 28. Since September 2004 until now, all patients with uncomplicated malaria has been either enrolled in efficacy trials or in the systematic cases´ follow up. By the time this abstract was written, more than 1200 patients have been evaluated. Gametocyte profiles and gametocyte clearance time in those patients will be presented and discussed. Information on public health decisions for policy makers also will be discussed specially for the Amazon region.

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MALARIA PREVALENCE AMONG TRIO AMERINDIANS IN SOUTHERN SURINAME

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The Global Fund Malaria project in Suriname is aiming to decrease the malaria incidence among people living in communities in the interior of Suriname. As part of the projects´s baseline, a malaria survey was carried out in an isolated Trio Amerindian village south Suriname called Kwamalasamutu, close to the borders with Guyana and Brazil. A community census carried out and malaria prevalence was determined by malaria smear and rapid test (Optimal) in the whole population. Positive cases received treatments according to national treatment guidelines. During February 2006, 734 inhabitants were recorded in 192 households. Malaria smears were taken in 598 people present at the moment of the visit to their house. There were 536 Optimal test results available among those 598 people. Malaria prevalence by malaria smear and Optimal test were very low 2% (10/598) and 0.37% (2/536). Malaria transmission in this village was low during the evaluation however; the 2005 malaria cases of Kwamalasamutu represented 2.3% of the total in the country. Interventions for controlling malaria in those isolated Amerindian villages will be discussed.

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MONITORING ANTIMALARIAL DRUG RESISTANCE IN VENEZUELA: A SIMPLE APPROACH FOR DEVELOPING COUNTRIES

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Venezuela has been part of a network for evaluating the antimalarial efficacy of first and second line treatments in the Amazon region (Red Amazonica de Vigilancia de la Resistencia a los Antimalaricos (RAVREDA)/AMI). Different sentinel sites were selected and used for monitoring drug resistance. However, after finishing drug trials very little is done specially in those sentinel sites. We proposed and evaluated a system aiming to include all patients with *Plasmodium falciparum* in a new routine approach called "Systematic cases´ follow up" in a sentinel site. This SCFu consists of informing all positive patients with malaria about the importance of returning for control visits during their consultation. A schedule visit card was given to all patients and a shorter version of the "*in vivo*" clinical record form were completed and kept at the sentinel site. From September 2004 until now (May 2006), there were 819 falciparum malaria cases enrolled. The majority of cases of *P. falciparum* (98%) were uncomplicated malaria and eligible for the SCFu. All patients received supervised ACT. The percentage of compliance to the follow up visits until day 28 was 12% (Adequate Clinical Response). Fever clearance time and parasite clearance time at day 3 were 99% and 99.5% respectively. Only 12% completed all control visits without extra financial resources. This routine approach was successful and fully in place at least in southern Venezuela. The Ministry of Health is evaluating extending the monitoring of antimalarial drug resistance using this approach. Issues on health education/health promotion and sustainability will be discussed.

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TWO-DAYS MEFLOQUINE-ARTESUNATE COMBINATION THERAPY FOR UNCOMPLICATED MULTIDRUG RESISTANT FALCIPARUM MALARIA AMONG GOLD MINERS IN VENEZUELA

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In the last decade, *Plasmodium falciparum* has shown significant clinical failures to chloroquine, Fansidar and quinine in Southern Venezuela. The Venezuelan Ministry of Health has decided in 2004 to switch to artemisinin based combination therapy with mefloquine plus artesunate for 3 days. However, the main goal is the supervision of all doses of treatment (DOTs). Since 30 to 40% of the malaria cases occur in mining areas, a two-days mefloquine-artesunate treatment was evaluated as an alternative for indigenous and illegal gold miners. The efficacy of the treatment was evaluated following the WHO *in vivo* protocol. Patients were followed up on days 1,2,3,7,14,21 and 28. Parasite clearance time and fever clearance time as well clinical and parasitological response were evaluated. During 2005, a total of 51 patients were enrolled into the trial. Most patients presented an adequate clinical response and there was one clinical failure on day 28. All patients became afebrile and cleared parasitaemia by day 2. Mefloquine-artesunate 2 días represents an alternative treatment for special groups such us gold miners or indigenous people.

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EMERGING PATHOGENS, INCREASED SOCIAL INTERCONNECTIVITY AND DEMOGRAPHIC COLLAPSE IN BAJA CALIFORNIA (1697-1830)

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When the Spanish friars established the first town in the peninsula of Baja California in October 25, 1697, the population of central Mexico was already decimated, as a result of the devastating epidemics, famines and wars of the sixteen and seventeen centuries that took 90% of the population. However, the population of Baja California remained intact at that time, with a robust size of 60 000 inhabitants. Yet, with the formal initiation of colonization and religious conversion of the peninsula in 1697, a rapid process of depopulation began. This process started 178 years after the arrival of the Europeans to Mexico. At this time, the coast of Sinaloa and Sonora, just one or two days across the Gulf of California, was densely populated and provided regular visitors to the coast of Baja California in the form of explorers, pirates, smugglers and soldiers. Why did the local populations resist the introduction of measles and smallpox until that time? This delay can be explained by how the native population was distributed throughout the region. The inhabitants of the peninsula were organized in small nomadic groups, fighting constantly with their neighbors for resources. This isolation worked as a barrier for transmission of human-to-human diseases. One of the first objectives of the Spanish priests was to build a major road associated to a chain of Missions along the territory and to concentrate the native nomadic groups in permanent settlements around the Missions. This proved fatal for the native population that was annihilated by a rapid succession of epidemics of measles and smallpox. Because the population size was not enough to sustain a permanent circulation among the settlers of the peninsula they were re introduced periodically from the continent, causing great mortality each time. After 133 years, the whole native population was extinct. The introduction of new human airborne pathogens simultaneous to an increased intercommunication among members of a previously isolated population resulted in a demographic catastrophe. Although remote in time, these events constitute an interesting model for today's world: The emergence of airborne infectious diseases, and increased intercommunication by means of trade and travel. Are we repeating history?

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VENTRICULO-PERITONEAL SHUNTING IN THE MANAGEMENT OF HIV-ASSOCIATED CRYPTOCOCCAL MENINGITIS

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Over 80% of cryptococcosis cases occur in HIV-infected patients, and cryptococcal meningitis (CM) is a frequent AIDS-defining illness. Fever, malaise, headache, and various neurologic symptoms are common, and mortality is high. Treatment requires antifungals and frequent lumbar puncture (LP) to manage increased intracranial pressure (ICP). The neurosurgical literature encourages the use of ventriculo-peritoneal (VP) shunt placement in managing ICP in HIV-infected patients with CM, but surgeons are often reluctant to do so. We report successful use of VP shunting in an HIV-infected patient with CM who had failed repeated LPs. Case Report: A 32-year-old Honduran female presented with a 3-week history of fever, headache and diplopia. Neurological exam revealed nuchal rigidity, left 6th nerve palsy and no papilledema. Cerebrospinal fluid (CSF) analysis was consistent with CM, showing elevated opening pressure and high titer of cryptococcal antigen. The CD4 count was 27cells/ml. Liposomal amphotericin (LA) was started with minimal improvement. Repeated LPs provided relief of headache and diplopia after each tap. She completed therapy with LA and was discharged on highly active antiretroviral therapy (HAART). Two months later, she returned complaining of headache and diplopia. Repeated LP again showed elevated CSF pressure, and temporary relief was reported after each. Imaging studies of the brain failed to show classical features of increased ICP such as periventricular diffusion of CSF or hydrocephalus. A right parietal VP shunt was placed with complete resolution of symptoms. She was discharged on maintenance fluconazole and HAART and is currently doing well. In conclusion, neurosurgeons often are reluctant to implant a VP shunt in patients with HIV-infection and CM. Reasons cited include: poor prognosis and life expectancy, risk of infection, shunt obstruction, and theoretical peritoneal seeding with infected CSF. Ventricular size is often normal despite increased ICP, and even after VP shunting no decompression occurs. It is postulated that cryptococci cause a "frozen" brain by coating it with polysaccharide, making the ventricles stiff and not readily able to change in size. A recent review in the neurosurgery literature encourages the use of VP shunting in managing ICP in HIV-infected patients with CM, and recommends it as a safe and reasonable treatment modality. Our case lends support to this approach.

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EFFECTS OF GLUTAMINE ALONE AND IN COMBINATION WITH ZINC AND RETINOL ON INTESTINAL BARRIER FUNCTION, DIARRHEAL DISEASES MORBIDITY AND GROWTH IN UNDERNOURISHED CHILDREN IN THE NORTHEAST OF BRAZIL

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The objective of this study was to study the impact of glutamine, zinc, and retinol alone or in combination on intestinal barrier function, diarrhea and growth in undernourished children in an urban Brazilian community. After parental consent children aged 2 months- 8 years with a height-for-age z-score less than the median (-0.06) were enrolled and

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randomized to receive zinc (40 mg twice weekly for 12 months), retinol (100,000-200,000 IU for children < or > 1yo every 4 months), both or neither (ie placebo) for 12 months. 324 children were enrolled. After 1 mo, half of each group (all having been randomized into 8 blocks) were given glutamine (Q, 16.2 g/day) or placebo (glycine 8.3 g/day) for 2 weeks and retested for absorptive function, Diarrhea morbidity, lactulose and mannitol excretion ratios (L/M), weight and height were measured. Patients were initially similar in age, sex, nutritional status (except for HAZ in one group) and L/M. Q or zinc alone significantly improved (decreased) the percent L excretion up to four and 1.5 months, respectively. Zinc plus retinol improved L excretion at long-term follow-up 4 months. Q alone significantly reduced the excretion of M during the follow-up period and retinol alone reduced the percent M excretion at 4 months. Zinc alone and other combinations of micronutrients did not change the percent M excretion. Q plus retinol significantly improved (decreased) lactulose: mannitol ratio throughout the whole follow-up periods. Glutamine plus zinc also improved L/M at long-term follow-up 4 months. Placebo controls, glutamine, zinc alone, Q plus zinc or zinc plus retinol had reduced HAZ z-scores during follow-up periods. However, Q plus retinol increased significantly HAZ z-score at 1.5 and 4 months periods of time. Q, zinc, retinol alone or Q plus zinc had a significantly improvement on WHZ z-score during the follow-up periods. The proportion of diarrhea reduced significantly only in the Q combined with zinc and retinol. In conclusion, Q, zinc, Q plus retinol or zinc plus retinol supplements repaired intestinal barrier function in undernourished children. Q, zinc or retinol alone or Q plus zinc reduced wasting in undernourished children. Q plus retinol, but not other micronutrients alone or in combination, reduced stunting. The combination of Q, zinc, and retinol decreased diarrheal diseases morbidity in undernourished children in the northeast of Brazil.

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POTENTIAL USE OF MULTIPLE FREQUENCY BIOIMPEDANCE FOR DIAGNOSIS AND MONITORING OF LYMPHATIC FILARIASIS LYMPHEDEMA

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An important innovation in general lymphedema research and treatment was the development of multiple frequency bioimpedance (MFBIA) technology that can simply and non-invasively yet accurately measure the volume of intra and extracellular fluid in a limb. A study of patients undergoing surgery for breast cancer showed that MFBIA could detect lymphedema up to ten months before it could be clinically diagnosed or detected by morphometric measurements. The instrument is portable, robust and able to be used in remote locations, making it ideal for diagnosing and monitoring lymphatic filariasis-associated lymphedema and trials need to be undertaken to access its usefulness in this setting.

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BURDEN OF MALARIA IMPORTED CASES IN VENEZUELA DURING 2004-2005

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Venezuela is a malaria endemic country with different circulation of Plasmodium spp. in different States. Identification of imported malaria cases has from endemic areas within Venezuela and from contiguous countries has implications for malaria control programs and travel recommendations. Through the national malaria program, Ministry of Health, all cases of malaria were analyzed to asses the importance of imported malaria cases. The study period was from January, 2004 to December, 2005. All cases were microscopically confirmed at least twice by a quality control system. During the study period, a total 92,331 cases of malaria were recorded (46,655 in 2004 and 45,676 in 2005). From this total, 91,572 (99.1%) cases were autochthonous and 759 (0.9%) imported. Cases came from Colombia (55.2%), Guyana (26.9%), Brazil (4.1%), Nigeria (0.5%), Mozambique (0.1%), Haiti (0.1%) and South Africa (0.1%). A mean of 7 imported cases per week was observed during the period. Distribution of autochthonous malaria cases through the period was as expected but with the imported cases occurred a mean of 2.11%, with 5 important peaks that were observed during weeks 5°, 23° and 46° of 2004 (these weeks accounted 20.63% of the year's imported cases) and 23° and 37° of 2005 (these weeks accounted 9.5% of the year's imported cases). In 2004, cases from Colombia, Brazil and Guyana were only reported, but in 2005, cases from other Caribbean and African countries were notified. Strengthened surveillance such as the carried out by this study is helpful to assess the potential introduction of other *Plasmodium* species or resistant strains that may subsequently spread through transmission of local mosquitoes from countries where malaria is endemic as occurs with all the border countries with Venezuela.

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SEROSURVEY AGAINST RICKETTSIA RICKETTSII AMONG HEALTHY INDIVIDUALS OF IN VILLETA, COLOMBIA

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Rocky Mountain spotted fever has re-emerged in Colombia near the same locality where it was first recognized in 1937. We have reported three fatal cases and confirmed that *Rickettsia rickettsii* was the etiologic agent. This is the first study based on a probability-sample in Colombia. To determine the prevalence of positive antibody titers against R. rickettsii in rural inhabitants of Villeta, Cundinamarca - Colombia and its relationship with some demographic and epidemiological characteristics. Sera from 392 randomly recruited healthy adults, a representative sample of the rural population of Villeta were analyzed by indirect immunofluorescent antibody assay to detect IgG against *R. rickettsii* as antigen. A cutoff titer of 1:64 was used. A structured questionnaire and informed consent was filled and signed by all people involved. The seropositivity rate for *R. rickettsii* was 40.56% (159 of 392 samples, CI 95%: 35.6-45.6). We did not find any association between a positive test and gender, age, occupation, education level, building material of the house, ownership or free circulation of domestic animals in and out of the house, time of permanence in the area, or number of people living in the house. Seropositivity was less frequent among those who referred previous contact with the nymph (OR:0.53, IC95%:0.32-0.89) or larvae stages (OR:0.59, IC95%:0.20-1.66). The prevalence of positive antibody titers against spotted fever group rickettsiae in this Colombian locality is high; however, it remains to be established whether *R. rickettsii* or other less pathogenic rickettsiae explain these findings. The lack of association between a positive test and several demographic and epidemiological characteristics could be a reflection of unique features of this area (including the high prevalence of infection). The lower frequency of positive tests among those who had contact with larvae and nymphs (as determined by their ability to identify them), suggests that learning to identify these immature forms is a preventive strategy that needs to be investigated.

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NUTRIKINE DYSREGULATION: A NEW PARADIGM FOR THE IMMUNODEFICIENCY OF MALNUTRITION

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Childhood malnutrition is associated with growth stunting, increased susceptibility to infection, and increased mortality. Thus, there may be a underlying immunologic relationship between growth failure and immunodeficiency. Previously, we have described a murine model of human weanling malnutrition which leads to: (1) increased visceralization after infection with Leishmania donovani; and (2) defective macrophage pro-inflammatory cytokine response (decreased levels of tumor necrosis actor-α, IL-10, and NO and increased levels of IL-6 after IFN-γ/LPS stimulation); and (3) an increased ratio of immunosuppressive prostaglandins compared to inflammatory leukotrienes (after LPS stimulation). There are particular molecular links between the nutritional status and immune function. We have coined a new term for these: nutrikines. Nutrikines include the adipokines (leptin, adiponectin, and resistin), insulin-like growth factor-1 (IGF-1), corticosterone, and gherelin. Relatively higher levels of leptin, IGF-1, resistin, and gherelin observed in a well-fed host would be expected to increase the pro-inflammatory response, whereas high levels of adiponectin and corticosterone (observed in malnutrition) would be expected to decrease the inflammatory response. Nutrikine dysregulation (abnormal levels of the aforementioned nutrikines) in the malnourished host may lead to defective macrophage priming, and a decreased pro-inflammatory response. Weanling mice received a diet deficient in protein, calories, zinc, and iron for 6 weeks and compared to mice on a control diet. Serum levels of leptin, IGF-1, and resistin were all significant lower in the malnourished mice, compared to controls (53, 71, and 22%, respectively). In contrast, levels of the immunosuppressive nutrikines adiponectin and corticosterone were significantly higher (60 and 287%, respectively) in the malnourished mice. Levels of gherelin were comparable. Thus, lower serum levels of immunostimulatory nutrikines (leptin, IGF-1, and resistin), and higher serum levels of immunosuppressive nutrikines (adiponectin and corticosterone) may be a factor in the defective macrophage pro-inflammatory response in the malnourished host and be a factor in the increased susceptibility to infection in the malnourished host.

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A MYCOBACTERIUM BOVIS BRAIN ABSCESS IN A 34-YEAR OLD HISPANIC MALE WITH AIDS

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Infection with *Mycobacterium bovis* has become rare in developed countries since the implementation of control measures in cattle and routine pasteurization of dairy products. In developing countries, however, it remains a significant proportion of mycobacterial infections. Consumption of unpasteurized dairy products from infected cows is the primary route of transmission to humans. Case: A 34-year old Hispanic male with AIDS (CD4 count 22/mm^3, HIV viral load 22,000 copies/mL) presented with a one week history of worsening left posterior headache, imbalance, and nausea with vomiting. The patient had emigrated from Mexico three years ago. He was from an urban area, with no direct contact with livestock and with no specific recollection of consuming unpasteuriazed dairy products. Physical exam was significant for a wide-based ataxic gait, dysmetria, and dysdiadochokinesia. MRI showed a ring enhancing bi-lobed right cerebellar mass with associated edema and midline shift. PPD and serologies for *Toxoplasma*, *Coccidiodomyces* were negative. Urine histoplasma antigen and cryptococcl antigen tests were negative A chest radiograph showed no acute disease. A SPECT scan of the brain did not suggest primary CNS lymphoma. Lumbar puncture was deferred due to the risk of herniation. Empiric therapy for toxoplasmosis was started. As no clinical improvement was seen, an MRI brain was repeated on hospital day 14 that showed interval enlargement of the cerebellar mass with new daughter lesions. Neurosurgery subsequently performed a craniectomy, with evacuation and drainage of the abscess. The smear of the pus was 4+ for AFB, prompting empiric rifampin/isoniazid/pyrazinamide/ethambutol therapy. Ultimately, *M. bovis* was identified in cultures from the abscess and sputum. The patient was subsequently treated with a rifampin/isoniazid/ethambutol/fluoroquinolone and trizivir regimen. In conclusion, disseminated *M. bovis* infection in patients with AIDS has not been commonly reported. Although the primary route of infection is via consumption of unpasteurized dairy products, infection can also occur by inhalation of infected aerosols from cows or aerosols generated during the handling of infected carcasses. *M. bovis* infection should be considered in any patient with these identifiable risk factors and also in patients from developing countries in which *M. bovis* remains endemic, even without these obvious risk factors.

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WHERE THERE ARE NO HEALTH POSTS: THE CHALLENGE OF COMMUNITY-DELIVERED INTRAVENOUS ANTIMONIAL THERAPY FOR LEISHMANIASIS IN NORTHEAST BRAZIL

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Leishmaniasis presents in various clinical forms in northeast Brazil, including cutaneous, mucosal and disseminated disease. In the Corte de Pedre endemic area of Bahia, the health post serves as the main referral center for the diagnosis and treatment of leishmaniasis and attends to 600 cases per year. The post is located in an isolated agricultural region and serves a population of approximately 500,000 people. Standard first line therapy is intravenous antimony (20mg/kg) delivered once a day for 20 or 30 days, depending on the clinical form of the disease. Due to long distances, poverty and limited access to transportation or feasible routes due to poor weather and road conditions, many individuals can not reach their nearest health post daily and instead acquire intravenous treatment through volunteers in their remote community. The informal delivery of intravenous therapy by community volunteers poses challenges to the safe and reliable treatment of leishmaniasis. Our experience has shown that community volunteers do not have formal training and may not consistently provide clean, safe injections of antimony, which is not a benign medication and may have serious adverse effects, such as cardiac arrhythmias. Community delivery of therapy also raises questions about the storage of antimonials, supply delivery, and the safety of community volunteers and their families with regards to the safe needle use and disposal. We have begun community-based trainings of volunteers who deliver intravenous antimony in the Corte de Pefra area. Volunteers are recruited with particular emphasis in areas of high prevalence. Lectures are delivered by health post staff on the basics of leishmaniasis, intramuscular and intravenous treatment for children and adults, safe needle use and disposal, and basic treatment of potential side effects. We also track the adherence to and delivery of antimonial therapy prescribed at the health post. Lastly, we follow up with trainees after 3-6 months in the field to reevaluate their performance and acquisition of knowledge and practice techniques. Community volunteers provide an important service in areas where there are no health care professionals to deliver intravenous therapy, and their support and training are crucial to leishmaniasis treatment in this rural area.

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DETECTION OF HUMAN FECAL MARKER, *CRYPTOSPORIDIUM*, AND *GIARDIA* USING IMMUNOMAGNETIC SEPARATION IN CONTAMINATED WATER SAMPLES

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Due to the increased prevalence of pathogens in contaminated water supplies, there is a pressing need for a rapid yet sensitive assay that detects human fecal contamination. We examined human-specific fecal markers as an indicator of water contamination and developed a method for sequentially detecting human fecal markers, *Cryptosporidium* and *Giardia* in water. Isolation of human fecal markers, *Cryptosporidium* and *Giardia* from 8 ml water samples was done using immunomagnetic separation, in which paramagnetic particles were coated with analyte-specific antibodies. Commercially available anti-*Cryptosporidium* and anti-*Giardia* antibodies were used to detect these pathogens, while a procedure developed in collaboration with TechLab, Inc. was used to detect human fecal markers. Human fecal markers were separated initially, followed by *Cryptosporidium* and *Giardia*. The sensitivities of the individual assays were compared to the sequential detection using a quantitative ELISA for fecal markers and quantitative PCR for *Cryptosporidium* and *Giardia.* Detection levels for human fecal markers, *Cryptosporidium* and *Giardia* were as low as 0.1 ppb (which approximates standards for fecal coliform levels), 10 oocysts/ml, and 100 oocysts/ml respectively. Preliminary testing done on drinking water samples taken from households in Fortaleza, Brazil revealed a number of highly contaminated samples (based on coliform counts) and showed that several were positive for human fecal markers and that one was positive for *Cryptosporidium*. In conclusion, antibody-coated immunomagnetic beads rapidly detect human fecal contamination in water samples less than 10mL. *Cryptosporidium* and *Giardia* can also be detected with this method. Using the assays together to rapidly detect human fecal contamination and transmissible enteric pathogens can provide an early warning of water supply contamination helping to limit disease transmission.

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CONTROL OF CLONORCHIASIS REQUIRES REPEATED CHEMOTHERAPIES

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The present study was undertaken to determine the best strategy of clonorchiasis control by repeated mass chemotherapies in China. Seven different control strategies were implemented, and all subjected residents (mass treatment) or egg positive cases (slective treatment) were treated with praziquantel repeatedly every 6 months, one or two years. The recommended dosage of praziquantel, 25 mg/kg, 3 times a day, was used. The egg positive rates in 5 heavy endemic villages were 44.8-70.0%. All subjected villages showed 72.8% to 92.0% reduction of egg positive rates after 2, 3 or 6 repeated chemotherapies. Mass treatments in 2001 and 2003 reduced the egg positive rate from 68.8% to 18.7% and 4 annual mass treatments reduced the rate from 44.8% (2001) to 8.7% (2004). Selective annual treatments reduced the rates from 50.8% (2001) to 13.8% (2004) or from 70.0% (2001) to 11.6% (2004), and two treatments in a year reduced the rate from 57.6% (2001) to 4.6% (2004). In moderate endemic areas, the egg rates were 22.6% and 28.3% in 2001, but 1.7% and 1.1% after 2 or 3 selective treatments. The repeated treatments reduced EPG counts significantly. Among treated residents, one showed drug eruption and was treated at a hospital. The present results confirm clonorchiasis is widely prevalent and heavily endemic along the rivers in Heilongjiang Province, China. The reduction of clonorchiasis by one mass chemotherapy was about 50% of pre-treatment positive rates. The more treated showed the better reduction but mass or selective treatments showed no differences in endemic areas. The chemotherapy of reservoirs hosts showed little impacts on the control effect. Mass or selective chemotherapy is effective to control clonorchiasis in heavy endemic areas when the chemotherapy is repeated while one selective treatment is effective enough in moderate endemic areas.

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PERSISTENCE OF BACTEREMIA BY *BARTONELLA BACILLIFORMIS* POST TREATMENT WITH CLORANFENICOL. ANCASH - PERU

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Bartonella bacilliformis (Bb) is an obligate intracellular bacterium that initially invades the endotelial cells and then the red blood cells, its target cells, in which it reproduces finally destroying them. Chloramphenicol was the antibiotic of choice for the treatment of the acute phase of Bartonellosis (Carrion's disease) in Peru: However there is not scientific evidence of its efficacy. The aim of this study was to determine the persistence of bacteremia by Bb after the treatment with chloramphenicol. A cohort study was performed in the Hospital of Caraz, Ancash department, located in the northeastern highlands of Peru, between July and December 2003. All patients who had a positive culture for Bb were enrolled. A clinical-epidemiological questionnaire was completed and blood samples were obtained for blood culture, thin smear, hematological and biochemical tests. All patients who after finíshing treatment with chloramphenicol PO for 14 days had a positive culture or positive PCR for Bb, or presented typical eruptive lesions of Carrion's disease were considered as persistence of Bb. A total of 66 patients were enrolled. Of them, 53% (35/66) were male, the median of age and the time of disease were 11 years (range 3 months-72 years) and 6.5 days (range 1-90 days) respectively. The samples for culture were taken between 36 and 408 days after the beginning of treatment with chloramphenicol. The half of patients (50%) persisted with the presence of Bb in blood, of whom 10.6% (7/66) had a positive culture for Bb, 28.8% (19/66) presented eruptive lesions, 7.6% (5/66) had a positive culture and eruptive lesions, and 3% (2/66) had a positive PCR. All thin smears taken after treatment were negative. The letality rate was 0%. In conclusion, chloramphenicol, the antibiotic of choice for the acute phase of Bartonellosis in Peru, produces clinical amelioration among cases, however it is not efficacious to eliminate Bb from blood. Patients may keep the viability of Bb in blood for months, increasing the risk of transmission in endemic areas.

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MAGNITUDE OF THE FIRST OUTBREAK OF DENGUE FEVER IN THE DISTRICT OF COMAS, LIMA-PERU

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Dengue fever (DF) is currently one of the most important vector-borne disease in Peru. Outbreaks from some departments of the northen coast and jungle of Peru have been reported. Native cases of DF in the city of Lima, capital of Peru, have never been reported. Our aim of this study was to determine the magnitud of the first outbreak of dengue in Lima city and identify the dengue serotype involved. We made an analytical cross-sectional study in the district of Comas, located in northern Lima, department of Lima, between April and May 2005. We selected a random sample of the population, collected blood samples in filter papers to determine antibodies IgM and IgG against the dengue virus and applied a clinical-epidemiologic questionnaire. In turn, a 5 cc blood sample was obtained for viral isolation from febrile patients visiting a Ministry of Health facility in the involved area. All specimens were tested in the National Institutes of Health of Peru. A total of 295 voluntary subjects were enrolled. Of them, 36.7% was male and the median of age was 32 years old (range 1-72 years). The 27.5% (21.0-34.0%) had IgM antibodies and the 26.5% (20.3-32.9%) IgG antibodies against dengue virus. All subjects that had IgG also had IgM antibodies. The estimated population in the study area was 42800 (CI 95%: 37140-48460) inhabitants and the number of DF cases estimated was 11780 (7810-16485); nevertheless only 845 cases had been reported in the surveillance system. From subjects with positive IgM only did the 29.5% fulfilled the dengue case definition and of them only did the 48% looked for medical attention. The serotype identified was dengue D3. In conclusion, the magnitude of this outbreak by dengue virus D3 was greater than did we expect. Less than 10% of infected people were reported by the surveillance system. This finding may be explained by the high frequency of subclinical or asymtomatic cases among infected people and the lack of expertise from the health personnel to identify this condition.

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EUROPEAN MALARIA CONSULTANTS IN THE US

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The Malaria Committe of the League of Nations was intrigued by the phenomenon of "anophelism without malaria". The members, Col SP James from England and Prof NH Swellengrebel from the Netherlands were charged to investigate the situation in the Southern States of the US, in 1927. The mission was to inquire about several points: 1. [the Americans] say that they drive away malaria with drainage of agricultural land. In Holland and in Italy the ditches are breeding places themselves. Is that not the case here and if so, why? 2. they claim to reach miraculous results with their "standard quinine treatment" of 8 weeks using 50 grams per cure. Others contradict this with pertinency. What is true? 3. what is the role of impounded water for electrical power stations in relation to malaria? 4. the antimalaria organisation is wholly financed by local governments (cities and counties). It is said to be simple but effective. What is the truth of it? In Memphis, anti-mosquito oiling activities were more aimed at *Culex* than at *Anopheles*, mostly to please the public. On the cotton plantations in Greenwood the picking period coincided with the malaria. In Jackson malaria was eliminated through drainage; some said because of drainage, others because of propaganda. Information of local physicians was unreliable and it appeared doubtful whether the decrease was as real as thought. In Mobile the drainage of marshes in 1922 was said to have stopped the malaria problem, but the ditches were grossly neglected and larvae easily found. Moreover, there were no statistics to demonstrate the decrease. In Montgomery, the State Health Officer was convinced that drainage had driven the malaria away. The visitors caught larvae under his eyes.. Only around Bamberg the dainage system was well maintained, but at enourmous costs. In the wet forests of Colombia *A. quadrimaculatus* bred ("quad"). Damming led to explosions of mosquitoes and malaria that had been rare before. Intensive oiling was said to have eased down the epidemic. In Suffolk Dr Boyd was met, and in Richmond Dr Williams, who both knew their profession. They agreed that specific antimalaria campaigns in the Southern States were an illusion: not aimed at the quad but at all nuisance mosquitoes, and combined with a general ignorance about malaria statistics. Swellengrebel departed with a firm idea about cause and effect. But the galley proof of the report written by James, stirred Swellengrebel's feelings. The malaria recession in the States had been due to "natural" causes and had started before active measures had been taken against it. Swellengrebel decided to write his own (1928). Contrary to the statement of the joint report in which the decline of malaria was described despite the abundance of anophelines, he concluded that it was "wholly due to Anopheles reduction by the extension of drained cultivated land; a single *quadrimaculatus* is more dangerous than a single *maculipennis* in Europe and destruction of the former is worth more than of the latter". The surgeon general remarked: "The US Public Health Service deeply appreciates the value if these very interesting analyses of the malaria problems of this country by the two eminent malariologists. It is unfortunate, but entirely understandable that the two reports should express diametrically opposed views on many points. The reports simply reflect how wide a divergence of opinion may exist.."

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ROLE OF HSP70 IN DENGUE VIRUS REPLICATIVE CYCLE

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Dengue virus is the causative agent of dengue fever, dengue hemorrhagic fever and dengue shock syndrome. Dengue virions are enveloped and icosaedrical containing a single stranded positive polarity RNA as genome. The unique open reading frame encodes three structural, C, prM and E, and seven nonstructural proteins. E protein is the most exposed protein and interacts with the receptor on the surface of the host cell. This first contact is a very important step in the establishment of dengue infection. Several authors have reported the interaction of dengue virus with different molecules such as heparan sulfate, DC-SIGN, a laminin receptor and some heat shock proteins such as GRP78, HSP70 and HSP90. Our group, using an affinity chromatography technique, found that HSP70 and HSP90 from neuroblastoma and monocytic cell lines bind to the E protein from dengue virus. Infection inhibition assays using antibodies against both proteins supported that HSP70 and HSP90 are part of the receptor complex in human monocytes and neuroblastoma cell lines. In an attempt to demonstrate firstly the function of HSP70 in dengue virus entry we used the interferent RNA technology to knock down the expression of this molecule. The iRNA-mediated knock down expression of HSP70 caused an important reduction in viral yield. Studing the ability of dengue virus to bind and enter into the silenced cells will allow us to propose a role of HSP70 in dengue virus replicative cycle and in viral entry.

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VECTOR COMPETENCE FOR DENGUE 2 VIRUS IN WILD COLLECTIONS OF *AEDES AEGYPTI* FROM VENEZUELA

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Aedes aegypti from 8 collections in Venezuela, were orally challenged with dengue 2 virus JAM1409 (DEN-2 JAM1409). In contrast with *Aedes aegypti* populations from Mexico, United States and Colombia, the vector competence of Venezuelan collections ranged from 77-95%. The presence or absence of a midgut infection barrier (MIB) and a midgut escape barrier (MEB) was determined in each collection. The percentage of mosquitoes exhibiting an MIB ranged from 2-15%, and those exhibiting an MEB ranged from 2-18% in the collections.

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CLINICAL AND MOLECULAR CHARACTERIZATION OF DENGUE PATIENTS COHORT IN RECIFE, BRAZIL FOR EPITOPE MAPPING AND IMMUNO PATHOGENESIS STUDIES

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Aiming to enable more efficient development of dengue vaccines and identification of dengue disease prognostic markers, we intent to characterize the human immunome elicited by dengue virus infection, focusing on the comparison between the dengue and dengue hemorrhagic fever syndromes. Our study cohort is composed of dengue fever and dengue hemorrhagic fever (DF/DHF) volunteers from the city of Recife-PE, Brazil that are enrolled among patients with clinical diagnoses of DF. Volunteers are examined on the first and fourth days of symptoms, with additional evaluations if necessary. Each case is collected 4 to 5 blood samples, from day 1 to day 30 from the start of the symptoms and one additional sample six months later. The clinical characterization includes clinical history and examination, hemogram, platelet count, liver enzymes, HLA typing, MBL2 and IL-18 SNPs analyzes, dengue serology (IgM and IgG), virus isolation and dengue quantitative PCR diagnosis and serum cytokine data (IL-2, IL-4, IL-5, IL-10, TNF–α and IFN-γ). The study data is integrated into a customized database that includes complete clinical data, research results and the respective inventories of cryopreserved samples of PBMCs, plasma and serum. Our cohort study enrolled 471 patients. The first serum samples from all patients were submitted to RT-PCR, virus isolation in C6/36 cell line, ELISA-IgM and ELISA-IgG. Serology (IgM and IgG) was performed in all additional serum samples collected, a total of 1512. Among these 471 patients, 239 (50.7%) are female and 232 (49.3%) male; age ranging from 5 to 84 years old, being 103 cases <15 years and 366 ≥15 years old. From a total of 230 (48.8%) laboratory positive cases, 88 (38.2%) were classified as primary and 89 (38.7%) secondary dengue infections. Among these 230 cases, 18 (7.8%) were classified as DHF, according to the WHO criteria and in contrast to what would be expected, 9 (50%) of our DHF cases were primary dengue infections. 62% of the RT-PCR samples collected before 5 days were positive, whereas only 38% of the samples collected after day 5 were positive. DENV-3 was the predominant serotype isolated and this fact has facilitated many of the studies. These samples and clinical data are being used for the development and validation of the dengue molecular diagnostics, epitope mapping of immune responses, dengue immunopathology, and the *ex vivo* evaluation of the immunogenicity of candidate vaccines.

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INACTIVATION OF DENGUE VIRUS USING HIGH HYDROSTATIC PRESSURE (HHP)

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Although several approaches are being investigated, no licensed vaccine to prevent dengue currently exists. In this study we investigated the use of High Hydrostatic Pressure (HHP) to inactivate a live dengue-2 virus. One advantage of HHP is that complete virus inactivation can be achieved under controlled conditions without exposure of the virus to radiation or chemicals such as formalin. DENV-2 was propagated in Vero cells and the culture supernatants were concentrated approximately 50 to 100-fold. The concentrated virus was sealed in polyethylene pipettes and subjected to different conditions of varying pressure, temperature, and time of inactivation. Following treatment, residual virus infectivity was determined by direct plaque assay and by a 14-day amplification assay on Vero cells. Viral antigenicity was measured by ELISA using monoclonal and polyclonal antisera. As a measure of functional activity, investigators determined the ability of the treated virus to hemagglutinate goose red blood cells. Of the 31 high-pressure conditions tested, 19 conditions resulted in complete virus inactivation from initial infectivity titers as high as 7 log10 pfu/ml. There appeared to be a small loss in antigenicity of the HPP-inactivated virus compared with untreated and formalin-inactivated virus; the loss was difficult to quantify. Both HPP- and formalin-inactivated virus also lost the ability to hemagglutinate goose red blood cells. Immunogenicity of HHP-inactivated virus will be tested in a murine model and compared with formalin-inactivated and vaccines.

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CHARACTERIZATION OF MICROVESICLES FROM IMMUNE CELLS INFECTED WITH DENGUE VIRUS

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Microvesicles were produced in large amounts after dengue virus infection of human immune cells. We characterized these particles by sucrose gradient ultracentrifugation and transmission electron microscopy. They are electron-dense particles with 70-90 nm in diameter. By Mass Spectrometry (MS), Flowcytometry and PAGE the microvesicles showed quantifiable differences bewteen uninfected and infected cells. Microvesicles were labeled with fluorescent probes for lipids (PHK-26, Sigma) and for RNA (SYTO® RNASelect, Invitrogen). Lipid probes were used to monitor microvesicle transfer to HUVECs. RNA labeling of microvesicles was resistant to RNase A treatment, and positive for dengue virus by qRT-PCR, suggesting virus particles were protected inside the microvesicle. All microvesicles derived from PBMCs, B, monocytes, myloid derived dendritic cells were positive for CD45 by Flowcytometry, and for each microvesicle type the cell surface markers present in the progenitor cell were also found in the corresponding microvesicle (CD14, CD19, CD56, CD1a, CD4, PECAM-1 for Monocytes, B, NK, dendritic cells and CD4-T and HUVEC respectively). All the microvesicles tested increased in net number after 48 hours of *in vitro* dengue infection at MOI of 1 but did not after IFN α treatment. Proteomics using MS quantitative analysis (iTraq) determined the proteins that were differentially present

in microvesicles of human B cells infected with dengue virus. Membrane, cytoplasmic and nuclear proteins were identified. Of those, plasma membrane proteins were confirmed by Flowcytometry. The implications of microvesicle formation and fusion during dengue infections could explain physiopathological conditions of the disease including activation of coagulation and immune modulation. Plasma and sera from acutely infected dengue patients with fever had 20 fold increased CD14 and CD19 positive microvesicles obtained with the procedure used for *in vitro* studies. Microvesicles from Monocyte and B cell resulted from the infection *in vivo*. Implications of microvesicle formation in dengue infection are discussed.

(ACMCIP Abstract)

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PRIMARY HUMAN MYOBLAST ARE TARGETS FOR DENGUE VIRUS INFECTION

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One of the main features of dengue fever is myalgia, involving the muscles of back and limbs. Therefore, the disease has been described as "Break Bone Disease." There is paucity of literature regarding the involvement of muscle during dengue viral infection. This study demonstrates dengue virus infection of primary human myoblasts. FACs analysis detected dengue virus in primary myoblasts (0.3-6%) infected for 48 hours at MOI of 1. In addition, electron microscopic studies show presence of virions in DV-infected primary myoblasts. These observations were supported by the TaqMan RT-PCR analysis of D2V. Increased gene expression levels of six innate response genes (Mda-5, CXCL6, IRF7, IDO, TRAIL and LGal3BP) and up-regulated surface expression of MHC-I suggests myoblast response upon infection. Previous studies have demonstrated that skeletal muscles in mice can be infected with adapted dengue virus leading to ultrastructural changes in skeletal muscles and biochemical changes physiologically (. Also, recent human study has observed mycositis in dengue patients during acute viral infection. Our study indicates that these observed changes in skeletal muscles might be an outcome of dengue virus infection of myoblasts.

(ACMCIP Abstract)

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TUMOR NECROSIS FACTOR-RELATED APOPTOSIS-INDUCING LIGAND (TRAIL) REDUCES DENGUE VIRAL LOAD IN HUMAN INFECTED CELLS

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TNF-related apoptosis inducing ligand (TRAIL) was identified from Affymetrix gene expression analysis of *in vitro* infections and from PBMCs of dengue patients as a potential link between the IFN type I, II and TNF-α response by comparing different cells permissive to dengue virus (HUVECs, Monocytes and B cells). A significant increase in dengue viral progeny was observed in anti-TRAIL Ab treated monocytes, B cells and HUVECs and conversely a decrease in viral progeny was seen in recombinant TRAIL treated monocytes. Type I and Type II interferons regulate dengue virus progeny. We found that IFN α mediated antiviral effect was partly dependent on TRAIL function. Thus, this study identifies TRAIL as a novel antiviral molecule able to limite dengue virus spread.

(ACMCIP Abstract)

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QUASI SPECIES OF DENGUE RNA: MOLECULAR DISSECTION OF DENGUE VIRUS INFECTIONS IN MONOCYTES-DERIVED HUMAN DENDRITIC CELLS

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Dengue is a single-stranded, enveloped RNA virus that infects human dendritic cells (DCs) primarily at the immature stage of their differentiation. In this study, the growth kinetics of dengue 3 virus in human DCs was assessed. We have shown that dengue 3 infects human DCs as a DC-SIGN, C-type lectin dependent process via dengue specific real-time RT-PCR quantification. The majority of dengue infections in dendritic cells were blocked by anti-DC-SIGN antibodies, but not by antibodies to other molecules on these cells. In contrast to real-time RT-PCR, agarose-gel based electrophoresis of dengue cDNA identifies genotypes within allotypes (based on size polymorphisms) derived from dengue infected DCs at 48, 96 and 144 hours post infection. The presence of various NS5 deletions (500-600s bps) in addition to the intact non-deletion NS sequences was identified from DCs 48 and 96 hours post dengue infection. However, no detectable NS5 deletion was detected 144 hours post dengue infection. Similarly, it was also found that the blocking of dengue 3 infections by anti DC-SIGN antibodies eliminated the heterogeneous dengue NS5 RNA species, i.e., RNA population with NS5 deletions. The observed quasi dengue RNA species is both time and DC-SIGN dependent. The rise and fall of the quasi dengue RNA species in DCs coincided with DC maturation. For example, the peak cytokine production was observed at 144 hours post infection. The presence of quasi dengue RNA species might reflect a strategy that dengue virus uses to minimize host responses.

(ACMCIP Abstract)

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MULTIPLEX DENGUE TAQMAN ASSAY (DEN1-4) FOR THE DIAGNOSIS OF ACUTE DENGUE SECONDARY INFECTIONS

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Dengue is an arthropod-borne flavivirus composed of single stranded RNA of approximately 11kb and is subdivided into four distinct serotypes (DEN1-4). Dengue is hyperendemic in Puerto Rico therefore most dengue infections are secondary infections. The Centers for Disease Control and Prevention surveillance system has determined the circulating dengue viruses in Puerto Rico for the last 30 years. The present study was a retrospective study of secondary dengue infections in which we randomly identified 100 candidates from each serotype that were confirmed using standard tissue culture isolation and immunofluorescent assay (IFA) for virus identification from the last ten years. These samples were used to validate the results of a multiplex TaqMan assay for all four dengue serotypes. In addition, we measured the antibody response of these sera using a MAC-ELISA at these early time points. These data determined that circulating viral titers during the first 5 days post onset of symptoms (DPO) were elevated throughout the entire 5 days determined by the multiplex TaqMan assay. In addition, the minimum cycle threshold value determined from the TaqMan assay for virus detection could not be related to the qualitative IFA results. Because virus isolation results were based on a qualitative determination of the amount of viral antigen present in serum infected cells, the results did not correlate. These results demonstrated the limitations of viral isolation during secondary infection because of the presence of cross protective neutralizing antibodies that may inhibit the infection of the cells used to amplify the virus. We concluded that the multiplex TaqMan assay was a valid assay for dengue serotype diagnosis in acute serum specimens and was more sensitive than virus isolation assay for secondary infections. However both assays should

be used for confirmation of a diagnosis of dengue due to the potential of contamination of the TaqMan assay because of its sensitivity and the decreased sensitivity of the virus isolation assay following secondary infections. In addition, because TaqMan assay is quantitative and relatively rapid test compared to virus isolation, viral load can be measured in the serum and could be used as an early and predictive measure of disease severity.

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SEROEPIDEMIOLOGY OF HOSPITALIZED DENGUE PATIENTS IN THE PHILIPPINES

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Dengue is a major health concern in the Philippines. Although dengue has been a notifiable disease in the Philippines since the 1950's, there remains a paucity of dengue data due to the limited diagnostic capabilities. We determined the seroepidemiologic profile of patients admitted with dengue-like illness to San Lazaro Hospital, a tertiary infectious disease referral hospital in Manila. Serotype determination was conducted using RT-PCR and nested PCR. Patients were characterized as having acute dengue virus infection (primary or secondary) based on the IgM/IgG ELISA. Between September 1, 2005 to January 17, 2006, a total of 275 patients were screened and 104 patients were enrolled in the study; 90 (87%) were confirmed to have dengue. There were 6 (7%) primary and 84 (93%) secondary dengue virus infections. Forty (38%) were female and 64 (62%) were male. The mean age was 18 years, with 50% of cases between 13-20 years, only 3% were < 7 years and 1% > 35 years. There was no significant difference in the clinical manifestations and laboratory parameters between the pediatric and adult age groups. Most study participants were diagnosed with DHF gr II (49%) and DHF gr I (25%). Fever was the most common presenting manifestation (100%) followed by loss of appetite, headache, and muscle pains. Hemorrhagic tendencies were seen in 86 (96%) patients. DEN-3 was the predominant serotype, though all 4 serotypes were identified during the study period. There was equal distribution between pediatric and adult groups; this may reflect changing epidemiology of dengue in terms of ages affected. Similar observations have been noted in Latin America and Southeast Asia since the 1980's. Studies have shown not only an increase in dengue incidence rates among young adults but also a shift in peak dengue mortality. This apparent shift suggests later exposure to dengue viruses. This epidemiological change may have important implications for health service planning and dengue control strategies. However, further study is warranted to determine if there is a real shift in modal age or whether such trend is the result of improved detection and diagnosis of dengue among the adult population.

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YUCATAN MINIATURE SWINE DEVELOP DENGUE VIREMIA AND ARE A POTENTIAL MODEL FOR DENGUE IMMUNOPATHOLOGY

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Evaluation of dengue vaccines has been limited to date by availability of adequate animal models, in particular for dengue disease and protective immunogenicity. Due to their physiological and immunological similarities to humans, we chose Yucatan Minipigs as a novel species in which to study dengue virus infection and resultant immune responses. We now expand upon our successful development of the first intermediate animal model for dengue viremia and report immunopathologic features of the swine model. We previously established a dengue viremia model using subcutaneous (SC) inoculation of swine. In the current work we expand the earlier results by evaluating intravenous (IV) inoculation as well as re-challenge of previously infected animals. Swine were inoculated with $1x10^7$ PFU of virus by central IV to evaluate the impact of administration route. Presence of viremia was tested using cell culture isolation and RT-PCR. In contrast to SC inoculation, no animals inoculated IV developed detectable viremia, though all animals developed antibody responses as determined by ELISA and neutralization assays. In subsequent experiments, previously inoculated animals (both SC and IV primary inoculation) were re-challenged by SC inoculation approximately 6 months after initial infection to assess the degree of immunologic protection. Unexpectedly, 5 of 6 animals previously infected SC, and 4 of 4 animals previously inoculated IV, developed a pronounced petechial rash upon SC rechallenge with virus. Cutaneous manifestations developed between day 3 and 4 post-inoculation and resolved by day 10. Histopathologic examination of skin biopsies revealed intradermal inflammation. Dengue immunoreactive immune complexes were detected in the sera of all animals that developed rash, but none of contemporaneously inoculated primary infection controls. In conclusion, Yucatan Miniature swine are susceptible to experimental dengue viremia and develop immune responses following dengue infection. Induction of viremia is dependent upon route of administration, suggesting the importance of initial target cells in initiating productive infection. Rechallenge of previously sensitized animals results in a cutaneous disease phenotype resembling skin lesions in human dengue infection, and the cutaneous findings are associated with dengue-containing immune complexes.

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ACTIVATION OF COAGULATION AND FIBRINOLYSIS IN DENGUE VIRUS INFECTION: RELATION TO THE BLEEDING SYMPTOMS

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Dengue virus infection may result in a wide range of clinical manifestations, from mild to severe with hemorrhage and vascular leakage. Blood mononuclear cells are targets of dengue virus and diminushed platelets count is a consistent finding. There are abnormalities in the coagulation and fibrinolytic processes that contribute to the pathogenesis and severity of the disease. We focused our study in the activation of coagulation process. A cohort of 113 patients with dengue (diagnosed by RT-PCR and serology) and 38 non-dengue viral infection was studied. Mean age was 25 +/- 16 (SD). Abnormal bleeding were petechiae, mucous bleeding, positive tourniquet test or vaginal bleeding and they were present in 77% of cases, 4 cases with serous leaking. The following tests were done for all cases at the febrile period until 72 h of apyrexia and at convalescence: platelet count, plasma thrombin time (TT), PT, aPTT, fibrinogen (Fbg), D-dimmer (DD), factor II fragment 1+2 (F1+2), von Willebrand Factor (vWF), thrombin-antithrombin complex (TAT) and soluble Tissue Factor (TF). For 70% of all dengue cases TT was higher with increased vWF and DD. In febrile phase there is a significant rise in TAT, at defervencens Fbg and platelets reached their minimal values. This characteristic behavior was more marked in the group with hemorrhagic manifestations. The TAT results suggests that activation of coagulation starts at the febrile (viral replication phase) followed by a fibrinolitic response (increased DD and a negative correlation of Fbg with DD). The hemorrhagic symptoms are a sing of severity; prolonged TT and TAT are precautious signs of outcome and prolonged TT can be used for differential diagnosis in dengue infections.

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DENGUE-2 AND YELLOW FEVER VIRUSES INDUCE GENE EXPRESSION CHANGES IN THE MIDGUT OF THE DISEASE VECTOR, *AEDES AEGYPTI*

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Dengue and yellow fever viruses are two flaviviruses that are extensively studied due to there ability to cause human disease. These viruses seem to be mainly studied in the mammalian system with little effort going towards the understanding of how these viruses act within the vector. By studying these viruses within the vector itself, we can dissect pathways that these viruses utilize within the host for entry, replication, and release. We are using *Aedes aegypti*, the vector that is largely responsible for transmission of these viruses, to further understand the pathways involved in viral infection. Using microarrays previously constructed in our laboratory, we compared both Dengue-2 (Jamaica 1409 strain) and yellow fever (Asibi strain) infected midguts with noninfected at 4, 7, and 14 days post infect. There were a few genes that showed a dramatic increase following viral infection, including a chitin binding protein and a synaptic vesicle protein. These genes are suggested to be involved in immunity and viral release, respectively. We are currently conducting studies on viral entry and release using specific inhibitors. These viruses are able to use host machinery for entry, replication, and release. By knowing what pathways are exploited by the virus, we may be able to block viral spread at the site of the vector.

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DEVELOPMENT OF A DENGUE ELISA-BASED MICRO NEUTRALIZATION ASSAY

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Dengue virus infections occur in most of the tropical and subtropical areas of the world, and dengue is considered as one of the most important infectious diseases. Various serological techniques have been used for the detection and quantification of neutralizing antibodies to dengue. The plaque reduction neutralization test (PRNT) is the gold standard to measure dengue-neutralizing antibodies. However this test is labor intensive, time-consuming, and carried out in 6 or 24-well plates, which limits its usage for epidemiological and immunological trials especially in the context of vaccine development and large-scale evaluation studies. To address the above shortcomings, we have developed an ELISA-based micro neutralization assay for the detection and quantitation of dengue neutralizing antibodies. The principle of this assay is similar to PRNT. However, the new assay is carried out in 96-well plates with optical density measurements and does not require viral plaque counting. This assay uses dengue virus serotype specific primary and peroxidase-labeled secondary antibodies with a colorimetric soluble substrate for viral antigen detection in the infected cell monolayer. We have optimized the ELISA based microneutralization assay for the four serotypes of dengue virus and demonstrated that this assay is sensitive and reproducible. A concordance study was conducted to evaluate the agreement between ELISA-based and PRNT methods with a panel of human serum samples. Our results indicate an acceptable relationship between the neutralizing antibody titers obtained by ELISA-based micro neutralization assay and standard PRNT.

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HIDDEN TRANSMISSION OF DENGUE VIRUS IN KAOHSIUNG SCHOOL CHILDREN DURING 2004-2005 AFTER THE 2001-2003 LARGE-SCALE EPIDEMIC OF DENGUE/DENGUE HEMORRHAGIC FEVER IN TAIWAN

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The severest epidemic of dengue fever (DF) and dengue hemorrhagic fever (DHF) occurred in Kaohsiung, Taiwan in 2002 [5387 DF, 242 DHF, 13 dengue shock syndrome (DSS), and 21 deaths]. It is interesting to investigate whether the dengue virus (DENV) might circulate silently among school children after this large-scale epidemic. This study was to measure the seroprevalence and seroincidence rates and the magnitude of silent transmission in primary school students and to investigate whether fever alert in communities might be feasible to detect the trends of DENV transmission in cities in advance. A prospective cohort was used to recruit grade 2-3 school children located in high or low 2002 epidemic areas and also considering population density of Kaohsiung. Serum samples from 8-to-11 year-old children were collected on June 29th, 2004 and Jan 17th 2005 for both pre-epidemic and post-dengue epidemic time points, respectively. The results found the overall seroprevalence rates of DENV infection before the 2004 epidemic season (1342 children:11 schools) and after the 2004 epidemic season (612 children:4 schools) were 4.55% (61/1342) and 5.56 % (34/612), respectively. Among the 174 paired serum samples, four children from three different schools were seroconverted as anti-dengue IgG (+). Thus, the overall seroincidence rate was 2.30% (4/174). Interesting, the geographical distributions of seroprevalence of DENV infection among these children's schools were parallel to the incidence rate of the dengue cases in 2001-2002. Besides, we used the five serological assays, including the three different ELISA assays at Taiwan-Centers for Disease Control and Prevention for capture dengue and Japanese encephalitis IgM and IgG and serotypes by NS1 indirect IgG plus plaque reduction neutralization test (PRNT) and western blot to confirm the results at the National Taiwan University. In addition, mostly ELISA positive samples were primary DENV-2 infection. The paired-serum samples show the consistency between PRNT and NS1 IgG ELISA serotyping. Most importantly, the five symptomatic DENV infected children showed different symptoms: fever, abdominal pain, cough, running nose, fatigue, and nausea. Risk factor analysis found that the family history of DENV infection was statistically significant with the children acquired the DENV infection (p=0.037). In conclusion, our seroincidence rate (2.30%) was much lower than those reported from Thailand in 1999-2000 and Singapore in 1996-97.

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DISTRIBUTION AND TISSUE TROPISMS OF THREE PHENOTYPICALLY DISTINCT YELLOW FEVER VIRUS STRAINS IN *AEDES AEGYPTI*

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Arbovirus dissemination from the midgut of a vector mosquito is a critical step in facilitating virus transmission to a susceptible host. We have previously characterized the genetic determinants of yellow fever virus (YFV) dissemination from the ***Aedes aegypti*** mosquito midgut using two genetically and phenotypically distinct strains of yellow fever virus (YFV): the wild-type, disseminating YFV Asibi strain and the attenuated, midgut-restricted YFV 17D vaccine strain. In our current study we examined the

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tissue tropisms of three YF viruses in ***Ae. aegypti***: Asibi, 17D, and a chimeric virus containing the Asibi M and E structural protein genes and 17D non-structural genes (17D/Asibi M-E). Mosquitoes were infected via intrathoracic inoculation or artificial bloodmeal and were sampled days 7 and 14 post-inoculation or days 3, 7, 10, 14, and 21 post-oral infection for paraffin embedding and serial sectioning. YFV antigen was visualized in the mosquito tissues by immunohistochemical staining. In orally infected Asibi and 17D/Asibi M-E mosquitoes, virus antigen was observed in the posterior and anterior midgut, cardia, salivary glands, fat body, and nervous tissues. As expected, 17D infection was limited to cells of the posterior midgut following oral infection. Differences in virus distribution in the posterior midgut between disseminating and non-disseminating viruses have been observed, and we have identified tissues important in virus amplification and dissemination. These observations will be discussed in the context of the hypothesized progression of events which occur during productive infection of ***Ae. aegypti*** with YFV Asibi.

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A MULTIPLEXED REAL-TIME QUANTITATIVE RT-PCR ASSAY FOR ARTHROPOD-BORNE FLAVIVIRUSES

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Arboviruses in the genus *Flavivirus* are major causes of human disease worldwide, and therefore it is essential to be able to rapidly and accurately test for them. Classical methods of detection using serologic or immunologic techniques are typically insensitive or require biological containment facilities in order to manipulate infectious cultures. Molecular detection assays based on broadly reactive (degenerate) primers also tend to have low sensitivity for many flaviviruses. In this study, we designed an assay to test for many flaviviruses using species specific primers in a single reaction. This real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR) assay combines flavivirus spp.-specific Taqman primers and probes (some previously described) into a single tube subjected to standardized thermocycling conditions. The multiplex assay contains specific detection for St. Louis encephalitis, West Nile, Dengue I, II, III, and IV, and consensus sequences for Tick-borne encephalitis complex groups: Russian Spring-Summer encephalitis and Central European encephalitis viruses. Viral RNA was extracted from infected cell-culture derived stock viruses, field samples, or was synthesized as subgenomic target RNA molecules. All flavivirus species and sample types were detected by the multiplex assay. A sensitivity analysis of the assay suggested that the multiplex was no less able to detect low virus titer samples than the single pathogen assay. This technique allows for a collection of specific assays to be used to screen for the presence of many flaviviruses of interest while saving labor and reagents, and without sacrificing sensitivity. The results demonstrated that these viruses can be screened for specifically using the rapid and sensitive method of real time qRT-PCR.

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HETEROLOGOUS INTERACTIONS OF WEST NILE VIRUS AND ST. LOUIS ENCEPHALITIS VIRUS: EFFECT ON REPLICATION AND VIRAL GROWTH KINETICS

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West Nile virus (WNV) has moved rapidly through the US since its introduction in 1999. Following the arrival of WNV to Florida in 2001, increased findings of WNV in the state surveillance system have coincided with a decrease in reports of Saint Louis encephalitis virus (SLEV). WNV is a flavivirus closely related to SLEV, which is endemic to Florida, and may be utilizing the same hosts and vectors as SLEV for transmission. These viruses may be interacting in a competitive manner for resources in this transmission system, and this interaction could partially explain the reduction in detected SLEV activity. To explore this possibility, we evaluated the effect of simultaneous and sequential infection on WNV and SLEV growth kinetics *in vitro*. When SLEV was introduced prior to WNV, SLEV infection was enhanced compared to when SLEV was introduced at the same time or after WNV inoculation. The same pattern was apparent for WNV, although WNV growth rates and titers were higher than those of SLEV when introduced simultaneously with equal titers of SLEV. We also tested asymmetric and equal multiplicities of infection (MOIs), monitoring viral titer over time. When introduced at asymmetric MOIs, the virus added at a lower MOI was inhibited by more than 10-fold versus control infections, with growth of the virus delayed over time. Results from *in vivo* co-infection studies will also be presented.

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FURTHER CHARACTERIZATION OF A WEST NILE VIRUS SMALL PLAQUE VARIANT ISOLATED IN NEW YORK, 2000

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A small plaque variant (SP) of West Nile virus (WNV) was selected from an isolate (WT) made from the kidney of a dead crow in New York in 2000. We previously reported growth of SP and WT viruses *in vitro* in Vero, DF-1 and C6/36 cells maintained at different temperatures, and *in vivo* in mosquitoes, chicks and mice. SP had lower rates of replication in Vero and DF-1 than WT, but grew equally well as WT in C636. In *Culex pipiens*, WNV SP had lower infection and dissemination rates following peroral infection and a higher ID_{50} after intrathoracic inoculation. The mean virus titers of WNV SP in mosquito bodies were significantly lower than WT. Chicks demonstrated delayed peak viremia and lower viral titers in blood following infection with WNV SP compared to WT. Current studies are investigating whether the differences previously observed in viral growth between SP and WT *in vitro* were due to temperature of incubation or cell line. Further studies in mosquitoes indicate decreased rate of viral replication and lower peak titers after three days post-inoculation. Adult house sparrows, natural hosts of WNV, were inoculated subcutaneously with 10^4 pfu SP or WT. The viremic response of the SP infected birds was variable, and the virus appeared to revert to WT plaque morphology. The average viremia titer of SP infected birds was lower than WT during the first 3 days PI, but then approximated the WT titers. Peak viremia for SP occurred at days 3-4, for WT day 2. C3H mice were inoculated SC in the footpad with 10^3 and 10^5 PFU WNV SP or WT. WNV SP had significantly lower morbidity and no mortality, and a lower viremic profile compared to WT. Virus was recovered with consistent high virus load from brains and footpads of all WT mice at the time of death in one experiment and on day 7 in a second experiment. No virus was recovered from brains of SP infected mice on day 7 pi demonstrating that SP has lower neuroinvasiveness compared to WT. Further studies are underway to clarify pathogenesis in sparrows and mice.

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POST HURRICANE JEANNE MOSQUITO BORNE INFECTIOUS DISEASE SURVEILLANCE AND HUMAN WEST NILE VIRUS INFECTION IN HAITI IN 2004

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We established laboratory-based fever surveillance in the three clinics providing health care in Gonaive, Haiti following hurricane Jeanne in 2004. Patients who were febrile (temperature $\geq$ 38.5° Centigrade) at the time of presentation were asked to provide blood for a serum sample and thick and thin malaria smears. The treating physician completed a brief medical history and physical on each of these patients and indicated the discharge diagnosis and treatment. All patients were asked to return in two weeks for a convalescent blood sample. Malaria smears were stained and read

using standard methods. To diagnose dengue, we used an IgM antibody-capture enzyme-linked immunosorbent assay (MAC ELISA) to detect anti-dengue IgM antibodies in all serum specimens. All the serum specimens were also tested for the presence of anti-dengue IgG antibodies to determine previous exposure dengue. In paired samples, a full titration of four-fold dilutions of serum specimen was used for the IgG ELISA and the end-point titration of these samples was used to assess seroconversion. To determine the presence of dengue viral RNA in acute serum samples (≤ 5 days post-onset of symptoms) we used both a nested polymerase chain reaction (PCR) and TaqMan assay. Because of the cross reactivity of flavivirus antibody tests, we utilized a plaque reduction neutralization test (PRNT90) to determine the specificity of the antibody response to the infecting virus. The serum was tested against West Nile virus (WNV), St. Louis Encephalitis (SLE) and dengue 1-4 viruses. In conclusion, of the 116 acutely febrile patients tested, three were diagnosed with malaria, two with acute dengue, and two with acute WNV infection. This is the first report of human cases of WNV in Haiti.

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PERSISTENCE OF WEST NILE VIRUS IN EXPERIMENTALLY INOCULATED ANIMALS

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Most RNA viruses cause a transient infection and are subsequently cleared from the host. Recently we found persistence of West Nile virus (WNV) RNA in house sparrows and pigeons six months after experimental inoculation. Our long-term research goal is to understand the mechanism of WNV persistence. Six-week-old, female C57BL/6 mice were inoculated subcutaneously (SC) with 10^3 PFU WNV, and eight to nine WNV-inoculated mice and one mock-inoculated mouse were sacrificed at monthly intervals. Brain, spinal cord, spleen, skin, lymph nodes, kidney and heart were harvested for virus isolation and WNV RT-PCR. Virus isolation was performed by co-culturing tissue homogenates on Vero cells, followed by two blind passages on Vero cells. At one month post-inoculation (p.i.), all eight WNV-inoculated mice had infectious virus in at least one of seven tissues. Infectious virus was found most frequently in the skin. WNV RNA was found more frequently than infectious virus, and all eight mice were positive for WNV RNA in the brain, spinal cord and skin. At 2 and 3 months p.i., no infectious virus was detected, but WNV RNA persisted in at least one tissue for all eight mice at 2 months and 8 of 9 mice at 3 months. WNV RNA was detected most frequently in the brain, spinal cord and skin. A similar study was conducted in one-day-old chickens inoculated SC with 10^3 PFU WNV. The seven surviving chickens and one mock-inoculated chicken were sacrificed at one month. In contrast to the mice, no infectious virus was detected in any of the tissues from the chickens. WNV RNA, however, was detected in at least one tissue for all seven WNV-inoculated birds and was detected most frequently in heart, brain and spinal cord. All tissues from mock-inoculated birds and mice were negative for infectious virus and WNV RNA. Sequence analysis and further studies to examine viral persistence in mice for up to one year post-inoculation are in progress. In summary, WNV can persist in diverse vertebrate hosts, and these results have potential implications in organ transplantation and overwintering of virus.

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EFFICACY, DURATION, AND ONSET OF IMMUNITY OF A WEST NILE VIRUS CHIMERA VACCINE

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The purpose of this research was to demonstrate the efficacy, duration and onset of protection of a chimera vaccine using a model of induced West Nile Virus (WNV) clinical disease in horses. Horses were inoculated one time with a 1.0 ml dose of vaccine virus that represented a 1X serial release dose for short term efficacy, duration and onset of immunity. Horses were monitored after vaccination daily for injection site reactions, general health, including temperature, pulse and respiration rate, and neurological condition. Efficacy and duration of the vaccine was demonstrated by challenge of vaccinated and control horses with virulent WNV using a model that induces signs of neurological disease that are consistent with those observed in horses infected with WNV under natural field conditions. Data from the efficacy studies with horses vaccinated one time with a 1.0 ml dose of vaccine showed a 95% protection against severe neurological disease, including encephalitis, compared to controls and a 100% protection against viremia compared to controls. Data from the duration of immunity studies with horses vaccinated one time with a 1.0 ml dose of vaccine showed a 95% protection against severe neurological disease, including encephalitis, compared to controls and a 100% protection against viremia compared to controls. Additional efficacy studies using the same challenge model demonstrated onset of immunity as rapid as 10 days post vaccination. A live West Nile Virus Chimera vaccine has been developed that provides protection of horses against severe neurological disease and viremia as demonstrated using a model of induced West Nile Virus clinical disease.

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CHANGES IN GENE EXPRESSION PROFILES IN *BRUGIA MALAYI* L3 INDUCED BY CULTURE AND RADIATION

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Brugia malayi (Bm) L3 are developmentally arrested in mosquitoes; they resume development in mammalian hosts. Irradiated L3 fail to develop normally in mammals and have a special ability to induce protective immunity. The purpose of this study was to examine changes in gene expression that occur when mosquito-derived BmL3 (L3i) are cultured for 2 days (L3c) under conditions that mimic the mammalian environment and permit molting (37C in MEM/NTSC with FCS) and also to examine how gene expression in L3c compares to that in L3 that are cultured after irradiation (L3ir). The Version 2 *B. malayi* oligonucleotide slide array contains 17,300 elements that are derived from filarial EST's and predicted ORF's that cover ~85% of total Bm genes. Arrays were co-hybridized with labeled L3 cDNAs (either L3i with L3c or L3ir with L3c). A gene was considered to have upregulated expression relative to the comparator if it showed a ≥ 2-fold hybridization signal with $P < 0.01$. 353 genes were upregulated in L3i relative to L3c. These included putative immune evasion genes (such as *Bm-Cpi-1*, *Alt-2*, *Bm-Val-1*, Serpin, *Bm-Mif-1*) and putative parasitism genes (ES22/24, SOD and TPX). 165 genes were upregulated in L3ir relative to L3c. Some 40% of these genes/clusters were also upregulated in L3i. The overlap genes include many novel genes with unknown functions. Most of the putative immune evasion genes were downregulated in L3ir, but genes for DNA repair and genes encoding the highly immunogenic *Ovegl* and *Juv-p120* were upregulated. This suggests that the immunogenicity of L3ir may be related in part to their failure to down-regulate certain genes that are highly expressed in L3i. 244 genes were upregulated in L3c compared to L3i. Some of these genes are involved in transcription, translation, and growth (e.g., ribosomal and RNA

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binding proteins and structural proteins such as actin and tubulin). These preliminary results suggest that expression profiling may shed light on the biological basis of irradiated L3 vaccines and on critical adaptations that occur when filarial L3 enter mammalian hosts.

(ACMCIP Abstract)

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VACCINATION WITH *ONCHOCERCA VOLVULUS* GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE (*OV*-GAPDH) USING THE MOUSE/*LITOMOSOIDES SIGMODONTIS* MODEL

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Recent studies identified *Ov*-GAPDH as a possible vaccine candidate molecule against human onchocerciasis. In this study the immunogenicity and protective potential of vaccination with *Ov*-GAPDH was tested in BALB/c mice infected with the rodent filaria *Litomosoides sigmodontis,* the mouse model of human onchocerciasis. BALB/c were vaccinated with either *Ov*-GAPDH.DNApl alone or in combination with the recombinant protein. Challenge of immunized and infection of naive mice with *L. sigmodontis* was performed 20 days post boost with 40 infective larvae by artificial infection (s.c. injection) or by natural infection via mites. Both types of vaccination led to protection in a subgroup of immunized mice affecting to a significantly higher degree female worms than males. The course of infection was associated with distinct ratios of *Ov*-specific IgG subclasses pre and post challenge. Apart from protection, enhanced susceptibility as well as pathology or combinations of both occurred. Pathology was observed in artificially infected mice irrespective of immunization. Recent studies in mouse models of autoimmune disease and inflammation emphasize the relevance of the relative proportion of antigen-specific IgG subclass responses with respect to Fc receptor-mediated effector mechanisms. Our results suggest that the same principle applies to a filarial infection, whereby the pattern of the antigen-specific IgG subclass response is relevant for the determination of protective immune responses, pathology or of both.

(ACMCIP Abstract)

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TISSUE MIGRATION OF *BRUGIA PAHANGI* THIRD STAGE INFECTIVE LARVAE: AN *IN VIT*RO MODEL

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Mosquito borne third stage infective larvae (L3) of filarial nematodes emerge from the mosquito labella into a drop of haemolymph during mosquito feeding. They reportedly migrate into the host through the vector produced wound and then through various connective tissues of the skin to a lymph or blood vessel. Earlier published studies have demonstrated the capacity of *Brugia* to penetrate a variety of tissue types rapidly. Nothing is known regarding the mechanisms associated with this process. An *in vitro* model was developed attempting to quantify this early prelymphatic migration in order to define the parasite molecules involved in this process. Skin from gerbils (*Meriones unguiculatus*) was cut into 1cm circles and inserted into the top of a blind well chamber and sealed. Different procedures were used to prepare the surface of the skin. These included: clipping, puncturing, shaving, tape stripping and combinations of each. RPMI 1640 without sera was used to fill the bottom of the chambers and 200L3 were placed in 100ul of media on the top of the chamber. Chambers were incubated for 1, 2 and 3 hrs at 37 C in 5% CO2. Larvae were counted in the upper and lower chambers after incubation. The total number that penetrated the skin was calculated. Large numbers (>80%) of L3 rapidly(1hr) migrated through the punctured skin and this system was discounted. L3 also migrated into and through the skin prepared using all other methods. Depending on the initial preparation this resulted in 69.9-29,7% entry and 17.2-14.4% complete migration of L3 through the skin. Histologic examination of the skin showed larvae in all tissues. It appears that larvae penetrate minimally disrupted epidermis and in some cases hair follicles. Initial attempts to alter this migration with anti-L3 excretory secretory antibodies were not successful.

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MAJOR SPERM PROTEIN: A POTENTIAL TARGET FOR ONCHOCERCIASIS VACCINATION AND DETECTION

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While a great deal of effort has been devoted to regional programs aimed at reducing the risk of onchocerciasis, significant obstacles remain which must be overcome prior to successful eradication. A key hurdle is the improvement of current diagnostic methods to monitor infection and possible recurrence. Great strides in detection technologies have been made using various assays including skin biopsies, detection of parasite DNA, and antibody-based strategies for specific parasite proteins. However, none of the current methods are capable of detecting sexually mature parasites. Major sperm protein (MSP) is the main component of nematode sperm and is critical for sperm motility. Because MSP represents one of the unique features of nematode reproduction, we propose that it is a potential target for diagnosis of nematode infection. In addition, while it is accepted that MSP polymerization is important for nematode motility, it is debated how this protein polymerizes. Evidence exists indicating that MSP dimer could be a single unit of MSP polymer, suggesting that antibody-mediated disruption of MSP polymerization could result in inhibition of nematode reproduction. We will present our results on the bacterial expression of O. volvulus MSP2 as well as the biophysical properties of this protein. Furthermore, our efforts aimed at isolating human monoclonal antibodies specific for MSP2 will also be discussed.

(ACMCIP Abstract)

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ANTI-WSP IGG1 ANTIBODY RESPONSE PREDOMINATES IN CHRONIC LYMPHATIC FILARIASIS

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Wolbachia have been believed to play an important role in the pathogenesis of filariasis. In order to evaluate their roles in the adaptive immune responses, we performed an ELISA to monitor IgG production (IgG1 and IgG4) against recombinant *Wolbachia* surface protein (rWSP) in patients with lymphatic filariasis. The effect of diethylcarbamazine (DEC) treatment of the disease on the antibody levels was also studied. Seventy-five serum samples were collected from the endemic areas of the western region of Thailand. The antibody responses against rWSP appeared to be IgG1 subclass, but not IgG4. The levels and seroprevalence of anti-rWSP IgG4 antibodies were not significantly elevated among filarial groups, while the levels and seroprevalence of anti-rWSP IgG1 antibodies were significantly elevated in individuals with chronic filarial symptoms. In contrast to filarial-specific IgG4 response which predominated in asymptomatic microfilaria-positive individuals, IgG1 antibody response to rWSP was not found to increase in neither asymptomatic microfilaria-positive and antigen-positive nor microfilaria-negative and antigen-positive individuals. DEC treatment had no effect on the levels of anti-rWSP IgG1 and IgG4 antibodies in microfilaraemic patients with adverse reactions. In

conclusion, the presence of anti-rWSP IgG1 antibodies was found to be associated to chronic filarial, but not to parasitological status in patients. The specific antibody response may play a role in the pathogenesis of lymphatic filariasis, but not in the adverse reactions.

(ACMCIP Abstract)

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TEMPERATURE-INDUCED DIFFERENTIAL GENE EXPRESSION PATTERNS IN THIRD STAGE *BRUGIA MALAYI* LARVAE

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Little is known about the changes in gene expression that occur as infective filarial larvae transition from the vector to the human host. To examine one aspect of this transition, 1,500 *Brugia malayi* L3 were cultured at room temperature in serum-free medium or at 37oC in the presence of human serum for 4 hours to simulate the mosquito vector and human host, respectively. Following RNA isolation, cDNA was amplified and labeled with Cy3 or Cy5 for hybridization to a slide array spotted with 65-mer oligos corresponding to 3569 clusters from the *Brugia malayi* EST database. Three technical replicates were performed, including one dye swap. Expression was detectable for 6,016 genes, of which 325 gave concordant values among all 3 replicates. 114/325 (35%) genes were upregulated by at least two-fold in L3 cultured at RT, and 33/325 (10%) were upregulated by at least two-fold in L3 cultured at 37oC in the presence of serum. Although a majority of the differentially regulated genes showed no homology to known genes, upregulation of genes involved in protein synthesis, including ribosomal proteins, was more common in the L3s cultured at room temperature (10 of 114 vs 1 of 33 upregulated genes, respectively). Confirmation of the array results by quantitative RT-PCR and further characterization of the differentially regulated genes is currently underway. Identification of genes involved in the transition of infective L3s from the insect vector to the human host could lead to the development of novel strategies to prevent filarial infection.

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CRYOPRESERVATION OF A. CANINUM, A. CEYLANICUM AND N. AMERICANUS INFECTIVE LARVAE AT -196°C

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Hookworm *Necator americanus, Ancylostoma ceylanicum* and A. *caninum* strains are maintained in our laboratories for research on human hookworm vaccine. To prevent an accidental interruption in their maintenance or to store infective L3 larvae as a backup for a strain not needed temporary (without associated effort and expense of continuous maintenance on laboratory animals), we studied the cryopreservation of their (ensheathed) L3 larvae. Several cryoprotectant agents and two cryopreservation protocols with different cooling speeds were studied. In all experiments, the cryovials with larval suspension complemented with cryoprotectant agent/s were first equilibrated for one hour at room temperature then cooled rapidly by direct immersion in liquid nitrogen at -196°C. Alternatively, prior to storage at -196°C larvae were cooled slowly at -80°C overnight in mechanic deep-freezer. The larval survival was assessed by L3 mobility test under stereomicroscope. The slow cooling protocol gave higher survival rates for infective larvae of all three hookworm strains than direct storage in liquid nitrogen. We found that white egg has an excellent cryoprotectant activity for all three hookworm strains. Overall, *A. ceylanicum* survival rates were higher than those of *N. americanus*. Cryopreserved *A. ceylanicum* larvae preserved the infective power for golden hamster. The L3 quality prior to freezing was of cardinal importance for larval survival regardless the cooling protocol or cryoprotectant agent.

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UNCIARIASIS IN PANAMA

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The objective of this study was to describe the epidemiology of geohelminthiasis in Alto y Bala, Veraguas, Panama. Previous voluntary acceptance of participation and informed consent, all subjects 2 years old and older were included in the survey. The study consisted in a questionnaire regarding various predisposing factors and visual evaluation of the house. Laboratory analysis included: complete blood count, cellular immunophenotypes and feces analyses (Kato-Katz and concentration). Alto y Bala is a rural community of 292 inhabitants allocated in 88 houses. A total of 228 (78%) subjects, 48% males and 52% females accepted to participate in the study. The mean age for the study group was 29.8±1.65 years; 42% were younger than 15 years old. The majority of the subjects were housewives, farmers or students. Mean school attendance was 2.7±0.20 years and the family income was less than US$100 per year. About toilet facility, 82.6% of the subjects defecated in latrines, but 47% referred open-field defecation. It was observed that 84% of the subjects claimed to wear shoes, but only outside the house. A total of 188 subjects (84%) were positive for the presence of intestinal parasites in the fecal exam. Helminths were observed in 72 subjects (32%), protozoos in 38 (17%), and mixed infections were detected in 78 (35%) of the participants. The most frequent helminth infection observed were caused by: *Necator americanus* [52%(117)] and *Ascaris lumbricoides* [33%(73)]. In all age groups evaluated, hookworm infection showed a prevalence of 40-61%. With respect to intestinal parasite infection, latrine use was a protector factor [0.41($IC_{95\%}$0.17-0.91)] and open-field defecation a risk factor [2.21($IC_{95\%}$ 1.04-4.74)]. In the group of subjects 12 years old and younger, mean hemoglobin concentration was 12.7g/L±0.13, in 10.5%(4/38) of the subjects the levels were lower than 12g/L, and all the positive subjects for intestinal parasites showed eosinophils percentages higher than 5. In conclusion, our study confirmed a high prevalence of intestinal parasite infections in the study area that correlated with eosinophilia. Lower Hb concentrations were observed more frequently in man than in women. Our preliminary findings support future studies for the control of soil-transmitted helminthiasis, based on regular anthelminthic treatment, health education and improved sanitation standards, until a vaccine is approved.

(ACMCIP Abstract)

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IMMUNODIAGNOSIS OF STRONGYLOIDIASIS: SCREEN WITH ELISA AND CONFIRM WITH IMMUNOBLOT USING A RECOMBINANT ANTIGEN

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The diagnosis of strongyloides remains difficult and the consequences of a missed diagnosis can be serious. The clinical features of infection can be subtle and non-specific. Both standard ova and parasite examinations and more specialized clinical laboratory concentration (Agar plate, Baermann extraction, Harada Mori culture, charcoal culture) are a biohazard and are insensitive compared to strongyloides serology. The sensitivity of the ELISA using crude antigen is high, however the specificity is low due to antibody cross-reactivity to antigens present in other parasites, such as filarial parasites, *Ascaris lumbricoides*, hookworm and *Schistosoma* spp. We compared three *S. stercoralis* antigens (one recombinant and 2 crude) with both ELISA and immunoblot. Sera from four clinical subsets were tested (*S. stercoralis* larva-positive patients, HTLV1 *S. stercoralis* positive patients, eosinophilic esophagitis cases and travelers/immigrants judged at risk for Strongyloides but negative for Strongyloides larvae). With the ELISA all three antigens demonstrated 100% sensitivity in the strongyloides larva-positive, (with or without HTLV1 positivity). The specificity for the crude batch 1, crude batch 2 and recombinant antigens was 67, 75 and 85 % respectively. The immunoblot using the recombinant antigen showed a specificity of 98%. Further measures of specificity with various helminthes are in progress. This recombinant antigen has the benefit of reproducibility. The ELISA with the recombinant antigen is sensitive and the Western blot appears specific.

(ACMCIP Abstract)

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DISSEMINATED HISTOPLASMOSIS IN AIDS PATIENTS IN GUATEMALA: PRELIMINARY RESULTS FROM A SYMPTOMATIC PATIENT COHORT

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Systemic fungal infections represent a diagnostic challenge to the growing immunocompromised patient population. In many countries where histoplasmosis is endemic, the burden of this disease in AIDS patients is unknown. As a part of a histoplasmosis diagnostic assay evaluation, we recruited symptomatic AIDS patients in Guatemala City to assess for the presence of histoplasmosis. We present the preliminary descriptive results of the clinical characteristics and epidemiology of histoplasmosis affecting this population. The prospective study was initiated at Clinica Familiar Luis Angel Garcia (CLFAG), where HIV-infected patients receive care in Guatemala City, in January 2005. Patients with symptoms of fever plus one of the following: weight loss, skin lesions, diarrhea, pancytopenia, hepatomegaly (+/- splenomegaly), or radiographic evidence of histoplasmosis were asked to participate (suspect histoplasmosis case). We performed routine diagnostic work-up which included blood and bone marrow cultures by regular methods, routine histopathology examination and histoplasmosis serology testing. We classified patients into confirmed, probable, and possible histoplasmosis case definitions based on results of these tests. Other diagnoses were infectious, non-infectious or unknown etiology. A total of 87 patients suspect histoplasmosis cases were identified; 52 were enrolled to date (60%). Their ages range from 18 to 54 years; 18 (34.6%) were female. The most common presenting symptom was fever (70%); 81% had pulmonary complaints, 56% had gastrointestinal complaints and 41% had AIDS. Ten (19.3%) met confirmed case criteria, by culture. Serology results were available from15; 5 met probable and 2 met possible histoplasmosis case definitions. Eleven (21. 2%) deaths were recorded in this cohort. In conclusion, in histoplasmosis endemic areas, among HIV-infected patients with systemic illness, the diagnosis of histoplasmosis is not infrequent and mostly achieved by culture. In settings where resources are limited, a rapid and reliable histoplasmosis diagnostic test is needed to decrease morbidity and mortality in this population due to delay in diagnosis and treatment.

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IMPACT OF THERAPEUTIC REGIMEN FAILURE IN THE RESISTANCE TO ANTIRETROVIRAL DRUGS IN NORTHEST BRAZIL

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Highly potent antiretroviral therapy is necessary to avoid viral replication in HIV patients, but also allows resistance mutations to appear. 41L, 67N, 70R, 210W, 215Y/F, 219E/Q, 44D and 118I are mutations defined as nucleoside analogous mutations (NAMs) because they compromise all Nucleoside Reverse Transcriptase Inhibitors (NRTI). 184V is important as it is associated with high level resistance to lamivudine. 103N is the mutation that occurs more frequently in Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs) causing cross resistance to this class. 33W/F, 82A/F/L/T, 84V and 90M are called protease inhibitor resistance-associated mutations (PRAM) because they cause cross resistance among Protease Inhibitors (PI). The objective of this study was to evaluate the development of resistance mutations and susceptibility profile to antiretrovirals in HIV-1 patients failing therapy. We evaluated 101 results of genotyping test from patients with therapeutic failure to 2- or 3-drug regimens with NRTI, NNRTI or PI. We utilized the Stanford Database for defined susceptibility profile. The samples were divided in three groups of failure: first (P), second (S) and multifailure (three or more fails) (MF) to antiretroviral regimens and correlated the groups with the profile of resistance and main mutations. Increased resistance mutations V82A/F/L/T, I84V, L90M, M41L, K70R, L210W, T215Y/F and K219Q/E were observed in MF ($p<.05$). High resistance detected to zidovudine, didanosine, estavudine and abacavir in MF ($p<.05$). There was not observed increase resistance to tenofovir ($p=0.28$) and lopinavir ($p=0.079$) in MF. There was predominance of high resistance to lamivudine and mutation M184V ($p=0.24$) in all patient groups. Predominance of high resistance to NNRTIs was also observed in all groups. In conclusion, accumulation of resistance mutations due to therapeutic failure will affect future options to HIV patients' treatment in Brazil since it allowed significant increase in resistance to actual antiretroviral drugs in use.

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VALUE OF THE NEOPTERIN CONCENTRATION AND THE NPT/CD4 RATIO AS PROGNOSTIC MARKES OF AIDS IN HIV INFECTED PATIENTS

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Neopterin (NPT: pteridine derivative of guanosine triphosphate) is released into circulation from interferon γ (IFN-γ) activated macrophages. Three categories of subrogate markers are used for prognostic evaluation in HIV infection: CD4 T-cell counts, viral load and markers of immune activation (cytokines, Î²-2 M, NPT). In the current study we evaluated the

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prognostic value of concentrations of plasma neopterin in the progression of the HIV infection and its correlation with others markers. We have evaluated CD4 T-cell counts, HIV viral load and neopterin levels in 108 blood samples including 88 HIV+ patients (with and without AIDS) and 20 HIV negative subjects. CD4 T-cell counts, HIV viral load and neopterin levels were evaluated using flow cytometry (Epics XL Coulter), the Amplicor HIV Monitor Test 1.5 (Roche) and by competitive ELISA (IBL), respectively. Mean neopterin concentration was 5.7 nmol/L in the control group, however, higher levels (45.8 nmol/L) were present in patients with AIDS without treatment. Strong direct correlation between CD4/CD8 and CD4/NPT ratios was shown (r = 0.973) in patients with AIDS under HAART. The observed CD4/NPT ratios were 182.2 (IC: 149.1-215.4), 57.9 (IC: 39.3-77.6) and 5.2 (IC: 0.6-9.8) in controls, patients with low viral loads (<100,000 copies/ml) and high viral loads (>100,000 copies/ml), respectively. Statistically significant differences ($p<0.0001$) in neopterin concentrations were obtained in AIDS patients with respect to treatment. Strong direct correlation (r = 0.814, Sperman rank) between neopterin level and viral load was found in patients without AIDS, not receiving HAART; however, an inverse correlation was observed between neopterin levels and CD4 T-cell counts (0.485) in the same group of patients. Our results demonstrated the possible benefit of measuring plasma neopterin and the CD4/NPT ratio as additive prognostic markers of progression in patients with AIDS.

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HIGH LEVEL OF POLYMORPHISM IN THE *GAG* REGION OF THE B-SUBTYPE HIV-1 STRAINS FROM NORTHEASTERN VENEZUELA

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HIV-1 strains around the world have shown a great genetic diversity. This high genetic variation is related to the emergence of drug resistant strains. The objective of this study was to determine the molecular characteristics of HIV-1 strains circulating in northeastern Venezuela and their relationship to the epidemiological characteristics of the patients. A total of 21 HIV-1 positive patients were chosen at random from three health care facilities in Sucre state. Viral RNA and proviral DNA were extracted using a commercial kit and the *env* and *gag* regions were amplified by nested PCR or RT-PCR, using the primers and protocols outlined by National Institutes of HealthAIDS. Molecular typing was carried out using the heteroduplex motility assay (HMA) and RFLP using *Fok* I restriction digestion. The studied patients were 12 males and 9 females, 71.4% were younger than 34 years of age and 20% had homosexual behavior. Most of the individuals showed a low educational level, poor knowledge about the infection and declared that never used condoms. The epidemiological survey determines that all patients were infected through sexual intercourse, and they were infected in Caracas (15%), Margarita Island (10%), Bolivar (10%), Delta Amacuro (5%), Anzoategui (20%) and Sucre (40%) states. We found that all HIV-1 strains were of type B for the *env* region, while 85% were of type B, two were undetermined and one sample did not amplify for the *gag* region. The undetermined strains and the one that did not amplify were amplified for the RT gene and sequenced, showing homologies of 95.0, 93.6 and 86.2% to the reference type B strain. The RFLP analysis showed that no "Brazilian" type B strains were present in the sample. Polymorphism was seen in 5% of the type B strains for the *env* region but 45% for the *gag* region. Statistical analysis associated the type-B polymorphic strains to patients infected in Anzoategui and Sucre states. Simple and complex quasispecies were seen in 50 and 15% of the samples for the *env* and *gag* region, respectively. The clinical characteristics of the patients such as time of diagnosis, type of treatment, CD4 counts and viral load were not associated with the molecular characteristics of the strains.

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MOLECULAR CHARACTERIZATION OF *SHIGELLA* AND *PLASMODIUM FALCIPARUM* CO-OCCURRENCE IN HIV-1 SEROPOSITIVE NIGERIAN CHILDREN

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Shigellosis and falciparum malaria are clinically distinct causes of morbidity and mortality in Nigerian children with or without HIV seropositivity. There is currently paucity of scientific data to justify their co-occurrence as opportunistic infections and mediate evolution of new control and management strategies in Nigerian children with immunity compromised by HIV virema. This study genotypes shigellosis and *Plasmodium falciparum* for plasmid and msp1 diversity in children with HIV-1 seropositivity. Serogroup diversity and antibiogram of the shigella isolates were also determined. A total of 27 consenting HIV-1 seropositive Nigerian children aged 5 - 14 years (mean age = 6.4yr) presenting with diarrhea and fever (axilliary temperature > 37.5^{o}C) were enrolled into the study. Stool and blood samples of each child were cultured on conventional media with thick blood films analyzed for *P. falciparum* parasitaemia. Plasmids were extracted from shigella isolates and characterized by alkaline lysis and electrophoresis respectively. While nested PCR was employed for msp-1 genotyping of *P. falciparum*. Of the 27 blood samples analyzed, 2 (7.4%) and 15 (55.6%) were positive for *S. sonnei* shigellemia and *P. falciparum* parasitaemia (GMPD = 13700 - 58500 parasites/µL) respectively. Stool cultures revealed mixed (3/27, 11.1%) and single shigella (8/27, 29.6%) ($P < 0.05$) constituted polymicrobial infections with *S. flexneri* exhibiting serogroup dominance (57.1% vs. 14.3 - 28.6%; $P < 0.05$) and isolates including *E. coli* and *Salmonella* serving as co-pathogens. Although all the isolates analyzed exhibited quinolone and fluroquinolone sensitivity, the shigella isolates were multidrug resistant with blood strains of *S. sonnei* exhibiting reduced susceptibility to ceftazidime (MIC = 10.0$\pm$6.0 vs. 3.2$\pm$0.7 µg/mL, $P < 0.05$) and ofloxacin (MIC = 0.2$\pm$0.06 vs. 0.08$\pm$0.01 µg/mL, $P < 0.05$) compared to strains recovered from stool cultures. The shigella isolates generally co-harbored low molecular weight plasmids of size 1.7, 2.0, 2.6 and 4.0kb but still display distinct extrachromosomal DNAs of 10.2kb in *S. flexneri* and 8.0kb in blood S. sonnei strains. The *P falciparum* isolates exhibited msp-1 diversity with block 2 RO33 family predominating and multiplicity of infection range of 1 - 5 was observed. Eighty-five percent of the parasites genotyped had the pfcrtT76 chloroquine resistance allele ($P < 0.05$). In conclusion, infections due to shigella and *P. falciparum* exhibit complexity and diversity of occurrence at molecular level in Nigerian children with HIV-1 seropositivity. The parasite pfcrtT76 dominance and display of serogroup discordance by blood and stool shigella flora strongly raise the possibility of chloroquine failure and antibacterial therapy with third generation cephalosporins being compromised in co-infected patients.

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CROSS REFERENCE BETWEEN CELLULOSE ACETATE ELECTROPHORESIS (CAE) AND PLOYMERASE CHAIN REACTION (PCR) IN *LEISHMANIA* DIAGNOSIS

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Effective diagnostic techniques for *Leishmania* are very important and critical in starting treatment of an infected patient. Leishmaniasis is a potentially life threatening and mutilating disease and various species are lethal if not treated on time and appropriately. PCR is becoming the ultimate tool in *Leishmania* diagnosis due to its rapidity in providing

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results, but is still lacking the full effectiveness of speciating all species of the *Leishmania* parasites. CAE is a technique that is been in place for a very long time, and it requires live parasites and a large number of promastigotes to work, but it remains the most effective technique in performing speciation of *Leishmania* parasites. Both techniques accommodate the diagnostic needs if used together, but until PCR is fully developed, Cellulose Acetate Electrophoresis remains the leading technique for *Leishmania* classification and speciation of all known's species of Leishmania parasites. In doing this project, my intention is to examine how far CAE and PCR can extend its similarity common grounds in *Leishmania* identification and speciation.

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BLOOD AGAR SUBSTITUTE IN GROWING LEISHMANIA

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Leishmania diagnosis is essential to determine the best course of treatment and foresee its effectiveness in the infected patients. Until new and more effective techniques are found, culturing the parasites is a key step in treating this disease. Currently there are many ways to grow and expand Leishmania parasites, but the technique most commonly used is growing the parasites using Novy- MacNeal- Nicolle (NNN, blood agar). This blood agar medium for culture is used to grow and expand all Leishmania species and is more effective when parasites are very sensitive and difficult to expand in other culturing media. NNN is also very difficult to use as a standard media. NNN is very expensive and time consuming in its preparation. It is also easily contaminated because of the rich nutrients that compose the medium, which allow other unwanted microorganisms to grow and interfere with identification of leishmania parasites. A control temperature environment is also needed to keep this culture medium active

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DIAGNOSIS OF HUMAN BABESIOSIS USING SELDI PROTEINCHIP TECHNOLOGY

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Babesiosis, an emerging zoonotic disease transmitted through the bite of *Ixodes* ticks and through blood transfusion, is caused by several species of hemoprotozoan parasites belonging to the genus *Babesia*. Prevalence of human babesiosis is worldwide but most cases have been reported in North America and Europe. The spectrum of disease manifestation is broad, ranging from silent infection to fulminant, malaria-like illness that may result in severe hemolysis, pulmonary, renal, or liver impairment, and occasionally in death. Laboratory diagnosis of babesiosis includes blood smears, indirect fluorescent antibody test (IFA) and PCR. These tests require highly trained personnel and are often expensive. Blood smears may be negative when patients first present to their physicians and it may be difficult to differentiate *Babesia* from *Plasmodium*. IFA requires babesia infected hamster erythrocytes as antigen that are difficult to procure and not suited to large scale testing. In addition, IFA can lead to misdiagnosis because of cross-reactivity with other parasites. To improve sensitivity, specificity, and provide better automated diagnosis of *babesia*, surface-enhanced laser desorption ionization time-of-flight mass spectrometry (SELDI-TOF-MS) serum protein profiling technology was used as a method to identify biomarkers specific to babesiosis infection. Serum samples were fractionated by pH using a Biomek 2000™ automated laboratory system and analysed by SELDI-MS (Ciphergen PBS IIc) using metal affinity (IMAC30) weak cation exchange (CM10) and hydrophobic (H50) protein-chip arrays. Analysis of sera from subjects with *babesia* infection alone (n=24), *Babesia* and Lyme disease co-infection (n=9), healthy controls (n=11), controls with flu-like illness (n=5), and other protozoan infections (n=10), led to the identification of a set of *babesia* specific polypeptide biomarkers in the range 2-150 kDa. Characterization of each of these *babesiosis*-specific markers by peptide mapping and tandem mass spectrometry is underway to determine potential biomarkers to be used to develop a faster, most cost-effective and accurate diagnostic test for this common, life-threatening parasitic infection.

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ANALYSIS OF BIOMARKERS ASSOCIATED WITH CHAGAS DISEASE USING ANTISERA TO NOVEL BIOMARKER EPITOPES

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Many parasites such as *Trypanosoma cruzi*, the causative agent of Chagas Disease (CD), can be transmitted by blood transfusion. Currently, there are no appropriate tests for screening blood products from Chagas infected donors. Surface-enhanced laser desorption ionization-time of flight-mass spectrometry (SELDI-TOF MS) is a new strategy for the discovery of biomarkers for parasitic diseases. We have applied SELDI-TOF technology to identify biomarkers that could potentially be developed as improved diagnostic tests for parasite infections, such as Chagas disease, as well as to understand pathogenesis events associated with infection. Protein profiling of Chagas sera and endemic control sera identified several biomarkers associated with infection, including fragments of human apolipoprotein A1 (ApoA1) and fibronectin (Fbn). Both N terminal and C terminal fragments of ApoA1 (NtApoA1: 13.6 kDa; CtApoA1: 24.7 kDa, respectively) and a C terminal fragment of Fbn (CtFbn, 28.7 kDa) were identified. Polyclonal rabbit antisera to the predicted novel peptide epitopes on these biomarkers were used to probe Western blots of sera from Chagas and uninfected control volunteers in order to confirm the identity of the truncation products in Chagas sera. The CtApoA1 sera detected the major truncation product near 24.7 kDa whereas the NtApoA1 sera detected a complex pattern of ApoA1 fragments. The CtFbn antisera detected a novel fragment near 29 kDa. Further studies will be reported evaluating these specific antisera against sera from other parasitic infections to determine whether these ApoA1 and Fbn truncation products are specific for Chagas disease. This would validate their utility as target antigens in an ELISA-based diagnostic test for Chagas disease.

(ACMCIP Abstract)

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EVIDENCE FOR GENOTYPE-BY-SEX INTERACTION IN THE GENETICS OF SEROPOSITIVITY TO *TRYPANOSOMA CRUZI* IN A BABOON MODEL

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Baboons housed at Southwest Foundation for Biomedical Research in southern Texas are exposed naturally to the insect vector that transmits *Trypanosoma cruzi*, a kinetoplastid protozoan that causes Chagas disease. About 3% of the baboons in the colony are seropositive for *T.cruzi*. Infected baboons can develop Chagas disease and exhibit disease progression and pathology, including myocarditis and EEG abnormalities, similar to that seen in human cases. Previous work has suggested that variation in serostatus to *T.cruzi* in the baboon is significantly influenced

by additive genetic effects, but these studies have assumed that the genes involved must act identically in males and females. In the present analysis we allowed for interaction between sex and genotype, such that different genes may be involved, and may have different effects, in males and females. In a sample of 1227 pedigreed baboons the heritabilities of serostatus in males (h^2=0.70, p=0.001) and in females (h^2=0.67, p=0.007) were each significant, yet not significantly different. However, the genetic correlation between males and females was significantly different from zero (r_g=-0.71, p<0.001) and negative. These results can be interpreted as indicating (i) that male and female baboons have in common a large proportion of the genes affecting their serostatus to *T.cruzi*, and (ii) that these genes have different effects in the different sexes.

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VIRULENCE AND IMMUNOLOGIC RESPONSE INDUCED BY A TYPE IIA NORTH AMERICAN ISOLATE OF T. CRUZI AS COMPARED TO THE TYPE I BRAZIL STRAIN

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Trypanosoma cruzi is widely distributed across North America, with Type IIa genotypes associated with placental reservoir hosts. While prevalence is high in sylvatic populations of raccoons *(Procyon lotor)*, pathology is largely absent. We compared a Type IIa North American isolate from St. Catherines Island (SC), Georgia with the Type I Brazil strain (BS) to determine whether differences in virulence could be determined. We found that *in vitro* the rate of cell invasion and percentage of cells becoming infected were statistically similar, but that the intracellular replication and emergence rates of the BS exceeded that of the SC isolate. When groups of BALB/c mice were infected with each isolate the Brazil strain induced consistently higher levels of morbidity and mortality, which was in agreement with the higher parasitemias noted in these mice. Infection with the SC isolate induced protection against a challenge infection with the BS. Western blot analysis showed similar antigenic recognition profiles although minor differences could be noted. This demonstrates that strain genotype may translate into significant differences in pathology. Comparisons with Type I isolates from North America will also be discussed.

(ACMCIP Abstract)

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QUANTIFICATION OF EVOLUTIONARY CONSTRAINTS OF SELECTED LEISHMANIA ANTIGENS AND THEIR IMPLICATIONS FOR VACCINE DEVELOPMENT

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A major obstacle to vaccine development against *Leishmania* comes from the fact that at least 18 species of *Leishmania* are pathogenic to humans, and even within species, antigenic diversity between strains has been reported. All this diversity needs to be taken into account for the development of a single vaccine with the boadest possible efficacy. Mutation analysis may be used to assess antigen diversity and evolutionary contraints due to structural, functional or immune requirements, and was thus used in this work to estimate the potential of leading vaccine candidates to provide broad protection against various strains and species of *Leishmania*. DNA sequences for *Leishmania* antigens glycoprotein 63 (GP63), cysteine proteinase b (CPb), histone 2B, thiol-specific-antioxidant (TSA) and nucleoside hydrolase 36 (NH36) from several strains and species were compared to evaluate their diversity. Aligned sequences were used for a sliding-window analysis of synonymous (Ks) and non-synonymous (Ka) mutations and the identification of selection pressures. GP63 and CPb were the most diverse antigens, and only small domains of the proteins were under negative (or purifying) selection (Ka/Ks<1), making it unlikely that they would provide broad cross-species immunity. Histone 2B and TSA were partially conserved, and exhibited some negative selection in some parts but not all of the protein, suggesting that escape mutants may occur. NH36 was the most conserved antigen, with a very strong negative selection pressure impeeding most amino acid changes. These results confirm that NH36 is a very good vaccine candidate for a *Leishmania* vaccine with broad species efficacy.

(ACMCIP Abstract)

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SHARED EPITOPES ON SURFACE OF *LEISHMANIA MAJOR RESPONSIBLE FOR VIRULANCE*

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Surface glycoproteins of *Leishmania Sp*. play an important role in the infectivity, survival, and developmental stages of the parasite. The two surface glycoproteins of *Leishmania major (L.major)* are the major surface protease (MSP or gp63) and parasite surface antigen-2 (PSA-2). Consistent with structural and functional homology between the two glycoproteins the species- specificity of shared epitopes on these glycoproteins was investigated by monoclonal antibody (mAb) specific for *L. major*. The results demonstrated that there is a shared repeated epitope on PSA-2 and MSP. The epitope on PSA-2 was repeated as 80-, 94-, and 96 kDa and mAb also recognized two shared epitopes as 61-, and 63 kDa on MSP. The expression of epitopes analyzed during the promastigote differentiation from logarithmic to stationary stages. Promastigotes were harvested from days 1-7 and subsequently analyzed by mAb. The results showed that PSA-2 epitope were unchanged whereas MSP expressed two forms of this epitope during differentiation, a 61 kDa expressed during logarithimic and stationary phase and a 63 kDa which was specific to metacyclic of MSP was detected only on day 3-7 of *L. major* differentiation.

(ACMCIP Abstract)

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DESIGN AND CHARACTERIZATION OF MONOCLONAL ANTIBODIES TO DISTINGUISH BETWEEN MSP ISOFORMS FOUND ON THE SURFACE OF *LEISHMANIA CHAGASI*

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Leishmania chagasi is the causative agent of visceral leishmaniasis in South America. The most abundant glycoprotein on the surface of *L. chagasi* promastigotes is the glycosylphosphatidylinositol (GPI) anchored protease MSP (major surface protease), also called GP63. The >18 tandem *MSP* genes are classified according to unique sequences at their 3' ends. The five *MSPS* genes (MSPS1, MSPS2, etc.) express 3.0 kb RNAs in stationary phase promastigote growth, the > twelve *MSPL* genes express 2.7 kb RNAs in logarithmic phase, and the single *MSPC* RNA is constitutively expressed throughout promastigote growth. *MSPS* and *MSPL* genes encode a C-terminal GPI anchor addition signal, whereas the C-terminus of the *MSPC* gene product is more suggestive of a transmembrane region with a short cytoplasmic tail. Little is know about MSP protein isoforms because they have a very similar amino acid sequence. We are interested in studying the protein expression and localization of MSPS, MSPL, and MSPC in the promastigote and amastigote stages of the *L. chagasi* lifecycle. Immunogenic peptides were generated to a divergent region on the C-terminal end of the MSP sequences. From these, we generated three monoclonal anti-peptide antibodies called AbMSPX, AbMSPS1L, and AbMSPC that (1) recognize all MSP isoforms in *L. chagasi*, (2) specifically recognize MSPS1 and MSPL1, or (3) recognize MSPC, respectively.

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According to immunoblots, AbMSPS1L is specific and will not recognize MSPS2, whereas AbMSPX recognizes isoforms migrating with MSPS2 and MSPS1. These monoclonal antibodies are being used to study the intracellular trafficking of MSP isoforms.

(ACMCIP Abstract)

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THE CHEMOKINE RECEPTOR CXCR3 IS NOT REQUIRED FOR HOST RESISTANCE TO MURINE VISCERAL LEISHMANIASIS

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Visceral leishmaniasis (VL) caused by *L. chagasi* and *L. donovani* is the most severe form of *Leishmania* infection and is fatal if left untreated. The CXC chemokine receptor 3 (CXCR3) is expressed on plasmacytoid DCs, NK cells and T cells and is involved in their trafficking to the site of inflammation. Three chemokines CXCL9 (Mig), CXCL10 (IP10) and CXCL11 (I-TAC) are the main ligands for CXCR3.Both CXCL9 and CXCL10 produced in high levels during *L. major* and *L. donovani* infections in man and mouse, and are believed to play a role in host immunity. We recently found that CXCR3 plays a critical role in mediating host resistance against cutaneous leishmaniasis (CL) caused by *L. major* by regulating trafficking of effector T cells to skin. Using an intravenous mouse model, we investigated the role of CXCR3 in the immune response to *L. donovani*. Here we report that C57BL/6 mice genetically lacking CXCR3 (CXCR3-/-) mount an efficient Th1 response following *L. donovani* infection, recruit significant number of CD4+ and CD8+ T cells to the liver and spleen and control parasite growth as efficiently as CXCR3+/+ mice. These results demonstrate that although CXCR3 controls effector T cell migration to skin during *L. major* infection, it is not required for trafficking of T cells to the liver and spleen during *L. donovani* infection. Furthermore, they also indicate that CXCR3 is not required for induction of Th1 response and host resistance to *L. donovani.*

(ACMCIP Abstract)

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IDENTIFICATION OF NOVEL *PLASMODIUM KNOWLESI* AND *P. VIVAX* MEROZOITE PROTEINS

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The erythrocytic stage of the malaria parasite (*Plasmodium*) life cycle is responsible for the clinical symptoms associated with malaria. The parasite merozoite form, expressed in the erythrocytic stage, is responsible for invasion of RBCs and propagation of this stage of the life cycle. Numerous proteins are expressed in these merozoites and these proteins may mediate crucial functions during merozoite invasion. Many of the merozoite proteins have not been identified, nor have their functions been defined. We sought to identify novel merozoite proteins in *P. knowlesi* and *P. vivax* using a strategic approach to more readily identify specific proteins expressed at the merozoite's apical end. *P. knowlesi* is a simian malaria parasite related to the human malaria *P. vivax* and has been used extensively as a model for merozoite invasion. Specific antibodies made against proteins expressed in *P. vivax* merozoites were used to identify homologous proteins in *P. knowlesi*. Identification of these proteins was accomplished using proteomics and screening of *P. knowlesi* genomic databases, followed by screening of the *P. vivax* genome database. Several proteins identified by these methods will be presented.

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ERYTHROCYTE INVASION BY *PLASMODIUM FALCIPARUM* MEROZOITES

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Host cell invasion and motility in apicomplexan parasites is powered by an actin/myosin motor complex that is anchored to the inner membrane complex (IMC). This complex consists of four proteins: a type XIV myosin, a regulatory myosin light chain, and two IMC anchoring proteins. Much of our understanding of invasion complex function has come from work in *Toxoplasma gondii* tachyzoites and *Plasmodium* sporozoites, but we currently know little about the motor complex that powers erythrocyte invasion by *P. falciparum* merozoites. This is of particular significance not only because it is the merozoite that directly causes 1-3 million deaths from malaria each year, but also because unlike other apicomplexan zoite stages, merozoites are not motile on inert substrates, suggesting that the regulation of its invasion/motility machinery may be unique. We have identified and characterized the *P. falciparum* invasion motor complex and established that all four complex proteins are transcribed, expressed, and localized in a manner consistent with a role in erythrocyte invasion. Critically, the same complex appears to be assembled regardless of which of the multiple alternative invasion pathways are being used by the parasite, making it an attractive target for the development of invasion-blocking intervention strategies. Ongoing studies are aimed at elucidating the means by which this invasion complex is assembled, and whether it requires activation once its assembly is complete, with a particular emphasis on the role of post-translational modification.

(ACMCIP Abstract)

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MODELING ANEMIA AND THROMBOCYTOPENIA IN RODENT AND PRIMATE MODELS OF SEVERE MALARIA

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Severe anemia is a common and serious sequela of malarial infection, and the relative contributions of direct parasite destruction of erythrocytes and host infection-response mechanisms have not been well understood. We have enhanced the original Jakeman-Saul-Hogarth-Collins (JSHC) anemia model designed to predict daily hemoglobin levels based on daily parasitemia and modified this model to include additional parameters such as daily reticulocyte and platelet counts. We then compared the modified model predictions to experimental data obtained from rodent infections with *Plasmodium yoelii* and *P. berghei* and rhesus macaque infections with *P. coatneyi* and *P. cynomolgi*. The model was used to estimate the relative contributions of direct erythrocyte destruction by parasites, destruction of normal erythrocytes, changes in erythropoiesis, and changes in platelet production in resulting anemia and thrombocytopenia. Using a predictive tool such as the modified JSHC model to analyze kinetics of infection parameters will allow evaluation of anemia and thrombocytopenia in different host/parasite experimental models and implicate novel virulence factors in induction of severe malaria.

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PLASMODIUM FALCIPARUM SYNTAXIN HOMOLOGUES

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Plasmodium falciparum, the causative agent of malaria, invades and occupies erythrocytes during is asexual lifecyle. Blood stage *P. falciparum*

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parasites traffic proteins of parasitic origin to the surface of the infected erythrocyte that mediate cytoadherance and play a crucial role in pathology of malaria. Mature red blood cells are terminally differentiated and do not posses the machinery necessary for protein synthesis and trafficking. How this parasite exports protein beyond the boundaries of its own plasma membrane to the surface of the erythrocyte remains an enigma. Syntaxins are responsible for the specificity of vesicle trafficking and different syntaxins are present on each organelle of eukaryotic secretory pathways. Syntaxins are thus ideal for serving as organelle specific markers. Nothing is currently known about the *P. falciparum* syntaxins, and we aim to use them as tools to dissect the secretory system that is hypothesized to form in the erythrocyte cytoplasm. We have identified six putative *P. falciparum* syntaxins and have generated antibodies to three of them. Using biochemical and molecular techniques, we demonstrate that *P. falciparum* exports a subpopulation of several syntaxins into the cytoplasm of the erythrocyte. We also show that co-staining with sera recognizing MAHRP-1, *a P. falciparum* protein that is exported to the Maurer's clefts, co-localizes with the exported syntaxins. Maurer's clefts appear to be an intermediate secretory compartment between the parasite and the erythrocyte membrane. Identification of syntaxins present in the Maurer's clefts is further evidence that transport to and from this novel organelle involves vesicular intermediates.

(ACMCIP Abstract)

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APICAL ORIENTED MEROZOITE PROTEINS WITH EGF-LIKE DOMAINS UPSTREAM FROM THE MSP-1 GENE ARE UNIQUELY EXPRESSED IN *P. VIVAX* AND *P. KNOWLESI*

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The release of *Plasmodium* merozoites into the bloodstream is responsible for the clinical symptoms seen in a malaria infection. Interventions to interrupt this step in the life cycle of the parasite require a thorough understanding of invasion mechanisms and molecular components that are involved. Here we present a unique gene encoding an apical pole protein, MAP-2 (Merozoite Apical Protein) in *P. vivax* and *P. knowlesi*. Using a gene fragment from the University of Florida EST database in conjunction with BLAST analyses, we identified two highly homologous contigs in the *P. vivax* and the *P. knowlesi* sequence databases. Three features make these genes of particular interest: 1. The map-2 gene is part of a synteny block that includes the msp-1 gene in *P. vivax* and *P. knowlesi*. 2. The map-2 gene is not present in *P. falciparum*. 3. The C-terminal region of the putative MAP-2 contains two EGF-like motifs oriented head-to-tail. To characterize these proteins we produced a recombinant fragment representing the central region of PkMAP-2 in the pET system and the soluble form of the protein was utilized to produce rabbit polyclonal antisera. Data on the localization and the biochemical characteristics will be presented. By using RT-PCR we determined that the *P. knowlesi* map-2 transcript is synthesized as a full-length product during the schizogony stage and ongoing studies will determine the exact timing for the protein expression on the merozoite. We will discuss the molecular characteristics of the map-2 transcript in the context of the synteny block containing the msp-1 gene in *P. vivax* and the simian malaria parasites

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PROTEOMICS DEFINES SPECIFIC *PLASMODIUM VIVAX* AND *PLASMODIUM KNOWLESI* INFECTED ERYTHROCYTE MEMBRANE ANTIGENS

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During their asexual development, malaria parasites induce a variety of structural and antigenic alterations in the membrane and cytoplasm of their host erythrocyte, including the development of caveola, caveola-vesicle complexes (CVC), clefts, and knobs. The alterations are different among the malaria species. For example, the human malaria, *Plasmodium vivax*, and simian malarias, P. cynomolgi and P. simium form caveola-vesicle complexes while *P. knowlesi* form just the caveola. *P. falciparum* and P. coatney form knobs on their outer surface membrane. The antigenic composition and function of the CVC is still unknown and has not been extensively explored. However some earlier research has shown that the CVC may be antigenic. This study is utilizing a proteomics approach to further identify and understand specific antigens of interest that may prove to be relevant for vaccine and drug development. Initially, a panel of available *P. vivax* monoclonal antibodies (mAbs) was used in Indirect Immunofluoresence Assays (IFA) on *P. knowlesi* schizonts. mAbs that showed cross-reactivity and specificity to caveola and other membrane structures were then used to immunoprecipitate corresponding proteins or protein complexes in *P. knowlesi* protein extracts. Proteomic tools were then used to identify the immunoprecipitated proteins in *P. knowlesi*. The protein sequences identified by mass spectrometry analyses were then blasted against all other Plasmodium databases and protein matches analyzed. This study is rapidly revealing the identity of new specific proteins for further investigations to understand the function of the infected erythrocyte membranes of *P. vivax* and possible targets of intervention.

(ACMCIP Abstract)

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FUNCTIONAL CHARACTERIZATION OF REFOLDED DBL1⟨ DOMAIN OF *PLASMODIUM FALCIPARUM* ERTHROCYTE MEMBRANE PROTEIN-1

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The ability of *Plasmodium falciparum*-infected erythrocytes to adhere with uninfected RBC (rosetting), other parasitized RBC (auto-agglutination), and to endothelial cell lining blood vessels (cytoadherence) is mediated by variant surface antigens, which are referred to as *Plasmodium falciparum* erythrocyte membrane protein -1 (PfEMP-1) and are encoded by var genes. The extracellular regions of PfEMP-1 contain multiple conserved cysteine-rich domains that are referred to as Duffy-binding-like (DBL) domains. The DBL1⟨ domain is a principle ligand identified on the parasitized RBC from children suffering from severe malaria often adhere to complement receptor 1 (CR1) on uninfected RBCs to form clumps of cells known as "rosettes". Here we show that DBL1⟨ domain from the PfEMP-1 protein containing nine disulfide bonds can be produced in *E. coli* and refolded into functional form. We expressed Pf DBL1⟨ (MC) in *E. coli,* refolded and purified the recombinant proteins from insoluble form. Refolded Pf DBL1⟨ (MC) exhibits functional binding to heparin in solution and to CR1 expressed on the CHO cell surface. Presently we are analyzing the

immuno-fuctional responses of DBL1⟨ as a potential vaccine candidate against malaria.

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DISSEMINATED INTRAVASCULAR COAGULATION IN A RHESUS MACAQUE EXPERIMENTALLY INFECTED WITH *PLASMODIUM COATNEYI*

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Disseminated intravascular coagulation (DIC) is a common complication of sepsis. DIC is mediated by a massive intravascular activation of the coagulation and fibrinolysis cascades. Intravascular fibrin deposits lead to a severe disturbance of organ perfusion resulting in multiple organ failure. Subclinical coagulopathy is a common finding in malaria-infected individuals but DIC is very rare. Here, we describe the first case of DIC associated with *Plasmodium coatneyi* experimental infection in a rhesus monkey. This simian malaria parasite shares several features with *P. falciparum*. Thus, we have used it as a model of severe anemia. An Indian rhesus macaque was experimentally inoculated with 2 x10^4 P. coatneyi-infected erythrocytes. The animal was closely monitored using daily clinical evaluation, temperature determination, parasite quantification, hemoglobin concentration, reticulocyte counts and platelet quantification. At day 10 after challenge, the animal developed anorexia and tachypnea and numerous petechiae were noted over the trunk and extremities. This clinical presentation was not correlated with hyper-parasitemia but with hypothermia and thrombocytopenia. Treatment with intravenous fluid support and complete blood transfusion was done. The animal also received artemether by intramuscular route. Three days after therapy the platelet counts returned to normal and parasitemia was abated. Nevertheless, both hands and the tail were gangrenous. In addition, acral discoloration was observed. Laboratory tests were compatible with DIC and euthanasia was opted due to poor prognosis. A complete panel of clinical laboratory analyses and histopathology data will be discussed in the context of the relevance for developing novel experimental animal models of severe malaria.

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IMMUNO-ELECTRONMICROSCOPY OF MALARIA MEROZOITE INVASION INTO RED BLOOD CELLS

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Electron microscopy has contributed substantially to our understanding of malaria merozoite invasion into red blood cells since the seminal studies of the 1960s and 70s. In recent years immuno-electron microscopy has been used to establish the cellular locations of a number of invasion-related antigens within merozoites, but our knowledge of their behaviour during the invasion process has advanced little. The only effective model that in the past has been used for this purpose is *Plasmodium knowlesi*, a parasite that infects rhesus monkeys. In this study we use this have used this approach to prepared specimens for both morphological and immuno-staining analysis by electron microscopy, and have re-examined this process in the light of molecular biological and proteomic advances. In this presentation we report the immuno-localization a range of merozoite surface and secretory antigens during the invasion process, including Merozoite Surface Protein-1 (MSP-1) whose final processing we confirm takes place at the moving junction with the red blood cell.

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CHARACTERIZATION OF *P. FALCIPARUM* MSP1-SPECIFIC MONOCLONAL ANTIBODIES WITH REGARDS TO REACTIVITY ON LIVE PARASITES, FINE SPECIFICITY, AND BIOLOGICAL FUNCTION

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Merozoite surface protein 1 is a major surface protein expressed on merozoites and its function is essential to the *Plasmodium falciparum* parasite life cycle. It is synthesized as a 195 kDa precursor that undergoes several proteolytic processing events. The primary event yields a noncovalent complex attached to the merozoite surface through the C-terminal MSP1-42 fragment. This fragment is cleaved secondarily to produce the MSP1-33 and C-terminal MSP1-19 fragments with the later bound covalently to the surface of the invasive merozoite. Previously, MSP1-19-specific, conformation-dependent mAbs were developed by immunizing with parasite derived Ag and were shown to either interfere *in vitro* with erythrocyte invasion (mAbs 12.10, 12.8), or have blocking activity (mAbs, 2.2, 7.5, 1E1) against the invasion inhibitory mAbs. These various mAbs have been used extensively to verify parasite-like structure on recombinantly expressed proteins thus serving as valuable tools for MSP1-42 vaccine development. To test the question whether recombinant proteins could also induce mAbs with biologically relevant activities, we immunized mice with recombinant MSP1-42/AS02A (GlaxoSmithkline Biologicals) and characterized antibody specificity using MSP1 fragment-specific ELISA's on p42, p33, p19, EGF-domain 1 and EGF-domain 2, as well as by IFA, western blot, flow cytometry and functional assays (GIA and PIA). Correlating the fine specificities of these new mAbs with biological function will not only assist in localizing protective epitopes within the MSP1-42 molecule but also serve as useful tools for measuring correct structure.

(ACMCIP Abstract)

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A DATABASE MANAGEMENT SYSTEM (DBMS) FOR MOTHER OFFSPRING MALARIA STUDY (MOMS)

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MOMS/DB is a DBMS that has been developed to store and track clinical and experimental data for a 5-year longitudinal cohort project to study severe malaria pathogenesis and protective immunity in mothers and infants. This study involves more than 2000 mother-infant pairs and is conducted in both Tanzania and the United States. Paper forms have been designed to collect clinical and experimental information (http://apps.sbri.org/gcgh/gcgh.htm) for this study. A barcode tracking system has been implemented to track all clinic and laboratory paper forms. The barcode system requires that preprinted barcode labels be placed on all forms before the data are entered into the database. An online data entry system (http://apps.sbri.org/cohort/) has been developed to help data entry persons to enter the information on the paper forms into the database which are hosted on a Microsoft SQL database server. The front end of this system was written in Java. A Linux server is dedicated as a web server. Double data entry technique, which is a way to verify the integrity of the data entry and protect data integrity from typography or subject errors, has been applied in the system. In order to track all samples collected in

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the study so we are able to link all experiment results to clinic information, a sample tracking system has been developed as well. Barcode labels are attached to all samples and boxes which are used to store samples. Experiment results, such as complete blood count and blood smear reading results, are captured through a Java standalone application and ultimately stored in a Microsoft SQL server database. MOMS/DB is compliant with the FDA guidelines of electronic records (21CFR11).

still limited. We tested the hypothesis that sulfalene/pyrimethamine/amodiaquine (SLP/AQ) is as efficacious and safe as artemether/lumefantrine; Coartem® (AL) in the treatment of uncomplicated *P. falciparum* malaria in multicenter study including Mali. We report here the results of this study carried from August to November 2005 in Sotuba and Kambila, in Mali. Treatment efficacy was assessed using 28 days WHO 2003 protocol. Safety was assessed clinically and biologically (hemogram and biochemistry). MSP1 and MSP2 and microsatellite CA1 were used to distinguish recrudescent from new infections. Of the total 450 required subjects for all the centers, 226 aged >= 2 years were enrolled in Mali, 113 in each treatment arm. SLP/AQ was given once daily for 3 days. AL was given twice daily for 3 days. The study was funded by Pfizer. Baseline characteristics of patients in the two treatment groups were comparable. The 28-day cure rate for SLP/AQ was 90.7% (n=107) compared to 83.5% (n=109) for AL; p=0.1. After correction for of re-infection, the 28-day cure rate was 98.1% for SLP/AQ and 98.2% for AL; p=0.99. SLP/AQ cleared fever faster than AL on day 1 (96.4% *vs.* 88.5% respectively, p = 0,024) while AL cleared faster the parasites on day 1 and day 2 compared to SLP/AQ (54.5% and 97.3% *vs* 4.6% and 50.9% respectively. p<0.001). Gametocyte carriage rate was similar in both treatments arms. No serious adverse events or significant laboratory abnormalities occurred. Adverse events rate were similar in the two arms except for abdominal pain, vomiting, anorexia and fatigue which were higher in SLP/AQ arm p<0.001. SLP/AQ is as effective as AL for the treatment of *P. falciparum* malaria, with a faster fever clearance but frequent digestive symptoms.

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EFFECTS OF PYRIMETHAMINE-SULPHADOXINE, CHLOROQUINE PLUS CHLORPHENIRAMINE AND AMODIAQUINE PLUS PYRIMETHAMINE-SULPHADOXINE ON GAMETOCYTES DURING AND AFTER TREATMENT OF ACUTE, UNCOMPLICATED MALARIA IN CHILDREN

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The effects of pyrimethamine-sulphadoxine (PS), chloroquine plus chlorpheniramine, a H1 receptor antagonist that reverses chloroquine resistance *in Plasmodium falciparum in vitro* and *in vivo* (CQCP), and amodiaquine plus pyrimethamine-sulphadoxine (AQPS) on gametocyte production were evaluated in 157 children with acute, symptomatic, uncomplicated falciparum malaria who were treated with these drugs. PS was significantly less effective than CQCP or AQPS at clearing asexual parasitaemia or other symptoms of malaria. Gametocyte carriage on days 3, 7 and 14 were significantly higher in those treated with PS. The ratio of the density (per µl blood) of peripheral young gametocyte (PYG), that is, ≤stage III to peripheral mature gametocyte (PMG), that is, stage IV and V, an index of continuing generation of gametocytes, rose to 1 by day 7 of treatment in those treated with PS, but remained consistently below 1 in the other treatment groups. PYG-PMG density ratio increased significantly from day 0-14 in those treated with PS and CQCP (2= 76, P= 0.000001and 2=42.2, P =0.00001, respectively) but decreased significantly in those treated with AQPS (2= 53.2, P= 0.000001). Both PS- sensitive and -resistant infections generated PYG (18 of 29 *vs.* 13 of 20, 2 = 0.04, P = 0.93) but PYG was present only in those with resistant response to CQCP. Combination of PS with amodiaquine (AQ), that is, (AQPS) resulted in less production of PYG, but in this setting, PYG was not indicative of response to AQPS. These data indicate that PS enhanced production or release of young gametocytes when used alone, but generated less young gametocytes when used in combination with AQ. PYG may be used as an indicator of response to CQCP but not PS or PS-based combination drugs.

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THE EFFECTS OF ARTEMETHER-LUMEFANTRINE *VERSUS* AMODIAQUINE-SULFALENE-PYRIMETHAMINE ON THE HEPATOMEGALY ASSOCIATED WITH *PLASMODIUM FALCIPARUM* MALARIA IN CHILDREN

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An open randomized controlled study of artemether-lumefantrine (AL) and amodiaquine-sulfalene-pyrimethamine (ASP) for the treatment of uncomplicated *Plasmodium falciparum* malaria was carried out in 181 children. In 79 children, the hepatomegaly reduction ratios (HRR) and the speed of resolution of hepatomegaly, the hepatomegaly resolution rates (HRSR) were calculated and compared between the two treatment groups. HRR and HRSR were similar in the two treatment groups. HRSR was 71% and 62% in AL- and ASP- treated children, respectively by 14 days of commencing treatment. There was no significant correlation between HRR and parasite reduction ratio (PRR) in the same patient. In children in whom parasitaemia cleared and hepatomegaly resolved within 14 days, recurrence of parasitaemia was associated with re-occurrence of hepatomegaly, suggesting that, the propensity for recurrence of infection drives the malaria-attributable hepatomegaly in children from this endemic area. Combination therapy may provide additional beneficial effects on patho-physiological processes and changes associated with falciparum malaria by rapid clearing of asexual parasitaemia and reducing the propensity for recurrence of infection.

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STAGE-SPECIFIC SURVIVAL OF *PLASMODIUM FALCIPARUM* TREATED WITH ANTI-MITOCHONDRIAL DRUGS

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The mitochondrion plays a critical role in the life and death decisions of many eukaryotic cells. Apoptotic pathways in metazoa and some unicellular organisms are initiated by the collapse of mitochondrial membrane potential. Atovaquone, an antimalarial drug, has been shown to inhibit electron transport, leading to the collapse of the membrane potential in malaria parasites. We are exploring effects of mitochondrial physiology inhibition by atovaquone alone as well as in combination with its synergistic drug, proguanil, in malaria parasites. Our results suggest that, in *P. falciparum,* a collapsed mitochondrial membrane potential does not necessarily induce a cell death pathway. The effects of atovaquone and atovaquone/proguanil are highly dependent upon the erythrocytic stage of the parasites exposed to the drugs. We have found that ring-stage parasites are the most resilient to drug treatment and can survive for periods longer than 48 hours. During treatment, however, survival of the parasite seems to depend on its ability to exist in a 'static' phase, once treatment ceases, the parasites 're-enter' the erythrocytic development cycle. To further understand this static state, we have analyzed the global transcription profile of treated parasites to determine the mRNA stability and any transcriptional changes that may occur during drug treatment. These studies begin to provide information on the physiological state of the parasite at which mitochondrial DNA mutations could arise leading to drug resistance.

(ACMCIP Abstract)

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BLACKWATER FEVER IN CHILDREN DURING CEREBRAL MALARIA: THREE OBSERVATIONS IN BAMAKO

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We carried out an exploratory study of cerebral malaria in the pediatric section of National Hospital "Gabriel Touré" at Bamako. The study was conducted during transmission seasons of years 2003 and 2004. The aim goal of the study was to successfully find out children tolerance of *quinine* during cerebral malaria. The population study consist by children from 6 month to 14 years old with a drop thick positive (*Plasmodium falciparum)* and/or Optimal positive IT, a "Score of Blantyre" less than 4, Repeated convulsions (at least 2 times per day followed by critical coma (for at least 15 minutes), Macroscopic haemoglobinurie after quinine administration. We observed 3 cases of macroscopic haemoglobinurie: 2,5% (3/119). Both cases had been treated by quinine. Haemoglobinurie was respectively observed 3 hours, 4 hours and 12 hours after treatment. About 2/3 cases, occurs complications as acute renal insufficiency with blood creatine level around 615,29 µmol and 719,10µmol. One of them died by respiratory distress which occurred 24 hours after admission in hospital. The treatment consists to immediately stop administration of quinine and replaced it by artemeter in intra muscular: 3,2mg/kg the 1st day and 1,6mg/kg/jour from 2nd to 5th day. Furosemide was administered to manage the complications: 2 mg/kg/jour. In most of the power country and specifically in Mali, people tend to get first self medication. Then, they are going to see a physician 3 to 5 days later in the case of medicine they were taken doesn't make well they feeling. That can be cause of many complications as the one observed during this study. Actually, detecting haemoglobinurie seems to be very easy, but as proved by this study, it is essential for a best perceptive in the treatment and prevention of cerebral malaria if we knew that patients always get hospital very late and used to take many kind of drugs as consigned by a tierce person (neighbors, parents etc…). Anyway, the first issue will be to stop immediately the medication by quinine.

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DEVELOPMENT OF QUANTITATIVE REAL-TIME PCR AS A SENSITIVE AND EFFECTIVE APPROACH FOR DETECTING PLASMODIUM-INFECTED HC-04 CELL LINE

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Plasmodium spp., the causative agents of malaria, results in the illness and death of millions world wide each year. The development of drug resistance has increased the urgency to develop new compounds against these parasites. Malaria prophylaxis targets blood and liver stage parasites. Development of drugs for blood stage parasites can be accomplished relatively easily compared to liver stage drug development because an *in vitro* system is available for rapid screening of compounds against blood stages. However, there has been no effective *in vitro* system available for evaluating compounds against the liver stage parasites of human malaria. A human hepatocyte cell line (HC-04) established in our laboratory can be optimized and used for *in vitro* screening of new compounds against liver stage parasites. This cell line maintains production of key proteins and enzymes which may be important for drug metabolization and parasite development. In addition, *P. falciparum* and *P. vivax* parasites can completely develop to mature merozoites in HC-04 cells that then invade red blood cells. To optimize this system, we need to develop a rapid assay that will allow us to follow parasite development in the liver cells without requiring microscopic examination. Quantitative PCR was considered for this purpose because of its rapidity and sensitivity. In this study, we developed universal genus specific primers for SYBR green probe and universal genus and species specific probes to 18s ribosomal RNA genes. The data indicated that the sporozoites required longer incubation time for cell lysis and the detection sensitivity was improved. There was no cross amplification of host and parasite when using primers for liver cell and *Plasmodium* spp. HC-04 DNA did not have a negative effect on the detection of parasites' DNA although the DNA quantity of HC-04 was many times higher. The established protocol for this rapid quantification of parasites in the liver cells will enhance the development of *in vitro* system for drug sensitivity screening of new anti malaria compounds.

(ACMCIP Abstract)

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MOLECULAR DIAGNOSIS OF MIXED *PLASMODIUM* SPECIES AND SUB-CLINICAL MALARIA IN MINING REGIONS IN THE BOLIVAR STATE, VENEZUELA

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We compared a nested PCR assay and microscopic examinations of Giemsa-stained blood slides in the diagnosis of mixed and sub-clinical infections of malaria in mining regions from Venezuela. A first group of 158 volunteers seeking care at Malaria Center Dr. Francesco Vitanza in Tumeremo, Bolivar state with positive malaria diagnosis by microscopy was evaluated by a nested PCR assay. 39% (61 *Plasmodium falciparum/ Plasmodium vivax,* 1 *P. falciparum/P. vivax/P. malariae*) of mixed infections were detected by the molecular diagnosis in contrast with 3% (4 *P.falciparum/P.vivax*) of mixed infections detected for the conventional microscopy. For each mixed infection identified by microscopy, the nested PCR detected 13 (R=13:1). A second group of 91 volunteer asymptomatic miners from the mining village Vuelvancaras, Sifontes District was studied for both methodologies. 34% (31 malaria cases) of the samples were detected positive to malaria by nested PCR, meanwhile the microscopic diagnosis detected only 7% (6 malaria cases). Also 4% (4 *P.falciparum/ P.vivax*) of mixed infections were diagnosed by nested PCR, that were not identified by microscopic assay. For each malaria case detected in the asymptomatic miners by microscopy, the nested PCR detected 5 (R=5:1). In our study, nested PCR showed a higher detection level than conventional microscopy in the diagnosis of mixed infections and sub-clinical malaria.

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FIRST REPORT OF NATURAL *PLASMODIUM KNOWLESI* INFECTION IN WILD MACAQUES, THAILAND

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We recently reported the first naturally acquired *Plasmodium knowlesi* malaria in a patient who possibly acquired the infection in southern Thailand. Concurrently, a large outbreak of human cases infected with this simian malaria parasite were identified in Malaysian Borneo. It is of note that all human cases so far encountered have been confined to these regions including Malaysian Peninsula where natural reservoir hosts such as crab-eating macaque, *Macaca fascicularis*, have been populated in these regions. Therefore, we conducted an epidemiological surveillance of *P. knowlesi* among macaque monkeys in 3 localities along western and southern Thailand. We collected blood samples from 138 monkeys after temporarily caught under the safety guideline protocol. Thereafter, all monkeys were released back to their original habitats without any eventful consequences. The macaque species were *M. fascicularis*, *M. nemestrina*, *M. arctoides* and certain hybrid or unidentified species. Each blood sample was searched for the presence of malaria parasites by the Giemsa-stained thick blood films. Results revealed that 36 samples (26.1%) contained ring stages of *Plasmodium* sp. while 4 monkeys (2.9%) were infected with *Hepatocystis* sp. Because ring stages of malarial parasites *per se* are not informative for species differentiation among simian malaria, we exploited the polymerase chain reaction method with *P. knowlesi*-specific primers targeting the 18S rRNA gene, followed by DNA sequencing for definite species identification. Of 36 ring stage-positive samples, *P. knowlesi* was detected in 9 *M. nemestrina* and none in other species of monkeys. We further examined the presence of other simian malaria potentially causing human infections by direct sequencing of mitochondrial genome. Of 7 samples analyzed, *P. inui* and *P. cynomolgi* were found in 5 and 2 isolates, respectively. The high prevalence of *P. knowlesi* and other simian malaria in macaque monkeys in Thailand emphasizes an important role of wild primate populations in malaria transmission to humans.

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PRECLINICAL PHARMACOKINETICS AND METABOLISM OF GW308678, A SECOND GENERATION 4(1H)-PYRIDONE ANTI-MALARIAL MITOCHONDRIAL ELECTRON TRANSPORT INHIBITOR

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4(1H)-Pyridones are a novel class of antimalarials that are selective inhibitors of *Plasmodium* mitochondrial electron transport. We have previously reported the preclinical pharmacokinetics and metabolism of GW844520. Subsequent safety assessment of GW844520 revealed a narrow therapeutic window due to cardiac toxicity in the dog but not in the mouse. Here we report the preclinical pharmacokinetics and metabolism of GW308678, a backup compound in this series that did not display cardiac toxicity in the dog or mouse under similar conditions. We have investigated the pharmacokinetics following single intravenous and oral administration to the mouse, rat, dog and monkey. Protein binding and blood cell association were investigated in the plasma or whole blood from preclinical species and human, *in vitro*. The routes and rates of metabolism of GW308678 were studied in animal and human liver microsomes and hepatocytes. Concentration- and time-dependent human cytochrome P450 inhibition, permeability and active transport were investigated *in vitro*. GW308678 had relatively low blood clearance ranging from 2 (in the dog) to 23% (in the rat) of liver blood flow, a long elimination half-life of 61 h in the dog, and a steady-state volume of distribution ranging from 2-4 times total body water in animals. The oral bioavailability following administration of a solution was high in all species (69-100%). GW308678 had high passive permeability and was not a P-glycoprotein substrate. Intrinsic clearance was low in liver microsomes and hepatocytes of preclinical species and human except in monkey hepatocytes. GW308678 did not associate appreciably with blood cells and had high plasma protein binding (>99%) in all species. GW308678 was an inhibitor for human CYP2C9 and a substrate of both CYP2C9 and 2D6. Two metabolites (mono-oxygenation and N-oxidation) were detected in human liver microsomes and hepatocytes, which were also detected in all preclinical species. Based on these data, we would expect GW308678 to have high bioavailability and low clearance in man making it suitable for the desired short duration of therapy by the oral route of administration.

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A SURVEY OF SYNTHETIC AND NATURAL PHYTOTOXIC COMPOUNDS AND PHYTOALEXINS AS POTENTIAL ANTIMALARIALS

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Discovery of antimalarial drugs with new modes of action is a primary focus of malaria research because the malaria parasite has developed resistance against almost every chemotherapeutic regimen available. Malaria parasites are members of apicomplexa and have a plastid organelle called the apicoplast. There are several pathways and functions present in both the apicoplasts and plant plastids which are fundamentally different to the analogous pathways and functions in humans that might be good targets for new antimalarial drugs. Apicoplast function is essential for survival and viability of *Plasmodium*, but all of the crucial apicoplast processes are not known. Many highly effective herbicides and natural phytotoxins target plastid processes. Therefore, such compounds might be effective against the apicomplexa. We have determined the activity of a variety of phytotoxic compounds, both natural and synthetic, against *P. falciparum*. We have also examined the activity of some stilbene-based phytoalexins. Most of synthetic herbicides possessed moderate antimalarial activity. Even the moderate action of these herbicides may be considered as useful lead in view of targeting of apicoplast pathways by the herbicides. The antimalarial potential of herbicides led to discovery of plant-like metabolic pathways and their essentially in survival of the apicomplexan parasites including malaria. Endothall showed promising antimalarial activity. It inhibits protein phosphatase in mammals, but its mode of action as a herbicide is unknown. The function of this enzyme is known to be essential for *P. falciparum*. Earlier work with glyphosate and triazines indicated significant interest in them as antimalarials but their activity could not be confirmed *in vitr.* Most of the natural phytotoxins were active, with anisomycin, cavoxine, cerulenin, and 19-trityl-12-oxo-acetonide being the most active. Antimalarial phytotoxins and phytoalexins may provide useful leads and probes to map the target pathways for new antimalarial drug discovery.

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SUNIVERSITY OF SOUTHERN CALIFORNIAEPTIBILITY OF *PLASMODIUM FALCIPARUM* TO CHLOROQUINE IN THE MALARIA-ENDEMIC VILLAGE OF MISSIRA IN MALI USING THE WHO *IN VIVO* TEST AND SEQUENCING

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Because CQ is the cheapest and least toxic antimalarial, monitoring the prevalence of CQ-resistant *P. falciparum* is an important aspect of malaria control. To estimate the prevalence of CQ-resistant *P. falciparum* in a village being considered as a test site for Phase 2 Studies of a candidate antimalarial, we used the WHO *in vivo* test in conjunction with molecular techniques to collect baseline information in the malaria-endemic village of Missira, Kolokani in Mali. Using the standard oral dose of 25 mg CQ base per kg over 3 days, we performed the WHO 14 day *in vivo* test to identify early and late therapeutic failures and to estimate the rates of adequate clinical and parasitological responses to treatment with CQ. PCR based on the polymorphic Block 2 region of *msp1* was used to distinguish between recrudescence and new infection. Point mutations of *pfcrt* gene within a region encompassing the 76 amino-acid position were identified using capillary sequencing. Results obtained for 27 children with uncomplicated malaria between 1 and 9 years of age included 4% early treatment failures, 11% late clinical failures, 33% late parasitological failures and 58% adequate clinical and parasitological responses. Using MSP-1 data after amplification of 16 specimens by PCR, we adjusted the overall data obtained with the *in vivo* test, prevalence of susceptible *P. falciparum* was 55.5% versus 44.4% of resistant parasites. In order to validate those data we increased the sample size of study population to 50 children, we didn't find a difference when the sample size was 27 children. To investigate whether mutation 76T is associated with resistance of *P. falciparum* to CQ, we sequenced four PCR products from infected subjects: one specimen from a subject that responded to CQ had K76; another specimen from a subject who did not respond to CQ had 76T, and 2 specimens from subjects who responded to CQ had 76T. Geometric mean parasite densities decreased of parasitemia from 10,113 on day 0, to 119 on day 4, and 128 on day 7, before rising to 1206 on day 14. This suggests that one could increase the dose of CQ to 30 mg/kg body weight in order to obtain clearance of resistant *P. falciparum* parasites that could emerge during the 3 days of the treatment. These results indicate that the prevalence of CQ-resistant *P. falciparum* is ~50% in rural areas such as Missira; it also indicates that some infections with 76T parasites respond to CQ in a semi-immune population.

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PRECLINICAL CARDIAC SAFETY PROFILE OF PIPERAQUINE PHOSPHATE AND CHLOROQUINE PHOSPHATE

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Many antimalarial drugs on the market have cardiac effects ranging from mild heart rate changes to excessive prolongation of the QT interval leading to lethal arrhythmias such as Torsade de Pointes (TdP). This study compared the preclinical cardiac safety of Piperaquine (QCP) and Chloroquine (CP). The QCP is an antimalarial drug being used as part of combination therapy reported to have improved clinical efficacy and tolerability, compared to CP. No effect on cardiac action potential duration (APD) was noticed with QCP up to 10 µM conc. in Canine Purkinje fibres as compared to maximum rate of depolarization and prolonged APD reported for CP at 0.3 µM conc. in cat Purkinje fibres. Effect on blood pressure (BP), heart rate (HR) and electrocardiogram (ECG) was assessed by administering single dose of 0, 5, 25 and 50 mg/kg of QCP and 0, 5, 10 and 20 mg/kg of CP by orally in 3 male and 3 female telemetered conscious dogs in a crossover pattern with a 7 day washout period. Blood samples were collected from all animals for pharmacokinetic assessment. No changes in mean, systolic and diastolic arterial pressure, HR or cardiac conduction times were observed at 5 and 25 mg/kg of QCP. However, tachycardia associated with decrease in PR and QC interval durations with long lasting increase in QTc interval (Bazett's, Frederica's formula and Sarma's method) and prolongation of ventricular repolarisation was noticed at 50 mg/kg dose. Tremors were observed in 3 dogs at 50 mg/kg dose. Similarly, CP treated animals showed significant and long lasting tachycardia associated with decreases in QC and QT interval, an increase in QRS complex duration and QTc interval at 20 mg/kg dose level, supporting an effect on the ventricular depolarization and repolarisation. Tremors (all dogs), seizure (one dog) and ataxia (4 dogs) were noticed in animals receiving 10 and 20 mg/kg dose levels. No disturbance in 6-lead electrocardiogram in the lead II and no change in the T wave morphology, attributable to the QCP or CP was seen at any dose. Plasma estimations for both the drugs showed dose related and gender independent exposure. The NOEL established for cardiovascular parameters was 10 mg/kg CP and 25 mg/kg QCP with systemic exposure (AUC_{inf}) 2377 and 46233 h·ng/mL, respectively indicating better cardiac safety of QCP in animals. The effect of QCP on humans compared to CP has not been determined.

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EVALUATING THE EFFECTS OF CHLOROQUINE AND AQ-13 ON CARDIAC QT INTERVAL

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We compared the effects of a candidate antimalarial, AQ-13, and chloroquine (CQ) on the cardiac QT interval using 2 formulas to correct the QT interval for heart rate: Bazett's ($QTc = QT/RR^{½}$), and Fridericia's ($QTc = QT/RR^{1/3}$), where QTc is the corrected QT and RR is the duration of the cardiac cycle in seconds. This report is based on Holter recordings from 27 volunteers (13 randomized to AQ-13, 14 to CQ). The drugs' effect was defined as the difference between baseline and maximal QT values (observed 4 hrs after the 2nd dose.) With both drugs, AQ-13 or CQ, both formulas performed similarly (Spearman's *r* from 0.17 to 0.31; *p* from 0.13 to 0.39 for the correlation between QTc and RR). However, when comparing the effect of the two drugs on the QTc interval, the results obtained with the two formulas were different. With Bazett's, average QTc prolongation was greater by 17 msec with CQ than AQ-13 (95% CI = 5, 29 ms). In contrast, with Fridericia's, both drugs had similar effects; QTc prolongation was only 6 msec greater with CQ than AQ-13 (95% CI = -8, 19 ms). The reason for this difference is that baseline RR interval in the AQ-13 group was shorter than the post-dose RR interval (median: 0.67 and 0.84 sec). In contrast, in the CQ group, baseline and post-dose RR intervals were similar (0.75 and 0.76 sec). This difference resulted in a disproportionately larger denominator for the baseline QT with AQ-13 when Fridericia's correction was applied because the smaller the number than 1 the larger the difference between its cubic and square roots. Therefore, in the AQ-13 group, baseline QTc calculated from cubic-root formula was much smaller than with square-root formula. This increased the difference between baseline and post-dose QTc intervals calculated with Fridericia's formula, made them similar to the differences observed with CQ, and thus led to the conclusion that there was no difference between AQ-13 and CQ in their effect on the QTc interval when Fridericia's (but not Bazett's) correction was used. Careful evaluation of the QT correction used should be performed when comparing the effects of different drugs on the QTc interval

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IDENTIFICATION OF AROMATIC SULFONYLS AS INHIBITORS OF B-KETOACYL ACP SYNTHASE III (PFKASIII) IN *PLASMODIUM FALCIPARUM* FATTY ACID SYNTHESIS

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Plasmodium falciparum rely on a Type II fatty acid synthesis pathway that is located in the apicoplast, an essential organelle that has no human counterpart, which makes this process a unique target for new anti-malarial drugs. Beta-Ketoacyl ACP Synthase III (pfKASIII) is one of five enzymes (MCAT, KASIII, KAR, HAD and ENR) that use ACP (Acyl Carrier Protein) as a substrate to initiate and elongate fatty acids within the malaria parasite. We developed a radioactive 96-well microplate assay that measures the inhibition of the pfKASIII enzyme by detecting the transfer of 14C-Acetyl-CoenzymeA to ACP, forming 14C-acetyl-ACP. In the presence of an inhibitor, the radiolabel will not be incorporated. Our pharmacophore and structure-based drug design program have yielded over 1000 compounds to screen in our assay. The KASIII assay has identified many promising chemotypes, most notably the aromatic sulfonyls, that have IC50s of <10uM against the enzyme and two strains of *Plasmodium falciparum* (W2 and D6). We have also identified thiosulfuric acids, sulfonic acids, and sulfonamides as prospective chemotypes. Almost all of these compounds were not toxic against two representative mammalian cell line screens. Molecular modeling and QSAR have greatly increased our ability to select compounds that are specific to pfKASIII. The homologue to pfKASIII in *E. coli* is FabH, and the active site differs from pfKASIII by 5 amino acids. Mutagenesis of the ecFabH active site to emulate pfKASIII will give us insight into the role of these particular amino acids in the binding of potential inhibitors.

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ORALLY ACTIVE ACRIDONES AS NOVEL AND POTENT ANTIMALARIAL CHEMOTYPES

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Malaria remains one of the world's pressing health problems, in large part due to the spread of drug-resistance. The desperate need for safe, effective, affordable and feasible new drugs remains unmet. In this presentation, we will describe the rational design and discovery of several novel antimalarial acridone chemotypes. Our preliminary studies reveal potent activity of these acridones *in vitro* against both chloroquine-sensitive and multidrug-resistant strains of *Plasmodium falciparum*. These water-soluble acridone derivatives exhibit great antimalarial efficacy *in vivo* with rapid parasite killing in *Plasmodium* (P. *yoelii* and P. *berghei*) infected mice, either by the oral route or intraperitoneal injection. T2, an example of our first generation acridone chemotype, has demonstrated oral efficacy with an ED_{50} of 27mg/kg in a 4-day suppressive test, and ED_{50} of 38mg/kg in a 3-day curative regimen against patent infection with P. *yoelii*. Another unique chemotype derived from this acridone development has shown synergy with quinolines (quinine, chloroquine, and amodiaquine), in addition to potent intrinsic antimalarial activity. In addition to *in vivo* oral efficacy, potential clinical utility is indicated by a favorable therapeutic safety index on the basis of *in vivo* and *in vitro* toxicity assessments.The likely drug target for these acridones may be the immutable heme. Lead compounds form soluble heme complexes, and inhibit aggregation of heme in our *in vitro* assay. On the basis of physicochemical properties, these acridones are predicated to accumulate in the acidic digestive vacuole of the parasites, causing toxic effects on the parasites through the inhibition of hemozoin formation. Details of the design, synthesis, chemistry, structure-activity optimization, and further investigation of the mode of action of several acridone chemotypes will be presented.

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ASSESSMENT AND CONTINUED VALIDATION OF THE MSF ASSAY FOR USE IN MALARIA DRUG SCREENING

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Traditionally, high-throughput *in vitro* anti-malarial drug screens have incorporated the use of radioactive substrates to measure the effect test compounds have on parasitic growth. Several alternative growth inhibition screening assays using fluorescent nucleic acid intercalating dyes have been recently published. In this study we evaluated the malaria SYBR Green I-based fluorescence (MSF) assay, described by Smilkstein et al, for its use in laboratory research and in support of the U.S. Army malaria drug resistance program and the Global Emerging Infection Surveillance and Response System (GEIS) objectives. We expanded upon Smilkstein's initial characterization and validation of the MSF assay to fit our program-specific drug screening needs by including antibiotics and antifolates in the drug panel and the testing of folic acid-free growth conditions. *Plasmodium falciparum* strains D6 and W2 were treated with a panel of known anti-malarial drugs and their respective IC_{50}s were determined using the MSF assay. The results were then compared to our historical IC_{50} data and/or side-by-side experiments generated using our standard [^{3}H]hypoxanthine incorporation assay. We also examined assay conditions that could potentially affect MSF assay readout, including assay length, starting parasite density and hematocrit levels, microtiter plate selection, and different culture medium components. The IC_{50} values from the MSF assay showed the expected pattern of drug resistance for both parasitic strains tested when compared to the values from the [^{3}H]hypoxanthine incorporation assay. One possible limitation of the MSF assay for some drug resistance applications is due to a significant edge effect observed independently of culture volume or drug tested, which could influence IC_{50} calculation. The MSF assay was easily amended for use with our robotic plate and handling equipment. Compared to our gold standard radioactive assay, the MSF assay is more cost-effective, simple, and less hazardous, while still allowing for accurate high-throughput, automated drug testing.

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STRUCTURE-ACTIVITY RELATIONSHIPS OF ORALLY ACTIVE ANTIMALARIAL ACRIDONES: SYNTHESIS, OPTIMIZATION, AND BIOLOGICAL ACTIVITY

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Several novel antimalarial chemotypes were discovered during our recent structural modification from xanthones to acridones. A series of acridone derivatives, functionalized to enhance accumulation and interference with hemozoin formation in the parasite digestive vacuole, were designed, synthesized, and evaluated for their *in vitro* and *in vivo* antimalarial activity. In vitro, these water-soluble acridones exhibit impressive activity with low nanomolar IC_{50}s against both chloroquine sensitive (D6) and multidrug resistant (Dd2) strains of *Plasmodium falciparum*. Interestingly, the shape of the dose-response curve or/and the combined action with quinolines varies between different positional isomers, suggesting that subtle chemical or physicochemical changes result in substantive mechanistic transformation. Importantly, lead compounds have demonstrated oral bioavailability and rapid parasite killing in our rodent studies, and *in vivo* and *in vitro* toxicity assessments indicate a favorable therapeutic safety index. Detailed structure-activity profiles of the acridones (i.e., *in vitro* and *in vivo* antimalarial activity, heme-binding constant, drug uptake and