release of sCD21 into the bloodstream, in malaria CD21 is removed from B cells (by the MPS) as part of the C3dg IC and therefore not released into circulation. Since both membrane-associated and sCD21 play a critical role in humoral immunological responses, their reduced expression probably contribute to the pathogenesis of complicated malaria.

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FUNCTIONAL ASSOCIATION BETWEEN RANTES-4151C/T PROMOTER POLYMORPHISM AND HIGH-DENSITY FALCIPARUM PARASITEMIA AMONG CHILDREN IN A HOLOENDEMIC MALARIA TRANSMISSION AREA

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RANTES (CCL-5) is a CC- (or β-) chemokine that regulates innate and adaptive immune responses. Genetic variation in RANTES conditions susceptibility to autoimmune, allergic, and infectious diseases. We previously demonstrated that suppression of RANTES in children with malarial anemia is associated with thrombocytopenia and enhanced monocytic hemozoin acquisition. However, the role of genetic variation in RANTES on conditioning disease outcomes in *Plasmodium falciparum* malaria is unexplored. The influence of a promoter variant (-4151C/T) on severe malarial anemia (SMA; Hb<6.0g/dL), high-density parasitemia [HDP, parasites>10,000/μL], and RANTES production was, therefore, examined in children (*n*=508) from a holoendemic malaria transmission area of western Kenya. Genotyping was performed using a Taqman 5-allelic discrimination assay and circulating RANTES concentrations were determined with a 25-plex inflammatory profile assay. The overall allele frequency was p=0.77 and q=0.23. Multiple logistic regression revealed that the -4151CC variant was associated with reduced risk of HDP (OR; 0.4, 95%CI; 0.2-0.9, *P*=0.028). Median (Q1-Q3) peripheral parasitemia levels were also lower in children with homozygous C allele [9612 (3618-39229)] compared to the CT [24564 (5901-65949), *P*=0.076) and TT [21360 (6021-46632), *P*=0.088) variants. No significant associations were observed between variation at -4151 and either SMA or hematological outcomes. Additional investigation showed that median (Q1-Q3) RANTES concentrations were higher for the CC variant [20.1 (11.4-47.8)] than the CT [10.6 (3.8-38.1), *P*=0.049] and TT [14.0 (2.7-76.9), *P*=0.235] variants. Results presented here demonstrate that variation at 4151 in the RANTES promoter is associated with protection against development of HDP and functionally higher RANTES levels in children with falciparum malaria.

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LEUCOCYTES AND CYTOKINE PRODUCTION IN PATHOGENESIS OF SEVERE MALARIA IN MALAWIAN CHILDREN

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Severe malaria encompasses three clinical syndromes; cerebral malaria (CM), severe malarial anaemia (SMA) and severe respiratory distress (SRD). The reasons why some African children develop SMA whilst others develop CM in response to *P. falciparum* infection are unclear. Acquisition of *P. falciparum*-specific antibody parallels immunity development. Could CM be due to prior priming of memory T cells causing immunopathology and SMA be an immunologically naïve response? Study participants were children aged 6 to 60 months presenting at Queen Elizabeth Central Hospital, Blantyre, Malawi, with SMA and CM, control groups with uncomplicated malaria and healthy controls. Eligibility for each group was determined by Blantyre Coma Score, malaria parasite slide positivity and haematocrit. HIV positive participants were excluded from the study. All malaria patients were followed up in convalescence. A 5 ml blood sample was collected which was used for full blood count analysis, determination of the percentage and absolute numbers of various lymphocyte subsets, percentage of activated lymphocyte subsets and the percentage of naïve and memory CD4+ and CD8+ T cells. Cytokine analysis was also done by CBAs on serum samples and by ICS in whole blood. Acute SMA was associated with lymphocytosis whereas acute CM was associated with lymphopenia which normalised in convalescence. Acute CM and SMA cases had higher proportions of activated α–β T cells than healthy controls. SMA patients had higher percentages of activated γ-δ T and NK cells compared to healthy controls. Most of the $CD4^+$ T cells in children with SMA were naïve whereas CM cases had a higher percentage of memory CD4+ T cells. Acute CM patients had higher levels of pro-inflammatory cytokines and IL-10 compared to SMA patients. Both CM and SMA patients had significantly lower percentages of cytokine producing monocytes during acute infection than healthy controls which normalised in convalescence of CM but were even lower in convalescence of SMA. In conclusion, results confirm that CM and SMA are different syndromes of severe malaria. The high activation in CM patients suggests that the immunological response to CM involves different cells. These findings suggest that CM is a result of immunopathological response resulting from T cells that are primed in earlier malaria episodes and that SMA is a naïve response to the infection.

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CYTOKINE PROFILE IN VARIOUS SEVERE FORMS OF FALCIPARUM MALARIA IN CENTRAL INDIA

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Severe malaria caused by *Plasmodium falciparum* infection is a syndrome that kills more than 2 million peoples annually. Recently, chemokine IP10 was reported to be associated with cerebral malaria and mortality associated with it. In this study, we investigated if plasma levels IP-10 and other cytokines involved in inflammatory pathway such as TNF-α, IFN-γ and IL-10 are different in various severe manifestations of falciparum malaria in a low endemic region of India. For this premilinary study, plasma samples from a total of 99 subjects which included 15 healthy controls (HC), 17 uncomplicated falciparum malaria (UFM), 25 cerebral malaria survivors (CMS), 16 cerebral malaria non survivors (CMNS), 18 severe malaria (SM) (patients with multiorgan involvement but with no cerebral malaria) and 8 severe malaria anemia (SMA) cases were analyzed. Commercially available ELISA kits (R and D systems) were used for cytokines estimation. Overall we found that plasma levels of IP10 progressively increased with the disease severity with an exception of SMA group. Highest levels were found in SM group (3047.76 pg/ml) followed by CMNS (2744.66 pg/ml), CMS (1487.64 pg/ml), UFM (657.09 pg/ml), SMA (327.14 pg/ml) and HC (192.85 pg/ml). Plasma levels of TNF-α measured highest among CMNS (138.54 pg/ml) and SM group (130.62 pg/ml), however, only the difference between HC and SM was significant ($p = 0.027$). IL10 levels were elevated in malaria patients compared to HC but no difference was observed between different severe disease groups. IFN-γ remained undetectable in plasma samples that were used

in the study. In summary, our study suggests IP-10 is a good marker to differentiate SMA from other severe forms of malaria including CM and confirms potential role of TNF-α in mortality associated with CM and SM.

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ASSOCIATION OF LOW CYTOKINE GENE POLYMORPHISMS IN RESISTANCE AND SUSCEPTIBILITY TO *PLASMODIUM FALCIPARUM* INFECTION IN ZIMBABWE

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Single nucleotide polymorphisms within the cytokine genes, TNF-α (-308 G/A), IFN-γ (+874 A/T), TGF-β (T/C codon 10 and G/C codon 25) and IL-10 (-1082 G/A and -819 T/C) associated with protection and susceptibility to parasitic infections were examined in samples from school aged children in the Eastern district of Zimbabwe. Whole blood specimens were obtained from 492 children between the ages of 5 - 16 years, of which 27.2 % were not infected and 72.8% infected with either malaria and/or different helminths. Genotyping was carried out using the Amplification Refractory Mutation System-Polymerase Chain Reaction (ARMS-PCR). The prevalence of samples with wild type TNF-α (GG) associated with low cytokine production was 76.1%, while 22.2% and 1.6% were predictors of medium and high production of TNF-α, respectively. For IL-10 (position -819) the distribution of wild-type, heterozygotes and homozygotes was 59.6%, 22.9% and 17.5%, respectively and a similar analysis of the polymorphisms on position -1082 for IL-10 revealed that most of the samples were of the wild-type genotype. For IFN-γ (+874 A/T), 70.5% were wild-type (AA) which is associated with high cytokine secretion, with 4.4% (TT) and 25.1% (AT) associated with low cytokine production. Limited analysis on the sample population also revealed that at the TGF-β locus (T/C codon 10) 88.5% were homozygous (TT) which predicts high production of the cytokine whereas 9.2% were homozygous (CC). Similar analysis at another locus of TGF-β (G/C codon 25) showed that only 2.3% of the sample population was heterozygous (GC) which would also predict high TGF-β production. There was no statistical significant difference in the frequencies of TNF-α, IFN-γ genotype polymorphisms among children infected with *Plasmodium falciparum* at baseline and at 6 weeks, 6 months and 12 months follow-up periods. Equal distribution of IL-10 (-819 G/A) and the rare occurrence of allele associated with low IL-10 (-1082 AA) production would suggest moderate to high IL-10 responses in the population analyzed. Finally, the high prevalence of TGF-β genotype (TT) predicting high cytokine production and the existence of homozygotes for IL-10 (high producer) might suggest the dominance of an anti-inflammatory environment when faced with acute *P. falciparum* infection in the samples analyzed.

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GENETIC HITCHHIKING, SELECTIVE SWEEPS, AND MULTIPLE ORIGINS OF DRUG RESISTANT *PLASMODIUM FALCIPARUM* IN THREE DISTINCT POPULATIONS

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The evolution and spread of drug resistant *Plasmodium falciparum*, particularly in Africa, and subsequent loss of affordable drugs are of great public health concern. Understanding how selection acts on the genome is of great interest to studies of drug resistance in *P. falciparum*. In order to broaden our knowledge of the impact of strong selection on the genome and the origins and relationships of drug resistant alleles we examined the patterns of genetic diversity surrounding drug resistance alleles from three ecologically distinct populations: Venezuela (low transmission), Kenya (holoendemic transmission), and Cameroon (moderate transmission). We characterized point mutations within and microsatellite markers surrounding two genes responsible for pyrimethamine and sulfadoxine resistance, dihydrofolate reductase (*dhfr*) and dihydropteroate synthase (*dhps*), respectively, as well as neutral microsatellite markers in the three *P. falciparum* populations. Strong selection has acted on mutant alleles of *dhfr* and *dhps* in each population as evidenced by skewed allele frequencies and microsatellite haplotype distributions, and also a reduction in variation or genetic hitchhiking surrounding both *dhfr* and *dhps*. We find multiple origins for highly resistant *dhfr* alleles in Africa, but also find that gene flow of a predominant haplotype has been an important factor contributing to the distribution of mutant lineages from Southeast Asia to Africa. We also find multiple haplotypes for mutant *dhps* alleles. Thus, there have been multiple, independent origins of the *dhfr* and *dhps* alleles in both African populations, indicating that selection may act differently on *dhfr* and *dhps* within a population. This genetic differentiation also yields support to the use of microsatellite markers to monitor the dissemination of resistance in natural populations with higher transmission intensities. Understanding how drug resistance originates and spreads under strong selection will be important for the sustained use of current and new antimalarial drugs.

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DISPERSAL OF DRUG RESISTANT DHPS REVEALS REGIONAL MIGRATION PATTERNS AMONG AFRICAN *PLASMODIUM FALCIPARUM*

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The first eradication campaigns showed that movement of malaria parasites by human migration can quickly undermine the interruption of transmission. The impact of an intervention will be maximised when it is applied at a geographical scale which encompasses regions of significant parasite exchange. Dispersal patterns of emerging resistance mutations can illustrate the extent and direction of contemporary parasite migrations. We have mapped antifolate resistance mutations in the *dhps* gene which have emerged only in the past 10-20 years and find clear evidence of population substructure within Africa. To create high resolution maps of the distributions of *dhps* alleles in Africa, we have generated new data for 20 countries and combined this with additional data from 7 published studies to cover a total of 50 sites in 27 countries. We examined the dispersal pattern of resistance mutants by characterising resistance allele lineages based on microsatellite variation flanking the *dhps* gene and then measuring the proportion of resistance allele lineages shared among populations sampled at 20 sites in Africa. We found 5 major lineages each of which had a unique geographical distribution. The extent to which allelic lineages were shared among 20 African populations revealed the existence of 4 regional parasite genepools within which parasites are well mixed, but between which genetic exchange is either more or less restricted. We observed marked differentiation between east and west African *Plasmodium falciparum* populations involving not only differences in the ancestry of resistance alleles but also in the resistance mutations themselves, indicating that qualitative differences in antifolate sensitivity and significant population structure exist at a regional level. We show that drug resistance mutations can disperse over large distances and when selection is present they quickly become established. High rates intra-regional of genetic exchange are therefore significant for the future strategic management of resistance, with the predicted consequence being that resistance once present in one country will quickly establish in others in the same region if selection is operating. We have mapped regions within which there are high rates of genetic exchange which is an indicator of rates of parasite exchange through migration. Defining such regions is a key step towards the design of effective international malaria control interventions.

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FIVE-YEAR SURVEILLANCE OF MOLECULAR MARKERS OF *PLASMODIUM FALCIPARUM* ANTIMALARIAL DRUG RESISTANCE IN KOROGWE DISTRICT, TANZANIA - ACCUMULATION OF THE 581G MUTATION IN THE *PFDHPS* GENE

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Antimalarial drug resistance has forced most malaria-endemic countries to change first-line malaria treatment from various monotherapies to artemisinin-based combination therapies (ACTs). In January 2007, Tanzania replaced sulfadoxine-pyrimethamine (SP) with artemether-lumefantrine (Coartem®) as first-line treatment against uncomplicated malaria. To examine the prevalence of molecular markers of resistance to SP and other antimalarials during and after widespread use of SP, we measured the frequency and prevalence of single nucleotide polymorphisms (SNPs) in the genes *Pfdhps, Pfdhfr*, and *Pfcrt* causing sulfadoxine, pyrimethamine and chloroquine/amodiaquine resistance, respectively, in parasite-positive blood samples from asymptomatic individuals between 6 months and 20 years of age. The samples were collected as part of longitudinal epidemiological and malariometric studies from 2003 to 2007 in two villages in Korogwe District in North-eastern Tanzania. The samples were analyzed by PCR, followed by a sequence-specific oligonucleotide probe (SSOP)-ELISA-based method. The frequency of the triple mutated *Pfdhps* haplotype, SGEGA, increased significantly from 8% in 2003 to 32% in 2007 (P<0.001), and the prevalence of the 581G mutation from 12% in 2003, to 56% in 2007. The triple *Pfdhfr* haplotype, CIRNI remained at approximately 90% throughout with only marginal differences from year to year. In contrast, the frequency of the sensitive *Pfcrt* CVMNK haplotype increased significantly from 6% to 30% (P<0.001) during the observation period. The dramatic increase of the *Pfdhps*-581G mutation resulting in triple *Pfdhps* mutant SGEGA haplotype indicate that sulfadoxine and/or other sulfonamide drugs still may exert a significant pressure on the parasite population. Further studies are needed to determine whether such changes in *Pfdhps* are endangering the continued use of SP for intermittent presumptive treatment of pregnant women (IPTp).

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THE INTRA-HOST DYNAMICS OF *PFCRT AND PFMDR*-1 ALLELES FOLLOWING ANTIMALARIAL TREATMENT IN SUDANESE PATIENTS

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Drug pressure is known to influence the selection of *Plasmodium falciparum* genotypes in favour of resistance associated alleles. However, the dynamics of these alleles within the host during the first few days of treatment and their impact on treatment out come have not been reported. This study investigates the intra-host dynamics of *pfcrt* alleles during treatment with chloroquine (CQ) and *pfmdr*-1 during treatment with artemether-lumefantrine (AL) in two independent clinical trials. A quantitative PCR (qRT-PCR) dual labeled probe assay was designed to detect three alleles (CVMNK, CVIET and SVMNT) associated with CQ resistance in the *pfcrt* gene in patient samples collected during CQ treatment on day 0,1,2,3,7 and 14 and measured against a *P. falciparum* international standard known to contain $2X10^6$ IU/µl of *P .falciparum* DNA for the CVIET allele. In addition a Sybgreen assay was employed to quantitate the CVMNK alleles employing the *P. falciparum* international standard. Within-host dynamics of the *pfcrt* gene in samples collected pre-treatment and during treatment day 1 to 14 show six classes of allelic patterns. Within these classes 18 types of combinations of alleles and treatment outcome were observed. Samples collected during the AL trial were genotyped for the all five alleles in the *pfmdr*-1 gene (N86Y, Y184F, S1034C, N1042D and D1246Y) by direct sequencing of day 0, day 3 and day failure samples for each patient where a sample was available Details of quantitative and quantitative analysis of this data will be presented.

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META-ANALYSIS OF MOLECULAR SURVEILLANCE STUDIES EXAMINING SULPHADOXINE-PYRIMETHAMINE (SP) RESISTANCE MARKERS IN AFRICAN *PLASMODIUM FALCIPARUM* POPULATIONS

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Mutations in the dihydrofolate reductase (*dhfr*) and dihydropteroate synthase (*dhps*) genes of *Plasmodium falciparum* are well characterized genetic markers for monitoring resistance to the antimalarial drug sulphadoxine-pyrimethamine (SP). However, there is no systematic review of literature reporting SP resistance mutations in Africa and their relationship to *in vivo* drug failure. Here, we report a meta-analysis of the reported prevalence of *dhfr* mutations (S108N, N51I, C59R) and *dhps* mutations (A437G, K540E, S436F) in Africa. Data are summarized as *dhfr* triple and *dhps* double mutant genotype frequencies in a database consisting of 181 reports in 25 countries published from 1997 to 2007. These frequencies were mapped to show geographic distribution of mutant genotypes and plotted against time to show changes in prevalence across the continent. Frequency data were correlated with rates of *in vitro* and *in vivo* drug resistance where available using the Genotype Failure Index (GFI) and Genotype Resistance Index (GRI) stated in the literature. The *dhfr* triple mutant is fixed in some areas and has swept the continent prior to the *dhps* double mutant. East Africa has been swept by both multiple mutant genotypes prior to the continent as a whole. Individual studies show strong positive correlation between mutant genotype frequency and clinical failure rate, but the pattern is not seen in the meta-analysis. This suggests the role of secondary factors influencing *in vivo* efficacy of antimalarials. The meta-analysis also underscores notable sources of bias in the literature such as: a) lack of uniform reporting of molecular data, particularly polyclonal infections b) molecular analysis limited to isolates from symptomatic patients as opposed to surveillance of the general population. These findings highlight the need to increase support for local surveillance capacity as well as establishing global networks, such as the World Antimalarial Resistance Network (WARN), to facilitate reliable molecular surveillance of antimalarial drug resistance.

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EMERGENCE OF A DHFR MUTATION CONFERRING HIGH-LEVEL DRUG RESISTANCE IN *PLASMODIUM FALCIPARUM* POPULATIONS FROM SOUTHWEST UGANDA

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The S108N, C59R, and N51I mutations in the *Plasmodium falciparum* gene that encodes dihydrofolate reductase, dhfr, confer resistance to pyrimethamine and are common in Africa. However, the I164L mutation, which confers high-level resistance, is rarely seen. We found a 14% prevalence of the I164L mutation among a sample of 51 patients with malaria in Kabale District in southwest Uganda in 2005 and a 4% prevalence among 72 patients with malaria in the neighboring district of Rukungiri during the same year. Surveillance at 6 sites across Uganda during 2002-2004 reported a single case of infection involving an I164L mutant, also in the Southwest, suggesting that this is a regional hot spot.

The spatial clustering and increasing prevalence of the I164L mutation is indicative of local transmission of the mutant. Targeted surveillance is needed to confirm the extent of the spread of the I164L mutation and to monitor the impact of I164L on the efficacy of antifolates for intermittent preventive treatment of pregnant women and/or infants with falciparum malaria.

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EVALUATION OF *EX VIVO* DRUG SENSITIVITY FROM *PLASMODIUM FALCIPARUM*-INFECTED SENEGALESE PATIENTS

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The emergence of drug-resistant has marginalized the utility of many previously effective drugs, constant surveillance for the development and spread of drug resistance is therefore necessary. To this end, we have adapted a high-throughput non-radioactive assay to evaluate the *ex vivo* efficacy of a series of clinically relevant antimalarial agents. Here we present broad drug efficacy profiles for chloroquine, quinine, pyrimethamine, amodiaquine, and artemisinin with respect to *P. falciparum* parasites derived from 44 Senegalese patients. Chloroquine and amodiaquine are thought to share a common molecular target and thus, we hypothesized that parasite sensitivity to these drugs would be highly concordant. Patients were enrolled from those presenting at healthcare facilities in Thies. Parasite proliferation was correlated with DNA content by staining with 4′,6-diamidino-2-phenylindole (DAPI) in 96-well plates. Broad drug sensitivity (i.e. sensitive, resistant, or very resistant) was evaluated using a series of eight duplicate drug concentrations. The half-maximal inhibitory concentration of chloroquine and amodiaquine was calculated using a dose-effect curve based upon a series of 24 duplicate drug concentrations. 252 patients with a thick smear indicative of infection by *P. falciparum* were enrolled in the study. 44 patient samples were broadly evaluated for *ex vivo* sensitivity to five clinically approved antimalarial agents. 31.8% of these isolates were resistant to chloroquine, 4.5% to amodiaquine, 22.7% to quinine, 47.7% to pyrimethamine, and 31.8% to artemisinin. Although a number of isolates demonstrated cross-resistance to unrelated compounds, we did not observe any clear cross-resistance between chloroquine and amodiaquine. A significant number of parasite isolates were found to be resistant to pyrimethamine, chloroquine, and quinine; however, remarkably little resistance was seen with regard to amodiaquine. Most troubling was the higher than expected resistance to artemisinin that was observed. In Senegal, artemisinin is generally used in combination with amodiaquine and thus, the clinical effectiveness of the cocktail has not been breached due to the preserved efficacy of at least one compound in almost all of the parasite isolates that were examined.

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CHANGES IN MICRORNAS EXPRESSED BY HUMAN MACROPHAGES AS A RESULT OF *LEISHMANIA CHAGASI* INFECTION

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The *Leishmania* spp. are intracellular parasites of macrophages, which survive in part by suppressing macrophage microbicidal activity. Factors that trigger microbicidal suppression may be set very early after invasion of the parasite into the host environment. Eukaryotic organisms utilize short noncoding RNAs called microRNAs (miRs) to repress gene expression. Each miR can regulate as many as 100 genes. We hypothesized that miRs are upstream trigger(s) responsible for the global changes in macrophage gene expression induced by phagocytosis of *L. chagasi*. We also hypothesized that *Leishmania* manipulate host miRs as a means of converting the macrophage into an environment compatible with its own survival. Using data generated from our prior mRNA microarray, we used prediction programs to identify miRs that might down-regulate expression of human macrophage genes during *L. chagasi* infection. Real time PCR was used to evaluate whether these candidate miRs were changed upon *L. chagasi* infection of the human macrophage-like U937 cell line. MiRs-7, 200a and 141 showed dynamic changes in expression between 1 and 24 hrs post infection, while miRs-125a, 146a, 155, 199b and 320 were not altered. Using a multiplex TaqMan human microRNA array containing 365 miRs and 2 snoRNA internal controls, we examined changes in miRs expressed in control versus *L. chagasi* infected human monocyte derived macrophages between 0.5 and 24 hrs after infection. Most miRs were unchanged or not expressed. One half hour post infection, 6 miRs showed increased expression. By 1 hr, a different set of 5 miRs were either up or down regulated. By 24 hrs, 7 miRs were either increased or decreased. The most striking changes were after 4 hrs, where 17 miRs were suppressed. Among the miRs that were down-regulated were four members of the let-7 family, one of which decreased by 11-fold. Predicted targets of these miRs include IL10, IL6 and TGFBR1. These proteins are necessary for *L. chagasi* progressive infection, and suppression of the let-7 family may allow their translation. This is the first study to date that has examined miR alterations during infection of human cells with a Kinetoplastid protozoan. We hypothesize that miRs function to suppress or enhance host gene expression during *Leishmania* infection, and as such underlie the global changes in gene regulation caused by these organisms. Such studies may provide targets for future directed siRNA therapy.

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A NOVEL HIT-DOMAIN PROTEIN HYDROLYZES M7GPPPM662′A, WHICH IS A TRYPANOSOME-SPECIFIC HYPERMETHYLATED CAP STRUCTURE

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The RNA cap of eukaryotic mRNAs is essential for function. For example, the cap guanylate protects mRNA from 5′ exonucleases, and the cap guanine N7 methyl group (m7G) facilitates translation initiation. Trypanosomes are parasitic protozoa that share a hypermethylated cap structure distinct from that of other organisms. The trypanosome cap 4 (m7Gpppm662′Apm2′Apm2′Cpm32′U) contains a 5′,5′-triphosphate bridge that links the m7G-cap to the first four transcribed nucleotides (A1A2C3U4). All four nucleotides are ribose-methylated, and the N6 position of A1 and the N3 of U4 are base-methylated. We identified and functionally characterized a trypanosome-specific dinucleotide pyrophosphatase, HIT-45, that hydrolyzes the m7Gpppm662′A portion of cap 4. HIT-45 contains an essential histidine triad (HIT) domain. Recombinant and endogenous proteins from the African trypanosome Trypanosoma brucei were purified and analyzed for substrate specificity using various 5′-capped and uncapped oligonucleotides. HIT-45 hydrolyzed specifically the β-γ phosphoanhydride bond to release m7Gp and ppG from several related 5′-capped dinucleotides. Most interestingly, HIT-45 was most active and had a higher affinity for m7Gpppm662′A among the substrates tested. m7Gpppm662′A is unique to trypanosomes and may be an intermediate in the decay of cap 4-containing mRNA.

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METABOLIC PROFILING OF CO-INFECTION OF *TRYPANOSOMA BRUCEI BRUCEI* STRAINS IN MICE

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Multiple-species and multiple-strain co-infections are common in parasitic infections. Co-infections have an important epidemiological and evolutionary influence on both host and parasite and have been shown to either increase or decrease the infection severity depending on the parasite species and the metabolic status of the host. The aim of the current study was to examine the metabolic profiles of urine and fecal water from mice with either a single strain of *Trypanosoma brucei brucei* (STIB777AE-G1 or STIB246BA-R1) or both strains using nuclear magnetic resonance (NMR) spectroscopy coupled with multivariate data analysis methods and to assess the parasite-induced metabolic response of the mice. 1H NMR spectra were acquired from urine and fecal water samples collected from 5 control, 5 T. bb STIB777AE-G1 ('green')-infected, 5 T. bb STIB246BA-R1('red')-infected and 5 red and green co-infected NMRI female mice 1 day prior to the infection and 1, 3 and 4 days post infection. No changes were found in fecal water profile. Regardless of the strain, the T. bb infection caused a characteristic alteration in the spectral signatures of urine at day 4 post infection, which consisted of increased concentrations of 2-oxoisocaproate, D-3-hydroxybutyrate, lactate, 4-hydroxyphenylacetic acid, phenylpyruvate and 4-hydroxyphenylpyruvate and decreased levels of hippurate, guanidinoacetate. However, reduced levels of creatine and creatinine were only observed in the red strain and co-infected mice at day 4. Although there were no marked differences in the nature of the metabolic signature observed in the T. bb red, green or co-infected mice, animals infected with the T. bb green strain demonstrated slower metabolic response than those infected with either the red strain or the combined strains, which suggests that the red strain is more severe than the green one. This study illustrates the potential of metabolic profiling in exploring the metabolic consequences of co-infection in a host-parasite model.

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APPLICATION OF A BIOLUMINESCENT *LEISHMANIA MAJOR* IMAGING MODEL TO THE DEVELOPMENT OF A NOVEL KILLED BUT METABOLICALLY ACTIVE WHOLE CELL VACCINE

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Established methods for studying infection in the murine cutaneous leishmaniasis model include measuring lesion size using calipers as well as limiting dilution analysis or quantitative-PCR (qPCR) applied to tissue recovered at time of sacrifice. We have developed and validated an *in vivo* imaging model for *Leishmania major* infections in susceptible BALB/c and resistant C57BL6 mice, to be reported. This strain was then used to study a new vaccine technology called Killed But Metabolically Active (KBMA) leishmania vaccines. UVA treatment of psoralen-treated *Leishmania* promastigotes generates metabolically active parasites that are fated to die due to permanent DNA covalent crosslinking. We present an *in vivo* application of the bioluminescent *L. major* model to determine safe doses of a specific psoralen, amotosalen, in the development of a KBMA whole-cell vaccine for cutaneous leishmaniasis. In mice infected with 10^7 *L. major*-luc promastigotes treated with 100nM amotosalen or higher and 5.4 J/cm^2 of UVA, parasites demonstrated metabolic activity for two weeks as determined by bioluminescent imaging, but died thereafter. Using luminometry on cultured *L. major*-luc, an amotosalen dose of 750nM yielded greater than 7 logs of killing. Bioluminescent *Leishmania* offer a useful complement to existing methods of studying experimental *Leishmania* infection in mice and vaccine studies against leishmaniasis.

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PARASITOPHOROUS VACUOLES THAT HARBOR *LEISHMANIA* PARASITES INTERACT EXTENSIVELY WITH THE HOST ENDOPLASMIC RETICULUM

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Leishmania are intracellular parasites that preferentially invade mammalian professional phagocytes wherein they replicate in membrane-bound organelles called parasitophorous vacuoles (PVs). Although numerous studies have been performed to elucidate the composition and characteristics of PVs, there are still many unresolved questions about the biogenesis and maturation of these compartments. In this study we followed up on observations that had detected interactions of PVs with the host cell's endoplasmic reticulum. Several approaches were employed to explore the extent of interactions of PVs with the ER. First, an ER integral membrane molecule (calnexin) and ER membrane associated molecules (Sec22b and Syntaxin 18) were tagged with GFP and transfected into macrophages. Studies that evaluated the recruitment of these molecules to PVs that harbor *L. pifanoi* or *L. donovani* parasites revealed that more than 90% of PV membranes were positive for calnexin, Sec22b and Syntaxin 18 through out the course of a 24h infection. In contrast, less than 20% of Zymosan phagosomes had membranes that were positive for these ER markers over the same course. Second, in studies with the toxin, ricin, which traffics to the ER through a retrograde pathway in route to the cytosol, ricin was found to accumulate in the PV lumen. Ongoing experiments with other endogenous ER luminal proteins complement this finding. Third, electron microscopic evidence has also been obtained that shows continuity of PVs with ER. Taken together, these results suggest that the biogenesis and maturation of PVs involves extensive interactions with the host ER. The implication of this observation will be discussed.

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NEW INSIGHTS IN THE PATHOGENESIS OF *LEISHMANIA BRAZILIENSIS* INFECTION: ROLE OF TNF-α, IFN-γ AND IL-17

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The type 1 immune response plays a pivotal role in control of leishmania infection. Absence or decreasing in IFN-γ synthesis is associated with parasite dissemination leading to a picture of diffuse cutaneous leishmaniasis and visceral leishmaniasis. However, in cutaneous and mucosal leishmaniasis there is a very strong production of IFN-γ and TNF-α but progression from infection to disease is not controlled and pathology is associated with a strong inflammatory response and TNF-α production. IFN-γ and IL-17 are cytokines that induce TNF-α, but while IFN-γ is usually associated with protection against intracellular infections, IL-17 has been associated with pathogenesis of chronic inflammatory and auto-immune diseases. In this study IL-17 levels were determined in supernates of lymphocytes and mRNA expression for IL-17 was determined in peripheral blood and in tissue of cutaneous and mucosal leishmaniasis. Cytokines involved in the differentiation of T17 cells and in IL-17 production were also determined in supernates of lymphocyte cultures and the ability of cytokines in down modulated TNF-α and IL-17 production were tested. IL-17 levels were higher in supernates of ML than in CL and mRNA for IL-17 were expressed in tissue of these patients. While IL-1 and TGF-β enhanced IL-17 production, IL-6 and TGF-β do not increase IL-17. There was a direct

correlation between IFN-γ and TNF-α as well as between IL-17 and TNF-α production. However neutralization of IFN-γ and IL-17 did not decrease TNF-α synthesis. Moreover, IL-10 and IL-27 failed to down modulate *in vitro* TNF-α and IL-17 in cutaneous and mucosal leishmaniasis.

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TUBULIN-BASED SUBUNIT VACCINE CANDIDATES SHOW PROMISE IN ANIMAL STUDIES

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African trypanosomiasis is fatal to humans and animals if left untreated. Infected ruminants serve as reservoirs for transmission of the parasite to humans causing serious public health threats. High mortality in cattle reduces milk and meat production, which are important economic commodities in sub-Saharan Africa. No effective vaccine has been developed against this disease. The trypanosome continuously changes the dominant variable surface glycoprotein (VSG) antigens that cover nearly the entire surface of the parasite, making it obsolete for vaccine development. To overcome this obstacle, we identified non-variable antigens of the parasite that can generate protective immunity. Tubulin, one such candidate, was shown to confer protection in mice when animals were challenged with homologous or heterologous strains of *Trypanosoma*. We have engineered regions of α and β tubulin of *Trypanosoma brucei* as fusions with the coat protein of a plant virus, *Alfalfa mosaic virus* (AlMV) and produced them as virus particles. Plant-produced recombinant AlMV particles displaying target peptides from α or β tubulin stimulated protective immune responses in mice and significantly delayed the disease onset in cattle.

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THE EFFECT OF SNP VARIANTS IN THE 3'-UTR REGION OF *IL-5* ON GENE TRANSCRIPTION AND MRNA STABILITY AND THEIR ROLE IN SYMPTOMATIC INFECTION WITH *SCHISTOSOMA JAPONICUM*

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Previous genetic studies have indicated that human susceptibility to infection with other schistosome species is controlled by genes in the 5q31-33 region of the human genome. We undertook a nested case-control was to identify SNP associations with human susceptibility to Schistosoma japonicum infection targeting the *IL-4*, *IL-5* and *IL-13* gene cluster in the 5q31-33 region of the human genome. Thirty one tagged SNPs were selected spanning these genes and were genotyped in 133 subjects susceptible for symptomatic schistosome infection and an equal number of putatively resistant individuals using the Sequenom MassARRAY platform. Tests of association between susceptibility to symptomatic infection and individual SNPs and haplotypes identified two individual tagged SNPs in the 3'-UTR region of *IL-5* and one haplotype to be globally significant ($p<0.05$). Here we present a mechanistic study which was undertaken to identify the effect of these SNPs on IL-5 transcription and translation and their possible effect on mRNA stability. In addition we present data on the possible effect of these variants on human predisposition to asthma and atopy to identify the relationship between allergy and symptomatic infection with *Schistosoma japonicum*.

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ASSOCIATION OF THE GENE POLYMORPHISMS IFN-γ +874 AND IL-13 -1055 WITH PATTERNS OF REINFECTION WITH *SCHISTOSOMA MANSONI*

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The most agreed upon immunologic findings that correlate with resistance in human schistosomiasis are high levels of IgE and low levels of IgG4. Genes that encode cytokines associated with regulation of these isotypes contain gene and promoter polymorphisms linked to producing high and low levels of the cytokines in other systems, but have not been studied in schistosomiasis. We have genotyped a cohort of men occupationally exposed to *Schistosoma mansoni* in western Kenya in regard to cytokine polymorphisms and resistance and susceptibility to reinfection after treatment with praziquantel (PZQ). In this cohort, genotyping of polymorphisms in the IL-10 promoter (-1082/-819/-592), the TGF-β gene (10/25), and the TNF-α promoter (1308) did not yield correlations with either resistance or susceptibility to reinfection. However, genotypic polymorphisms in IL-4 (-590T high IgE), IL-13 (-1055T high producer) and IFN-γ (+874A high producer) have demonstrated several correlations. Resistance to reinfection was significantly correlated with the heterozygous genotypes IL-4 -590 C/T (RR 1.8, CI 1.15, 2.81 compared to T/T) and IL-13 -1055 C/T (RR 1.8, CI 1.13, 2.99 compared to C/C), and the homozygous IFN-γ +874 A/A genotype was significantly correlated with susceptibility to reinfection (RR 2.9, CI 1.2 3.8 compared to T/T). Analyses of these 3 loci together, using binomial regression and model comparison with Akaike's information criteria confirmed the correlations of the IFN-γ +874 A/A and IL-13 -1055 C/T loci with susceptibility and resistance, respectively, but found them to be independent of one another, not combinatorial. It is clear that these polymorphisms do not by themselves confer resistance or susceptibility, but we propose that they allow the phenotype to be expressed upon suitable immune exposure. Based on the literature, these polymorphisms contribute to the regulation of their respective cytokines, likely leading to downstream differences in the production of critical defense mechanisms.

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COMPARISON OF POTENTIALLY PROTECTIVE HUMAN TH2 RESPONSES AGAINST DIFFERENT SCHISTOSOME SPECIES

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Parasite specific IgE is associated with lower re-infection with all three major schistosome parasites of man. In *Schistosoma mansoni* infection, IgE responses to worm, including worm tegument antigen, and the tegumental antigen SmTAL1 (previously known as Sm22.6), are at increased levels post-treatment and these IgE levels post-treatment are associated with resistance to re-infection. Other correlates of resistance to re-infection include peripheral blood eosinophilia, and IL-5 and IL-4 cellular responses to parasite Ag *in vitro*. IL-5 is produced *in vitro* in response to worm antigen in adults before treatment but not children. This IL-5 production is correlated with worm-IgE 7wks post treatment; indicating that an individual's ability to mount a protective IgE response to released *S. mansoni* antigens is associated with pre-treatment IL-5 responsiveness. IL-5 is principle in proliferation, recruitment and survival of eosinophils, however, we have data that suggests that eosinophils themselves may be

an important cellular source of IL5. An important aim of our work is to link these correlates of immunity in treatment-reinfection studies, focusing on IgE, Th2 and regulatory cytokines and eosinophils. Here we report on and contrast these Th2 associated mechanisms and their control in three studies of comparable design in three differing ecological systems: a high *S. mansoni* transmission area on Lake Victoria in Uganda, a seasonal, lower *S. mansoni* transmission riverine area in Kenya and a high transmission *S. haematobium* area on the River Niger in Mali.

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THE ROLE OF HYGIENIC BATHING AFTER DEFECATION IN THE TRANSMISSION OF *SCHISTOSOMA MANSONI*

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Transmission of *Schistosoma mansoni* depends on fecal eggs reaching water, but the way this happens is poorly understood. We studied the role of hygienic bathing after defecation in the contamination of water with *S. mansoni* eggs. Individuals in an endemic community in Northern Senegal (N=991) were examined for *S. mansoni* infection and a random sample (22%) was interviewed about stool disposal practices and hygienic behavior. We assessed the presence and viability of *S. mansoni* eggs adhering to the peri-anal region of 13 infected volunteers, by counting the miracidia in the water they had used for hygienic washing; for 10 of them (77%) miracidia were demonstrated. From the population infection distribution, average number of defecations per day, proportion of individuals bathing after defecation, and association between miracidial counts and infection intensity, we calculated a daily population miracidial output of ~30,000 through hygienic bathing. For comparison, one complete stool reaching the water was calculated to yield ~2500 miracidia. Thus, 12 individuals in this population should defecate into the water every day to produce the same number of miracidia as through hygienic bathing. Our results suggest a major role of hygienic bathing after defecation in the transmission of *S. mansoni*.

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CYTOKINES PROFILES IN SPLEEN CELLS AND EXPRESSION IN HEPATIC GRANULOMAS BEFORE AND AFTER CHALLENGE WITH *SCHISTOSOMA MANSONI* IN C57BL/6 MICE VACCINATED WITH MICE AND HUMAN ANTI-IDIOTYPES

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Human anti-idiotypic vaccines (Human anti-Id) and mice IgG1 subclass monoclonal antibodies MAbs (Mice anti- Id MAb, M 3F9C and M 9F5D) in experimental schistosomiasis achieving varying degrees of resistance to challenge infection with *Schistosoma mansoni* cercariae. To further characterize the mechanisms involved in the induction of protective immunity associated with such vaccines model, mRNA transcripts for Th1 (IL-2 and INF-γ) and Th2 (IL-4, IL-5, IL-10 and IL-13) cytokines were assessed in the liver granulomas using the molecular technique of in situ hybridization and these cytokines were also measured from the supernatant of spleen cells cultured with SEA using ELISA technique before and after challenge with normal *S. mansoni* cercariae in mice vaccinated human anti-Id alone; Mice anti-Id alone and both of the two vaccines together. Vaccination of C57BL/6 mice with M anti-Id was resulted in ~10% to 18% protection and with H anti-Id resulted in ~31% to 36% in two experiments of resistance to infection. Spleens and livers were collected prior to challenge at weeks 6-post initial immunization and after challenge at weeks 7-12 post *S. mansoni* infection. The study showed that there is significant increase in Th1 cytokines and significant decrease in Th2 cytokines expression (in the liver granulomas) and production (from supernatant of splenocytes cultured with SEA) and also there is significant decrease of granulomas formation in mice vaccinated with Mice anti-Id. These results suggested that mice anti-Id monoclonal antibodies combined with human anti-Id could mimic at the T cell level the properties of a protective antigenic epitopes of the irradiated-cercariae vaccine with marked reduction of the immunopathology that resulted in liver granulomas formation and subsequent hepatic fibrosis.

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CIRCULATING CD23+ B CELL SUBSET LEVELS IN ADULTS WITH *SCHISTOSOMA MANSONI* INFECTIONS

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Resistance to human schistosomiasis is associated with high schistosome-specific IgE levels. The production of IgE in response to *Schistosoma mansoni* infection may be regulated in part by B cell surface receptors such as CD23. We recently found that the expression of membrane CD23 (mCD23) on B cells and higher levels of plasma soluble CD23 (sCD23), the cleaved form of mCD23, correlate with the development of resistance and other markers of resistance to re-infection, such as eosinophilia. Using flow cytometric analyses, we have also defined the distribution of CD23+ B cells and their levels of CD23 expression by examining persons with different *S. mansoni* exposure histories compared to uninfected persons, as well as how these levels change following praziquantel (PZQ) treatments. Expression of mCD23 on B cells was higher in un-exposed persons (82.35 ± 22.21; n = 18) and in those exposed to *S. mansoni* primarily as adults (69.27 ± 17.3; n = 26) than those exposed throughout their lives by being raised in fishing villages along Lake Victoria (53.83 ± 18.54; n = 18) [$P = 0.0219$], suggesting that long-term chronic schistosome infections may dampen the expression of CD23 on B cells. Isolated B cells from infected subjects did not respond to crude schistosome egg or adult worm antigens by alteration of their B cell mCD23 expression. Persons who were followed through several treatments with PZQ and reinfections (n = 24) were seen to slowly develop a gradual, statistically significant [$P = 0.04$], re-bound trend of increased CD23+ B cells over a 20-month period. B cells produce anti-schistosomal IgE, and in other settings are known to be both effective antigen presenting and immunoregulatory cells. Continued definition of B cells and their surface receptor molecules (antigen-specific and non-specific) from people with differing exposure and treatment histories is likely to be critical for a comprehensive understanding of potentially protective humoral responses during the development of resistance to reinfection by schistosomes.

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TREMATODE INDUCED CHANGES IN THE BRAIN METABOLIC PROFILE

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The metabolic effects of trematode infection on the brain were assessed in three trematode-rodent models using a 1H NMR spectroscopic based metabolic profiling strategy. The aim of this study was to describe the remote effect of a blood fluke (*Schistosoma mansoni),* a liver fluke (*Fasciola hepatica*) and the intestinal trematode *Echinostoma caproni* on the biochemical micro-environment of the brain. Metabolic profiling has been used for successful identification of disease biomarkers in previous experimental set-ups with these host-parasite models but focussing mainly

on biofluids and tissue, associated directly with the pathology. Here we show clear infection induced effects on the metabolic profile of the brains of mice and rats infected with *S. mansoni* and *F. hepatica*, respectively, whereas infection *with E. caproni* did not result in any perturbation of the biochemical profile of the brain. These findings are an important contribution to exposing the mechanism of neuro-schistosomiasis, and moreover they signify a completely new revelation in the case of non-ectopic influence of *F. hepatica* infection on the host central nervous system, and thus encourage further studies on the impact on parasitic infection on brain.

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SEASONALITY, WATER QUALITY VARIABILITY AND DIARRHEAL DISEASE IN NORTHERN COASTAL ECUADOR

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Seasonality plays a key role in determining incidence of infectious diseases. Diarrheal diseases in particular show seasonal trends, with bacterial pathogens usually peaking in warmer months and viral pathogens peaking in cooler, dryer months. However, studies of the impacts of water quality on diarrheal disease are usually undertaken cross-sectionally, over a short period of time. In this study, we explore how seasonality affects diarrheal disease incidence in a rural area of northern coastal Ecuador, using longer-term datasets. We use water quality data (as measured by E. coli counts) for both source and in-home water samples collected on a weekly basis over the course of one year in one village. We test the relationship between weekly variability in water quality and diarrheal disease incidence, water treatment and water storage practices in the home. We find that peaks in geometric mean values of microbial contamination of source waters often correspond to peaks in weekly village diarrhea incidence in the wet season, but not in the dry season. We also find that perceptions of villagers about water cleanliness do not correspond to levels of microbial contamination; people are more likely to treat their water in the dry season, whereas microbial contamination of source waters peaks in the wet season. We relate these findings to a broader analysis of the relationship between weekly rainfall and diarrheal disease incidence in 21 villages across a larger region over the course of five years. Our findings suggest that seasonal variability may be a key factor in the relationship between water quality and waterborne disease. A consideration of seasonality can help guide public health interventions, by targeting messages about water treatment to the times when people are most at risk for waterborne disease.

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SHIFTING PREVALENCE OF MAJOR DIARRHEAL PATHOGENS IN PATIENTS SEEKING HOSPITAL CARE DURING FLOODS IN 1998, 2004, AND 2007 IN DHAKA, BANGLADESH

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Bangladesh, a cholera endemic densely populated country located in the fertile Ganges-Brahmaputra Delta, is prone to flooding during seasonal monsoons. After the 2007 monsoon season, Bangladesh experienced severe flooding that led to diarrhea epidemics of extensive scale. We compared the clinical features and microbiological characteristics of patients from this recent flood (2007) with floods in 2004 and 1998 using the existing systemic surveillance system which monitors every 50th patient attending the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B). There was increased severity of dehydration from diarrheal illness in 2007 compared to the 2004 and 1998 floods ($p<0.001$). In 2007, the most prevalent diarrheal pathogens were *Vibrio cholerae* O1 (33%), rotavirus (12%), and enterotoxigenic Escherichia coli (ETEC) (11%). V. cholera is usually more prevalent during the floods; however, there was no significant difference between the 2007 flood (33%) and 2006 non-flood (35%) periods. Both *V. cholerae* O1 Ogawa (44%) and Inaba (49%) were isolated in 2007, while mainly the Ogawa serotype (98%) was isolated in 1998. Characteristics of ETEC strains also differed between the 2007 and 2004 floods; in 2007, 51% were LT producing ($p<0.001$ compared to 2004), 22% were ST ($p<0.001$), and 27% were ST/LT ($p=0.238$) producing ETEC. There was a shift of common colonization factor (CF) types expressed by ETEC; the major CF identified in 2007 was CS7 (20% in 2007 compared to 6% in 2004; $p=0.05$), while CFA/I, the prior predominant type, was identified less frequently (12% in 2007 versus 20% in 2004; $p=0.14$). In conclusion, our findings demonstrate alterations in the clinical and microbiological characteristics of major diarrheal-disease causing pathogens in Bangladesh during the recent 2007 flood. Surveillance of changing etiological agents of acute watery diarrhea, *V. cholerae* and ETEC, is important for successful public health interventions to prevent and control diarrhea related morbidity and mortality.

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SHIGA TOXIN GENE TYPES OF SHIGA TOXIN-PRODUCING *ESCHERICHIA COLI* (STEC) ISOLATED FROM PERUVIAN CHILDREN

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Shiga toxin-producing *Escherichia coli* (STEC) cause significant morbidity and mortality worldwide; however, they are not routinely sought as stool pathogens in many countries. The ability of STEC to cause disease is related to their capacity to secrete shiga toxin 1 (Stx1) and/or Stx2 and intimin (*eae*). The epidemiology of STEC infection is unknown in Peru. The aim of this study was to determine the distribution of virulence genes of STEC isolated from Peruvian children. Five *E. coli* colonies/patient were analyzed by a multiplex Real-Time PCR to identify the presence of *stx1*, *stx2* and *eae* in stool samples from children with and without diarrhea from three previous cohort studies in Peruvian children. All studies were in the community setting, 485 children (0-36m) were followed in 1987 in Huaraz-Ancash; 313 (6-18m) in 2004 in Villa El Salvador-Lima; and 1025 (0-12m) in 2007 in Chorrillos-Lima. We have analyzed 3231 samples (1200 from Huaraz, 556 from Villa El Salvador and 1475 from Chorrillos). STEC strains were detected in 0.7% (23/3231) of all samples, including 0.6% (13/2331) in diarrheal samples and 1.1% (10/900) in controls. Isolation rates were similar in all three studies. The median age of the children with an isolated STEC strain and diarrhea was 9m (4-21m) and 11m (1-32) in controls. Only one STEC sample was from a patient with bloody diarrhea. Among the isolated STEC strains, s*tx1* was the most common gene, present in 77% (10/13) of diarrhea cases and 70% (7/10) of controls; *stx2* was present in 23% (3/13) and 20% (2/10), and *eae* was present in 69% (9/13) and 50% (5/10), respectively. All *eae* genes were present only in *stx1*-STEC samples. Among diarrheal samples, STEC strains were detected throughout the year. In conclusion, STEC were isolated infrequently from Peruvian children with diarrhea. However, this study shows that these pathogens are present in the community in Peru, and therefore children could potentially develop more severe disease and complications associated with STEC infection. The majority of the STEC strains in the community setting were *stx1* and *eae* positive. The absence of haemolytic uremic syndrome may relate to the low frequency of stx2 positive strains.

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AGE-RELATED SUSCEPTIBILITY TO INFECTION WITH DIARRHEAGENIC *E. COLI*

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Diarrheagenic *E. coli* are being recognized as important childhood enteropathogens worldwide. However it is unclear whether there are differences in age-related susceptibility to infection, especially among infants. The purpose of this study was to determine the association of isolation of diarrheagenic *E. coli* with age and symptom status in Peruvian infants. We conducted a nested case controlled study in a cohort of 1025 children from 2 to 12 months of age in Lima, Peru. Stool samples from all diarrhea episodes that required medical attention were collected as well as samples from a randomly selected group of 40 children per month without diarrhea. Stools were analyzed for common enteric pathogens. Five *E. coli* colonies/patient were studied by a multiplex real-time PCR to identify: Enterotoxigenic (ETEC), Enteropathogenic (EPEC), Shiga toxin-producing (STEC), Enteroinvasive (EIEC), Enteroaggregative (EAEC), and Diffusely Adherent *E. coli* (DAEC). We have studied 1330 stool samples: 906 diarrhea and 424 controls; 41.8% (556) were from children < 6m and 58.2% (774) from children 6-12 month of age. The diarrheagenic *E. coli* were isolated in 29.6% (268/906) of diarrheal episodes and 32.8% (139/424) of control samples. The most commonly isolated pathogens in both groups were EAEC and EPEC. No significant differences were found in frequency of diarrheagenic *E. coli* as a group in children < 6m (OR:0.71, 95%CI [0.46-1.1]) or in children 6-12m (OR:0.97, 95%CI [0.71-1.32]). However, within the diarrheagenic *E. coli* pathotypes there was a significant association of DAEC with diarrhea in children 6-12m (OR:3.48, 95%CI [1.18- 10.27]) but not in children < 6m (OR:1.32, 95%CI [0.47- 3.67]). ETEC tended to be associated with diarrhea in children 6-12m, (OR:3.12, 95%CI [0.91-10.76]) more often than in children < 6m (OR:1.58, 95%CI [0.33- 7.44]). In conclusion, diarrheagenic *E. coli* were found with similar frequency in children with and without diarrhea, showing that infants are frequently exposed to these pathogens in this setting. DAEC was associated with diarrhea primarily in older infants. We speculate that the high prevalence of these pathogens in infants without diarrhea could be explained by protection through transplacental mechanisms and/or breastfeeding, which was 81.4% in the study population. We plan to follow the cohort in their second year of life, when most of these factors are no longer present.

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FACTORS ASSOCIATED WITH ORAL REHYDRATION THERAPY UTILIZATION FOR CHILDHOOD DIARRHEA MANAGEMENT AMONG PRIMARY HOUSEHOLD CAREGIVERS -- ASEMBO, KENYA 2007

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Diarrhea is a leading cause of death in Kenyan children <5 years old. Use of life saving oral rehydration therapy (ORT) for diarrhea treatment declined by 32% in Kenya from 1998 to 2003. We identified factors associated with ORT use by primary caregivers of children with diarrhea in Asembo, a rural area in western Kenya. Trained fieldworkers administered structured questionnaires and conducted in-depth interviews with caregivers of children <5 years old with diarrhea (≥3 stools/day) during the preceding 2 weeks, who were identified through active surveillance. ORT was defined as use of commercially-prepared oral rehydration solution (ORS), homemade sugar-salt solution, or increased fluids. We interviewed 371 caregivers using structured questionnaires; median age was 1.3 years for children and 28 years for caregivers. The majority (61%) used ORT during the recent illness. Factors associated with ORT use were care-seeking at a health facility (OR_{adj}=3.5, 95%CI=2.1-5.7), knowing how to prepare ORS (OR_{adj}=2.5, 95%CI=1.2-5.2), belief that herbs had no effect or were harmful (OR_{adj}=2.0, 95%CI 1.1-3.8), and belief that the child could have died from the recent diarrhea episode (OR_{adj}=1.8, 95%CI 1.0-3.0). We conducted 24 in-depth interviews. Caregivers reported that ORS could be reliably obtained at a health facility, but not from other sources; distance and cost present obstacles to reaching health facilities. Caregivers also reported lacking confidence in treating diarrhea at home and being reluctant to administer ORS without the recommendation of a health worker. In conclusion, in rural Asembo, health worker recommendations are important to caregivers' use of ORT for diarrhea treatment; however access to health facilities is limited by distance and cost. Reinforcing health workers' recommendation of ORT, providing accurate information on the role of ORT, empowering caregivers to use ORT upon diarrhea onset with or without health worker recommendation, and ensuring community availability of commercially-prepared ORS packets would improve household diarrhea management in rural Kenya.

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MANAGEMENT OF DIARRHEAL ILLNESS IN YOUNG CHILDREN OF RURAL WESTERN KENYA - FINDINGS FROM A HEALTH UTILIZATION AND ATTITUDES SURVEY, 2007

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Diarrheal illness is a major cause of childhood morbidity and mortality in rural western Kenya. We surveyed caretakers in western Kenya to determine how attitudes and practices concerning management of childhood diarrhea influenced severity of illness and health seeking behaviors. We randomly selected 1,425 households with a child in one of three age strata (<12 months, 12-23 months, and 24-59 months old) from the population of 20,000 children <5 years old in the Kenya Medical Research Institute/Centers for Disease Control and Prevention Demographic Surveillance System. In a cross-sectional survey we interviewed caretakers from the selected households about household characteristics, perceptions of illness, any recent diarrheal illness in the selected child, and health care utilization. Mild diarrhea was defined as ≥3 loose stools during a 24-hour period. Moderate-to-severe diarrhea was defined as diarrhea with at least one of the following: sunken eyes, wrinkled skin, intravenous rehydration, dysentery, or hospitalization. From April to May 2007, 1,049 caretakers of children <5 years old participated; 276 (26%) reported a child with diarrhea during the previous 2 weeks; 183 (66%) of diarrheal episodes were classified as moderate-to-severe diarrhea based on caretakers' report (39% were <12 months old, 39% 12-23 months old, and 22% 24-59 months old). Children with moderate-to-severe diarrhea were more likely to receive reduced amounts of food or fluids during illness than children with mild diarrhea (91% vs. 78%, p<0.05). Among those with moderate-to-severe diarrhea, 156

(85%) sought health care, including 97 (53%) who reached a licensed practitioner or clinic (formal health care). Children with moderate-to-severe diarrhea who reached formal health care were more likely to receive oral rehydration salts (74% vs. 13%, p<0.05), antibiotics (51% vs. 5%, p<0.05), intravenous fluids (18% vs. 3%, p<0.05), and to be hospitalized (17% vs. 0%, p<0.05) than children reaching informal care. Insufficient home-based fluid and nutritional management of children with diarrhea may have contributed to progression of illnesses to moderate-to-severe diarrhea. Access to formal health care resulted in differences in management which likely influenced outcome. Education of caretakers about proper food and drink intake at home and health care seeking during diarrheal illness is urgently needed.

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FACTORS ASSOCIATED WITH RECOMMENDATION OF ORAL REHYDRATION THERAPY FOR DIARRHEA TREATMENT AMONG HEALTH WORKERS IN KENYA, 2007

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Health workers (HWs) play a key role in community diarrhea case management. Diarrhea is a leading cause of death for children <5 years old in Kenya. Use of oral rehydration therapy (ORT), the recommended treatment for non-bloody diarrhea, has declined dramatically in recent years in Kenya. We conducted a cross-sectional survey of HWs in rural Asembo and Kibera, an urban slum, to assess ORT recommendation for diarrhea management. We defined ORT users as HWs who recommended ORT for both a hypothetical case of watery diarrhea with some dehydration, and for the HW's most recently treated case of watery or mucoid diarrhea, with some or no dehydration, according to WHO criteria for dehydration. We performed logistic regression to identify factors associated with ORT use. Of 333 HWs interviewed, 8% were clinicians (medical or clinical officer, nurse), 38% were herbalists (including traditional healers), and 35% were community health workers (CHWs). ORT was felt to be the most effective treatment for watery diarrhea by 64% of clinicians, 59% of CHWs, and 5% of herbalists,. ORT was recommended by 54% of HWs for the hypothetical case. ORT users were less likely to be herbalists than ORT non-users (OR_{adj}=0.1, 95% CI=0.03-0.4). ORT users were more likely than non-users to consider ORT among the five most effective treatments for watery diarrhea (OR_{adj}=4.5, 95% CI 1.3-15.3) and believe that dehydration was a potential complication of diarrhea (OR_{adj}=5.6, 95% CI 1.3-24.2). Among HWs other than herbalists, ORT recommendation was associated with being a clinician (OR_{adj}=5.4, 95% CI=1.1-27.8) and believing that ORT is among the most effective treatments for watery diarrhea (OR_{adj}=5.2, 95% CI=1.3-20.4). In conclusion, ORT recommendation for a patient with watery diarrhea was infrequent among Kenyan HWs, particularly herbalists. Interventions to raise HW awareness of ORT effectiveness may increase ORT utilization and thereby improve community management of childhood diarrhea; interventions should target clinicians, herbalists, and CHWs, all of whom provide guidance to caregivers of ill children in Kenya.

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EXACERBATION OF ANEMIA IN *PLASMODIUM FALCIPARUM* MALARIA AND GRAM NEGATIVE BACTEREMIA CO-INFECTED CHILDREN IS ASSOCIATED WITH ELEVATED INFLAMMATORY MEDIATORS

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Although blood-borne bacterial infections are known to enhance malarial disease severity, the role of inflammatory mediators in conditioning disease outcomes has not been reported. We previously have shown that children with malaria and Gram[-] bacteremia co-infection (n=24) had significantly lower hemoglobin (P=0.009) and parasitemia levels (P<0.001), compared to either Gram[+] co-infected children (n=17) or those with malaria mono-infection (n=407). Since a pro-inflammatory milieu is known to significantly affect erythropoiesis, a bead-based Multiplex assay was used to measure 25 soluble mediators in these three groups of children. Study participants included children less than four years of age from a holoendemic *Plasmodium falciparum* transmission area and were HIV-1 negative. Multiplex analyses revealed significant increases in IL-1β (P=0.011), IL-1Ra (P=0.030), and IL-2 (P=0.034) in Gram[-] co-infected children compared to the malaria-only group. Gram[-] co-infected children also had significantly higher levels of inflammatory mediators known to affect erythropoiesis, such as IFN-γ (P=0.008), IL-15 (P<0.001), and MIG (P=0.006). In addition, both Gram[-] and Gram[+] co-infected children had significantly higher levels of IFN-α (P=0.025 and P=0.001), IL-4 (P<0.001 and P<0.001), IL-5 (P=0.002 and P=0.001), IL-7 (P=0.001 and P<0.001), and IL-12 (P=0.003 and P=0.016) relative to the malaria mono-infected group. However, IL-6 (P=0.009), and IL-10 (P=0.004) were significantly lower in the Gram[+] co-infected group relative to the malaria mono-infected group, while TNF-α was decreased in both co-infected groups relative to malaria alone. Taken together, results presented here show that acute inflammatory mediators (IL-1β, IL-1Ra, and IL-2), as well as those known to effect erythropoiesis (IFN-γ, IL-15, and MIG), are uniquely associated with Gram[-] bacteremia and malaria co-infection, and may directly and/or indirectly, exacerbate anemia seen in these children.

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MARKED DECLINE IN CHILDHOOD MORTALITY IN THE WESTERN KENYA DSS: EVIDENCE FROM LONGITUDINAL DATA, 2003-2007

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For over a decade, the high child mortality rates in western Kenya declined only modestly, from 257 deaths among children <5 years per 1,000 live births in the early 1990s to 227 deaths per 1,000 live births in 2002. Recent data, however, have shown a remarkable decline in both infant and under-five mortality since 2003. We investigated yearly changes in all-cause and cause-specific child mortality from 2003 to 2007. We used data on births, deaths, verbal autopsy, and duration of residence from the KEMRI/CDC Demographic Surveillance System (DSS) from 2003 to 2007 and life table techniques to compute annual infant death rates, under-five survival probabilities, and life expectancy at birth. We then used the period rates to assess trends in all-cause and cause-specific mortality. The overall infant mortality rate for the area dropped from 131 to 74 deaths per 1,000 in 2007, a 43% drop over five years. In the same years under-5

mortality dropped from 239 to 137 deaths per 1,000 live births. Between 2003 and 2007, deaths per 100,000 live births declined from 26 to 19 in neonates and from 105 to 58 in the post-neonatal period. Consequently, life expectancy at birth increased from 38 years (36 for males and 40 for females) in 2003 to 49 years (48 for males and 51 for females) in 2007. Although verbal autopsy data showed malaria and pneumonia remained leading causes of death among children age 1-59 months throughout these years, there was a decline of 16% and 58% in the cause-specific rates of mortality for these diseases, respectively. There was however 23% increase in the cause-specific mortality rates for neonatal sepsis, the leading cause of neonatal mortality, from 11 to 13 deaths per 1,000 in the period under review. In conclusion, child mortality decreased markedly and significantly between 2003 and 2007, mostly due to a decline in deaths associated with pneumonia and malaria. Further analysis is ongoing to assess factors contributing to this decline.

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AN OPERATIONAL MALARIA OUTBREAK IDENTIFICATION AND RESPONSE SYSTEM IN MPUMALANGA PROVINCE, SOUTH AFRICA

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Focal increases in malaria cases often preceding an epidemic by weeks and early detection of these increases is advocated by WHO. A simple system was developed in the low malaria transmission province of Mpumalanga Province, South Africa, for early identification of malaria outbreaks and guiding integrated public health responses. Using five years of case notification data two binomial-thresholds were determined for each primary health care facility in the highest risk malaria area of Mpumalanga province. When thresholds were exceeded at health facility level, health staff notified the malaria control programme, which then confirmed adequate malaria treatment stocks to manage potential increased cases. Cases in these areas were followed up at household level to determine source of infection and review thresholds at village/ town level to determine whether additional response measures were required. Binomial outbreak threshold performance was evaluated against other currently recommended thresholds using retrospective data. Its acceptability at primary health care level was evaluated through structured interviews with health facility staff. Ninety-two percent of health facilities reported outbreaks within 48 hours with an appropriate response achieved within 24 hours. When compared to other epidemiological systems for a specified malaria season the binomial thresholds produced one false weekly outbreak, while the cumulative-sum, twelve and the mean plus two times the standard deviation methods produced nine. In conclusion, the malaria outbreak surveillance system using binomial thresholds achieved its primary goal of identifying outbreaks early, facilitating appropriate local public health responses aimed at averting a possible large-scale epidemic.

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A PHASE 2, OPEN LABEL, NON-COMPARATIVE TRIAL OF AZITHROMYCIN 2G PLUS CHLOROQUINE 600 MG BASE DAILY FOR THREE DAYS FOR THE TREATMENT OF UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA

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This study was undertaken to evaluate the efficacy and safety of a 3-day regimen of 2 g azithromycin (AZ) plus 600 mg chloroquine (CQ) base (AZCQ) in the treatment of symptomatic uncomplicated *P. falciparum* malaria in adults in Tumaco, Colombia and Goa, India. A 1 g AZ plus 600 mg CQ 3-day regimen had earlier demonstrated 64% and 71% efficacy at these sites (day 28) respectively. After obtaining informed consent, adults with fever, a positive peripheral blood smear and a positive rapid diagnostic test for *P. falciparum* were administered AZCQ regimen for 3 days. All subjects were hospitalized and monitored closely for a minimum of 3 days, until 3 consecutive smears were negative for asexual *P. falciparum* parasitemia and until the investigator deemed discharge was appropriate. After discharge, patients were evaluated weekly from Day 7 through Day 42. The primary efficacy endpoint was the Day 28 asexual *P. falciparum* parasitological clearance (PCR corrected). A total of 110 subjects were assigned to and completed the 3-day AZCQ treatment. AZCQ was generally well tolerated. There were no deaths, serious or unexpected adverse events (AE) or discontinuations due to AEs. One subject at Goa reported a severe AE of vomiting on the first day of dosing. Most AEs were gastrointestinal and mild in severity. A total of 109 subjects were evaluable for the Day 28 primary endpoint analysis. There were no Early Treatment Failures. At Tumaco, 53 of 55 evaluable subjects (96.4%) cleared parasitemia at the Day 28 primary endpoint (PCR-corrected); 2 subjects had recrudescence, 1 on Day 27 and 1 on Day 28. At Goa, 51 of 54 evaluable subjects (94.4%) cleared parasitemia at the Day 28 (PCR uncorrected); 3 subjects had recurrence of parasitemia, 1 on Day 19 and 2 on Day 21. PCR data to differentiate recrudescence from reinfection for Goa site is pending and will be presented at the conference. In conclusion, a 3-day regimen of 2g AZ plus 600 mg CQ was safe, well-tolerated and effective in the treatment of acute uncomplicated falciparum malaria in adults in Tumaco, Colombia and Goa, India.

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EPIDEMIOLOGY OF IMPORTED MALARIA IN HOUSTON CHILDREN: 1994-2007

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Imported malaria occurs in US children despite availability of chemoprophylaxis. Failure to receive malaria prophylaxis contributes to the burden of imported pediatric malaria. We sought to describe the epidemiology of pediatric malaria in Houston and to determine the extent to which failure to receive prophylaxis contributes to malaria acquisition in children. A review was conducted of children 0-17 yrs of age evaluated from 1994 through 2007 at 4 referral centers (Texas Children's, Ben Taub General, Lyndon B. Johnson and Hermann Children's Hospitals). Children were identified by Infectious Disease service record and ICD-9 code review. Malaria diagnosis was by positive smear. Data were analyzed using STATA 9. There were 100 children with malaria with a median age of 7 yrs (range 19 days to 17 yrs). A mean of 7 cases were diagnosed yearly (range 3 - 13). Of 67 children for whom country of birth was specified, 28 (42%) were US born. Of all children, 39% had moved to Houston from malaria-endemic regions and 61% were known to or presumed to have traveled

to endemic regions. Species were *Plasmodium falciparum* (53%), *P. vivax* (36%), *P. malariae* (1%), *P. ovale* (1%), mixed (4%), non-falciparum (1%) and unknown (4%). Of 61 travelers, 87% acquired malaria in West Africa. Cases peaked in January (15%) and July/August (34%). Prophylaxis was documented in 52% of 49 children traveling from the US but was appropriate to region in only 25% of 28 with regimen known. Severe malaria, manifesting primarily as hyperparasitemia, comprised 20% of cases. 2 infants had congenital malaria. There were no deaths; 1 child relapsed with *P. vivax*. Appropriate prophylaxis did not differ for children with severe vs. uncomplicated malaria (p=0.38). In conclusion, malaria occurs in both native and foreign-born children in Houston and disease often is severe. A majority of children have not received appropriate prophylaxis, suggesting that reduced morbidity can be achieved by effective chemoprophylaxis, particulary through targeting travelers to West Africa.

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PROSPECTIVE ANALYSIS OF HOSPITAL ADMISSIONS, DIAGNOSIS, DISEASE AND OUTCOMES FOR MALARIA IN JAYAPURA, PAPUA, INDONESIA

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We describe a prospective study of patients admitted with malaria at a large hospital at Jayapura in northeastern Papua, Indonesia. We have analyzed 3 months of enrolled subjects totaling 104 admissions. The proportion of subjects admitted with a PCR-confirmed diagnosis of *Plasmodium falciparum* was 61% (63), 17% (18) by *P. vivax*, and 22% (23) by both species. The proportions of subjects classified as having severe disease among admissions was 65% for *P. falciparum*, 83% for *P. vivax*, and 61% for mixed infections. The dominant basis of classification to severe disease was hyperparasitemia for all infections, accounting for 85%, 80% and 86% of severe cases, respectively. Severe anemia (<6 or <7g/dL for children and adults), a reportedly dominant feature of severe malaria in other reports from the region, proved surprisingly rare, appearing in only 3(7%) of the severe falciparum cases, none of the vivax cases, and 1(7%) of the mixed infection cases. Severe hypoglycemia (<70mg glucose/dL) appeared in 6(14%), 6(40%), and 2(9%) of falciparum, vivax and mixed species malarias, respectively. Severe jaundice (>3mg bilirubin/dL) appeared in 6(14%), 2(13%) and 1(5%) of PF, PV and mixed infections. Evidence of renal failure (<3mg creatinine/dL) appeared in only 2 (5%) patients with falciparum malaria. Respiratory distress appeared in only 1 patient with vivax malaria, and seizures were noted in 3 patients with falciparum malaria, 1 with vivax malaria and none with mixed species. Patients with vivax mono-infection more often presented with more than one criteria of severe disease (40% vs. 25% and 22%).

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A RANDOMISED TRIAL OF AN EIGHT-WEEK, ONCE WEEKLY PRIMAQUINE REGIMEN TO PREVENT RELAPSE OF *PLASMODIUM VIVAX* IN PAKISTAN

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Vivax malaria remains a major cause of morbidity in the subtropics. Undermining the stability of the disease requires drugs that prevent relapse and provide reservoir reduction. A 14-day course of primaquine (PQ) is effective but cannot safely be used in routine practice because of its interaction with glucose-6-phosphate dehydrogenase (G6PD) deficiency for which testing is seldom available. Safe and effective use of PQ without the need for G6PD testing would be ideal. The efficacy and safety of an 8-week, once weekly PQ regimen was compared with current standard treatment (chloroquine alone) and a 14-day PQ regimen. 200 microscopically confirmed *Plasmodium vivax* patients were randomly assigned to either once weekly 8-week PQ (0.75mg/kg/week), once weekly 8-week placebo, or 14-day PQ (0.5mg/kg/day) in North West Frontier Province, Pakistan. All patients were treated with a standard chloroquine dose and tested for G6PD deficiency. Deficient patients were assigned to the 8-week PQ group. Failure was defined as any subsequent episode of vivax malaria over 11 months of observation. There were 22/71 (31.0%) failures in the placebo group and 1/55 (1.8%) and 4/75 (5.1%) failures in the 14-day and 8-week PQ groups respectively. Adjusted odds ratios were: for 8-week PQ vs. placebo - 0.05 (95%CI: 0.01-0.2, p<0.001) and for 14-day PQ vs. placebo - 0.01 (95%CI: 0.002-0.1, p<0.001). Analysis restricted to failures occurring in the 9 month post-treatment period confirmed that the 8-week regimen was superior to current treatment. Only one G6PD deficient patient presented. There were no serious adverse events. In conclusion, a practical radical treatment for vivax malaria is essential for control and elimination of the disease. The 8-week PQ course is more effective at preventing relapse than current treatment with chloroquine alone. Widespread use of the 8-week regimen could make an important contribution to reservoir reduction or regional elimination where G6PD testing is not available.

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BASOPHILS AND IGE AMPLIFY THE IMMUNE RESPONSE TOWARDS *LITOMOSOIDES SIGMODONTIS*

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Filariae induce a type 2 immune response characterized by eosinophilia, elevated serum levels of Ag-specific and polyclonal IgE, and increased T-cell production of type 2 cytokines. In this study, we tested the hypothesis that IgE-mediated basophil activation and subsequent IL-4 release amplifies type 2 responses in filarial infections once Ag-specific IgE is present. To accomplish this, normal and IgE-depleted BALB/c mice were infected with *Litomosoides sigmodontis* (Ls) and assessed at various time points for serum polyclonal and Ag-specific IgE levels, frequencies of activated and IL-4 releasing basophils in blood, circulating eosinophil and basophil counts, splenocyte cytokine production, and splenocyte proliferation in response to Ls Ag. Timecourse studies in infected mice show that Ls Ag-driven basophil release of IL-4 occurs once circulating Ls Ag-specific IgE is present and is shortly followed by amplification of circulating eosinophils. Both the peaks of circulating eosinophils and basophils as well as the highest frequencies of basophil activation occurred at 8 weeks p.i., when circulating microfilariae appear. Interestingly, basophil activation becomes substantially attenuated after 10 weeks p.i., even though Ls Ag-specific IgE levels are maximal at that time point, suggesting that IgE-mediated responses become downregulated during chronic infection. Treatment of Ls-infected mice with anti-IgE decreased circulating IgE levels below the level of detection by ELISA and resulted in elimination of splenic IgE-producing B cells as detected by ELISPOT. While depletion of IgE did not affect worm burdens or microfilaria counts, it did result in decreased numbers of circulating basophils and eosinophils, and in decreased LsAg-driven splenocyte proliferation. In conclusion, basophil activation and release of IL-4 begin when levels of helminth-specific IgE are detectable, become maximal at time of initial microfilaria production, and then become attenuated late in the course of chronic filarial infection. Additionally, IgE, possibly through IgE-mediated activation of basophils, serves to amplify basophilia, eosinophilia, and parasite-antigen driven cellular proliferation during filarial infection.

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INDUCTION OF TRAIL- AND TNF-α-DEPENDENT APOPTOTIC CELL DEATH IN HUMAN MONOCYTE-DERIVED DENDRITIC CELLS BY *BRUGIA MALAYI*

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Dysregulation of professional antigen-presenting cells has been postulated as a major mechanism underlying antigen-specific T cell hyporesponsiveness in patients with patent filarial infection. To address the nature of this dysregulation, dendritic cells (DC) and macrophages (MΦ) generated from elutriated monocytes were exposed to live microfilariae (mf), the parasite stage that circulates in blood and is responsible for most immune dysregulation n in filarial infections. DC exposed to mf for 24-96 hours showed a marked increase in cell death ($p = 0.008$) and caspase-positive cells compared with unexposed DC, while mf exposure did not induce apoptosis in MΦ. Interestingly, 48 h exposure of DC to mf induced mRNA expression of the pro-apoptotic gene TRAIL and both mRNA and protein expression of tumor necrosis factor (TNF)-α. Monoclonal antibodies to TRAIL-R2, TNF-R1, or TNF-α partially reversed mf-induced cell death in DC, as did knocking down the receptor for TRAIL-R2 using small interfering RNA. Mf also induced gene expression of BH3-interacting domain death agonist (Bid) and protein expression of cytochrome *c* in DC; mf-induced cleavage of Bid could be shown to induce release of cytochrome *c*, leading to activation of caspase 9. Our data suggest that mf induce DC apoptosis in a TRAIL- and TNF-α-dependent fashion.

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ANTI-*WOLBACHIA* ANTIBODIES MAY DECREASE THE LIKELIHOOD OF ACUTE ADENOLYMPHANGITIS IN LYMPHATIC FILARIASIS

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In addition to elephantiasis and hydrocoele, acute adenolymphangitis (ADL) characterized by transient swelling and pain of a limb or male genitalia with fever is a significant cause of morbidity in persons with lymphatic filariasis (LF). The pathogenesis of ADL is not well understood but the filarial endosymbiont bacterium *Wolbachia* has been implicated its development. This study aims to examine how human adaptive immunity to *Wolbachia* relates to development of ADL. A cohort of 106 individuals was identified from a database that describes the impact of Mass Drug Administration (MDA) on transmission, infection and disease attributable to LF in Papua New Guinea. Complete survey data and available longitudinal blood samples were the criteria for inclusion. ELISA using recombinant *Wolbachia* surface protein (wsp) was used to measure anti-*Wolbachia* IgG antibodies. Antibody levels from individuals with episodes of ADL (ADL group, N=32) observed over a 5-year period were compared with those with no ADL (no ADL group, N=74). Age, sex, microfilaremia and chronic disease did not significantly differ between groups. Filarial antigenemia was higher in the ADL group (p=0.0027). Antibody levels for the ADL group were lower (median=20.95 units, p=0.0024 by Kruskal-Wallis test) than the no ADL group (median=39.82 units) in the year preceding treatment. Antibody levels did not differ between the groups after MDA-mediated reduction in transmission, and increased in both after the first and subsequent treatments with MDA (mean difference=17.00, p=0.036 by paired t-test). In conclusion, individuals with higher anti-*Wolbachia* wsp antibody levels have a lower risk of developing LF-associated acute disease. Treatment with MDA results in a significantly higher antibody titer over time. These findings support an association between the pathogenesis of acute LF and adaptive immune responses to *Wolbachia*.

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FILARIAL LYMPHATIC PATHOLOGY IS CHARACTERIZED BY AUGMENTED PRO-INFLAMMATORY CYTOKINE PRODUCTION IN RESPONSE TO TLR2 AND TLR9 LIGANDS

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Lymphatic filariasis can be associated with the development of serious pathology in the form of lymphedema, hydrocele and elephantiasis in a subset of infected patients. Toll-like receptors (TLRs) are thought play a major role in the development of filarial pathology. To elucidate the role of TLRs in the development of lymphatic pathology, we have examined the cytokine responses to different Toll-ligands in individuals with filarial pathology [CP] (n=11) and compared them to asymptomatic, infected individuals [INF] (n=11) or uninfected, normal individuals [EN] (n=11). The ligand for TLR2 - the synthetic lipoprotein, Pam3CSK4 induced significantly elevated production of the prototypical Th1-type cytokines - IFNγ and TNFα; and the Th1 inducing cytokine - IL-12; but not Th17 cytokines in CP patients in comparison to both INF ($p = 0.0256$ for IFNg; $p = 0.0005$ for TNFα and $p = 0.0004$ for IL-12) and EN ($p = 0.0058$ for IFNγ; $p = 0.0086$ for TNFα and $p = 0.0001$ for IL-12). Similarly, a different ligand for TLR2 - heat killed *Listeria monocytogenes* (HKLM) also induced significantly elevated production of IFNγ, TNFα and IL-12 in CP patients in comparison to both INF ($p = 0.0181$ for IFNγ; $p = 0.0418$ for TNFα and $p = 0.0181$ for IL-12) and EN ($p = 0.0025$ for IFNγ; $p = 0.00048$ for TNFα and $p = 0.0151$ for IL-12). The ligand for TLR9, CpG oligonucleotides (ODN2006), on the other hand, induced significant increased production of Th1 and Th1 inducing cytokines (IFNγ, TNFα and IL-12) as well as Th17 and Th17 inducing cytokines (IL-17, IL-23, IL-1b and IL-6) - in CP patients in comparison to both INF ($p = 0.0086$ for IFNγ; $p = 0.0126$ for TNFα; $p = 0.0008$ for IL-12; $p = 0.0151$ for IL-17; $p = 0.0086$ for IL-23; $p = 0.0151$ for IL-1b and $p = 0.0418$ for IL-6) and EN ($p = 0.0002$ for IFNγ; $p = 0.0010$ for TNFα; $p = 0.0005$ for IL-12; $p = 0.0002$ for IL-17; $p = 0.0002$ for IL-23; $p = 0.0302$ for IL-1b and $p = 0.0418$ for IL-6). Cytokine responses to the other Toll ligands - TLR3 ligand (PolyIC), TLR4 ligand (LPS), TLR5 ligand (Flagellin) and TLR6 ligand (FSL-1) - were not significantly different between the three groups. Our data therefore, strongly suggests an important role for TLR2 and TLR9 mediated pro-inflammatory cytokine induction in the development of pathology in human lymphatic filariasis.

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ELEVATED PLASMA ANGIOGENIC AND LYMPHANGIOGENIC FACTORS ARE ASSOCIATED WITH INFECTION PER SE RATHER THAN CLINICALLY APPARENT DISEASE IN HUMAN FILARIAL INFECTION

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Lymphatic dilatation, tortuosity and secondary dysfunction is a hallmark of patent lymphatic filariasis (LF), even in those with subclinical infection associated with microfilaremia (MF). Lymphangiogenesis occurs often as sequelae to this lymphatic pathology presumably in response to lymphedema when collateralization of lymphatics occurs. This process is mediated by the vascular endothelial growth factors-C and -D (VEGF-C and VEGF-D) that can interact with the lymphatic endothelial cell (LEC)-

specific receptor VEGFR-3. These factors control the assembly of tubes and functional vessels. Previously, we have shown that LECs when stimulated in-vitro with filarial antigens induce proliferation and tube formation, associated with release of pro angiogenic factors. To assess their role in filarial infection, we assayed a number of pro-angiogenic factors in plasma of well characterized patients from filaria-endemic populations. We then assessed the relevance and contribution of these factors to filarial disease. When compared with endemic controls (EN, N=36), MF individuals (N=36) or those with lymphedema or elephantiasis (CP, N=36) had significantly elevated levels of VEGF-A (Geometric Mean [GM] 82 vs 124 vs 171 pg/ml $p<0.0001$), VEGF-C (GM) 96 vs 138 vs 158 pg/ml; $p<0.0001$) and VEGF-D ($p<0.007$). However, no differences were detected in the levels of soluble VEGFR-1 levels among the 3 groups. The levels of the pro-angiogenic molecules Ang-1 and Ang-2 were also significantly higher in the MF and CP individuals compared to the EN ($p<0.0001$), as were the associated molecules PlGF ($p<0.04$) and b-FGF ($p<0.003$). Correlations between the levels of VEGF-C and other markers proved to be highly significant. Interestingly, the levels of b-FGF (associated with fibrosis) were even more significantly elevated in the CP group when compared to those with MF (GM 15 vs 5 pg ; $p<0.007$). To assess the role of Wolbachia in the induction of these pro-angiogenic factors, plasma from mf+ patients with Loa loa (no Wolbachia, N=36) were compared to mf+ patients with *Wucheria bancrofti* There was no difference in any of these factors between the two infected groups, suggesting a minimal role if any for the endosymbiont Wolbachia. Our data support the notion that lymphatic filarial infection is associated with elevated levels of lymphangiogenic (and angiogenic) factors but only a few may be mediating the serious pathologic consequences of LF.

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INCREASED IMMUNE STIMULATION AFTER MACROFILARICIDAL THERAPY

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More than 150 million humans in tropical countries are infected by filarial nematodes which harbor intracellular bacterial endosymbionts of the genus Wolbachia (Rickettsiales). As previously shown, depletion of Wolbachia leads to a higher percentage of adult worm death compared to ivermectin, which is primarily microfilaricidal. This study examined the development of *Onchocerca volvulus*-specific immune responses before and after antiwolbachial treatment in onchocerciasis patients in Ghana, West Africa. We provide evidence that cellular immune responses increase after a 6-weeks treatment course with doxycycline. In detail, levels of IL-5 in whole blood assays after stimulation with *O. volvulus* crude extract were elevated after macrofilaricidal therapy compared to microfilaricidal treatment. This was underscored by a higher IL-5/IL-10 ratio as observed locally at the mRNA level in onchocercomas from doxycycline treated patients. Interestingly, patients with doxycycline therapy displayed significantly fewer new onchocercomas after 14 months than those treated with ivermectin. These data are in line with the current hypothesis that macrofilaricidal therapy removes the source of immunosuppressive molecules.

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A *LOA*/BABOON MODEL FOR INVESTIGATING THE MECHANISMS OF ENCEPHALOPATHY FOLLOWING IVERMECTIN ADMINISTRATION

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An experimental model using baboons was developed in investigate the pathogenesis of *Loa*-associated encephalopathy following ivermectin treatment in humans. Eighteen baboons were splenectomised and each infected with 600 infective larvae of Loa loa (human strains) and parasitological, haematological, immunological and biochemical indicators monitored. Twelve animals with microfilariae loads > 30000mf/ml of blood were divided into four experimental groups: a control group not receiving drug, a second group receiving only ivermectin only, a third group receiving ivermectin and aspirin, and lastly a group given ivermectin and corticosteroid. Monitoring of animals for side effects took place every 6 hours post treatment until autopsy. Pre and post treatment data were collected for parasitological, biochemical, haematological and immunological assessment. At the autopsy, organs were collected for histological studies. Close to 80% of the baboons develop microfilarial loads greater than 30000mf/ml of blood with 40% of them having very high microfilarial load (> 100000mf/ml of blood). The duration of patency was between 5 to 6 months. Eosinophils and γ-GT levels showed a positive correlation with the microfilarial loads. However, the white blood cell, red blood cell and lymphocyte levels were not affected by the infection. Haemoglobin levels increased in all animals. Creatinine levels were also significantly increased and SGPT, SGOT and glucose levels varied during the course of infection. There was a significant drop in microfilaeremia counts in the animals post treatment with ivermectin ($p<0.005$) while no significant drop was observed in control animals. Microfilariae were also present in the cerebrospinal fluid, peritoneal and pericardial fluids and bone marrow, most of the time after treatment with ivermectin. One animal died 5 hours after ivermectin administration and the other treated animals all recorded clinical manifestations such as rashes, itching, diarrhoea, haemorrhages in various organs (including the brain), lymph node enlagrement, dizziness and restlessness. A wide range of tissues and body fluids were collected at autopsy from all animals and processed for histopathology. The findings to date will be interpreted in terms of the mechanisms of encephalopathy following ivermectin treatment of high microfilaraemic patients.

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DENGUE AND THE DEMOGRAPHIC TRANSITION

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An increase in the average age of individuals experiencing dengue hemorrhagic fever (DHF) has been reported from several settings across Southeast Asia. The cause of this increase is not known. We propose that a shift in demographics in Thailand toward lower birth rates and death rates has brought about a reduction in transmission of dengue and a lengthening of the interval between large epidemics. To test this hypothesis, we estimate the force of infection (the per capita acquisition of infection in the population) using the full age distribution of cases from 72 provinces of Thailand for the last twenty years and associate it with age structure, birth rates, climatic and socioeconomic indices over this

period. We find that the force of infection has declined roughly linearly over the last twenty five years by approximately 2% each year since a peak in the late 1970's and early 1980's. The strongest predictor of the change in force of infection is the median age of the population, with provinces that have experienced greater increases in median age showing greater declines in the force of infection. Median age is also strongly associated with mean forces of infection over the entire time interval. Using mathematical simulations of dengue transmission, we show that the reduction in birth rate and the shift in the age structure of the population can explain the increase in the periodicity of multi-annual oscillations of DHF incidence, reduction of the force of infection and shift in the age distribution of cases in the absence of other changes. The shift toward lower birth and death rates increases the longevity of immune individuals and their relative abundance compared to susceptible individuals. Immune individuals absorb potentially infectious mosquito bites, effectively protecting susceptible individuals around them. The changes in the force of infection suggest that clinical management should prepare for a continued increase in the average age of dengue cases as Thailand's population continues to age. The mechanism of this change implies that other countries in the region, lagging behind Thailand in the demographic transition, may experience the same declines as their age structure shifts toward older age groups. Though the impact of demographic changes on the force of infection has been hypothesized, to our knowledge, this is the first observation of this phenomenon.

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SAFETY AND IMMUNOGENICITY IN CHILDREN AND ADULTS FROM ENDEMIC COUNTRIES AND ADULTS FROM NONENDEMIC COUNTRIES OF A TETRAVALENT, LIVE ATTENUATED DENGUE VACCINE

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The safety/immunogenicity of tetravalent, live attenuated dengue (DEN) vaccine with 5 log10 TCID50 of chimeric DEN1, 2, 3, 4 viruses (DV) was assessed in 3 randomized, controlled, blind-observer phase I trials conducted in the US, Mexico (MX), and the Philippines (PH). Participants (N=318) from the US (aged 18-45 years, n=66), MX (aged 2-45 years, n=126), and PH (aged 2-45 years, n=126) received either 1 dose of DV or control (placebo [US], yellow fever [YF] vaccine [MX], or typhoid fever vaccine [PH]); all participants received 2nd and 3rd doses of DV at month 3 or 4 and month 12, respectively. Safety data was collected after each dose through day 28; serum was collected at baseline and every 2 days to day 20 (US) or weekly through week 2 (PH, MX) after each DV dose. At baseline, 20% were flavivirus-naive in PH; more were naïve in the US (97%) and MX (92%). No vaccination-related serious adverse events were reported in any study, and DEN-like syndrome was not reported. US and MX participants receiving DV had more systemic reactions than controls, but the active YF control had a more similar rate (79% vs 58% US; 50% vs 43% MX); some transient white blood cell count changes of no clinical significance were observed in those who did not receive placebo. By qRT-PCR, viremia was predominantly DEN4, and 2 (1 US and 1 MX child) had a DEN4-positive status by plaque assay. Viremia was less common after the 2nd and 3rd DV dose and in those who previously received YF vaccine. Seroconversion (Ab concentration ≥10 1/dil) and GMT increased at the initial dose and subsequent doses. Seroconversion against 3 serotypes after 3 DV doses in the US trial was 82-100%, depending on the assay laboratory. In the MX trial, seroconversion against at least 3 serotypes after 3 DV doses was 63-82%, while in the active control (1 dose YF, 2 doses DV) seroconversion against 3 serotypes was 68-88%. Complete postdose 3 data from all studies will be presented. It appears that previous DEN infection does not substantially modify the safety/immunogenicity profile of DV, while prior flavivirus (i.e. DV and YF) exposure may have a priming effect.

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IMMUNE RESPONSE TO TETRAVALENT DENGUE VACCINATION IN MEXICAN SUBJECTS: THE EFFECTS OF YELLOW FEVER VACCINATION

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Dengue endemic areas are often also endemic for yellow fever (YF) so target populations for a tetravalent dengue vaccine (TDV) may be YF-seropositive following vaccination. It is important to test the safety and immunogenicity of sanofi pasteur's TDV in these target populations. This study in Mexico evaluated sanofi pasteur's TDV in different age groups with and without prior YF vaccination. A randomized (2:1) controlled, multicenter study in Mexico City and Estado de Mexico enrolled 126 subjects. Different age groups were vaccinated sequentially using a stepwise approach: 18 aged 18-45 years, 36 aged 12-17, 36 aged 6-11 and 36 aged 2-5. Group 1 received a first injection of TDV and Group 2 yellow fever (YF) vaccine, and both groups received TDV injections 3.5 and 12 months later. $PRNT_{50}$ antibodies were determined before and 28 days after each vaccination against parental dengue strains. Of 126 subjects (84 in Group 1, 42 in Group 2), 8% were dengue seropositive at baseline. 108 subjects (73 in Group 1, 35 in Group 2) received all 3 injections and completed the study. Four SAEs, all considered as not related to the study vaccine, were reported. The reactogenicity profile after TDV was comparable in the two groups. After two doses of TDV, 50.0%, 74.0%, 72.2% and 87.2% were seropositive against dengue virus serotypes 1, 2, 3 and 4 respectively in Group 1, and 85.3%, 87.9%, 91.2% and 94.1%, in Group 2. In addition higher GMTs were recorded in Group 2 than in Group 1 after the second dose of TDV for serotypes 1, 2 and 3. Previous YF vaccination did not increase the reactogenicity of sanofi pasteur's TDV. Subjects receiving YF vaccine prior to two doses of TDV showed higher humoral immune responses against the 4 dengue serotypes (measured by seropositivity and GMT), suggesting a YF priming effect. These results are very promising in the context of dengue vaccine immunization programs for Latin American countries.

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INCIDENCE OF SYMPTOMATIC AND SUBCLINICAL DENGUE IN A FOUR-YEAR PEDIATRIC COHORT STUDY IN NICARAGUA

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To study the natural transmission patterns and epidemiology of dengue in Nicaraguan children, we are conducting a prospective, community-based cohort study of ~3,700 children aged 2-12. Enrollment of healthy children began in August of 2004. Children are followed prospectively for every primary medical appointment, and serum samples are collected every July/August to asses exposure to dengue virus (DENV). Symptomatic DENV infection is confirmed by RT-PCR, viral isolation, or serology in paired acute- and convalescent-phase samples. Asymptomatic or subclinical infections are defined as those patients who did not present to the study Health Center with suspected dengue or undifferentiated fever but who seroconverted or displayed a ≥4-fold increase in anti-DENV antibodies, as evidenced by Inhibition ELISA, in their annual serum sample. Between September 2004 and December 2007, 4,742 children participated in the cohort, contributing a total of 12,271 person-years of time. There

were 159 symptomatic cases of dengue, with 9 classified as dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS), all in 2007. Overall, the incidence rate of symptomatic dengue was 13.0 per 1,000 person-years (95% CI 11.1, 15.1) over the four years of the study. The yearly incidence rate of symptomatic dengue varied from 3.5 to 17.6 cases per 1,000 person-years, with peaks in 2005-6 and 2007-8. The burden of dengue varied by age, with 10-year-olds having the highest symptomatic dengue burden of 22.8 cases per 1,000 person-years. A majority of symptomatic cases occurred during the dengue season (August-January); however, cases did occur in every month of the year, suggesting that there is low-level transmission year-round. The overall estimated incidence of DENV infection was 77 infections per 1000 person-years over the first three years. By age 12, virtually all children (99%) had been exposed to DENV, as evidenced by anti-DENV antibodies. Incidence of asymptomatic/subclinical infections was estimated at 68.4 infections per 1000 person-years. The ratio of symptomatic to asymptomatic DENV infections varied widely, from 1:6 in 2005-6 to 1:13 in 2004-5 and 2006-7. Interestingly, a dramatic shift in disease severity was observed over time, with significantly more DHF/DSS cases during the 2007-2008 season. This large-scale prospective study is the first to determine the incidence rate of dengue and DENV infection in Central America over multiple years.

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A PROSPECTIVE STUDY OF PRIMARY DENGUE VIRUS INFECTIONS DURING INFANCY: PRELIMINARY FINDINGS

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Primary dengue virus infections during infancy produce a higher incidence of dengue hemorrhagic fever (DHF) compared to primary infection in older children and adults. The current hypothesis for this phenomenon is that passively acquired maternal anti-dengue antibodies mediate enhancement of viral infection in these infants. We are conducting a prospective study of dengue virus infections during infancy to directly measure pre-illness anti-dengue humoral and cellular immune responses and their association with clinical protection or disease severity. We have approximately 5,000 mother-infant pairs enrolled in the study in San Pablo, Laguna, Philippines. During surveillance for acute febrile illnesses during the 2007 rainy season, we identified 40 study infants with symptomatic primary dengue virus infections and at least 10 with asymptomatic primary dengue virus infections. The vast majority were dengue virus type 3 infections. We will present a systematic examination of pre-infection anti-dengue humoral immunity (neutralizing and enhancing capacity), anti-viral innate cellular immune responses, viremia, and disease severity in this cohort. The findings from this study will have implications for dengue vaccine development and testing, and further our understanding of DHF pathogenesis.

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SUBSTANTIAL UNDERREPORTING OF DENGUE DEATHS IN AN ASIAN DENGUE ENDEMIC COUNTRY

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Dengue is a growing global public health problem. Though dengue is a notifiable disease in many endemic countries, under-reporting of dengue cases has been widely documented. Reporting of dengue deaths, however, is believed to be relatively reliable. Accurate estimates of dengue deaths are critical for disease burden, as one dengue death at infancy represent the equivalent of at least 1,500 dengue cases in terms of healthy days of life lost. Therefore, we tested the hypothesis that dengue deaths are also substantially under-reported. First, we selected a low-income country in Southeast Asia hyper-endemic for dengue with an independent data source for dengue deaths. Second, among respondents to the country's complete Demographic and Health Survey (DHS), we identified the number of live births who reportedly died from dengue during a four-year period of 2002 through 2005. Third, we adjusted the number of reported dengue deaths using verbal autopsies from the DHS. Fourth, we derived person-years of risk in the DHS and calculated death rates. Fifth, we extrapolated this estimate to the entire country based on the average annual number of live births in the country over these years. Sixth, we compared the calculated number of dengue deaths against the officially reported number of dengue deaths among children under 4 years of age. Finally, we estimated the level of under-reporting of deaths and corresponding expansion factor. DHS reported 13 possible dengue deaths (mostly diagnosed by a health worker) among 3,048 live births born from 2002 through 2005. Based on combination of symptoms, verbal autopsies attributed 72.4% to likely dengue deaths. As the average period of risk of death per child in the cohort was 2 years, there were 6,096 person-years of risk. The resulting dengue mortality rate in children under 4 was 1.5 per 1,000 person-years. With 400,000 annual live births and 1.48 million children under 4, the calculated number of dengue deaths in this age group was 2,220. As the officially reported number was 44 per year, the study found that only one out of 50 likely dengue deaths in this age group was reported. In conclusion, in this country, an expansion factor of 50 would be necessary to estimate the number of early childhood dengue deaths accurately. Dengue burden based only on officially reported deaths would be a substantial underestimate. Similar patterns may occur in other dengue-endemic countries.

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AN ESTIMATION OF THE DISEASE AND ECONOMIC BURDEN OF DENGUE IN SOUTHERN VIETNAM

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Dengue causes significant morbidity and mortality resulting in a large health care expenditure in endemic countries and those with periodic outbreaks. We estimated the disease and economic burden associated with dengue in southern Vietnam based on the combination of results from a prospective cohort study and of a multicenter cost study. Dengue burden in southern Vietnam was estimated from the age-specific and severity-specific incidence of dengue reported by routine surveillance in the region between 2004 and 2006. Reported incidences were compared with the incidence rate of laboratory-confirmed dengue infection obtained during the same period from active surveillance of febrile episodes in an ongoing prospective cohort study of school children in Long Xuyen. The comparison identified the degree of underreporting by passive surveillance. Cost data, expressed in 2006 US dollars (US$), were obtained from a prospective cost study conducted in 2006-2007 in 4 different sites including a children's hospital in Ho Chi Minh City, a provincial hospital in Long Xuyen and 2 district hospitals (Phu Tan and Chau Phu), describing medical and indirect costs associated with dengue severity and age. The average annual incidence reported for all dengue cases between 2004 and 2006 was 441/100,000 for children up to 14, and 98 for those aged 15 and above. The comparison of the reported incidence with that observed in the prospective cohort study led to an underreporting factor of 1.2 for dengue shock syndrome, 5.4 for dengue hemorrhagic fever and 15.7 for dengue fever. Consequently, the annual number of dengue cases in southern Vietnam was estimated to vary over the period from 290 000 to 460 000. Total costs incurred for managing these dengue cases represent on average 26 million US$ per year for the Vietnamese economy. In conclusion, combining the results of passive and active epidemiological surveillance of dengue with those obtained by cost studies enables to derive the actual economic consequences associated with this disease.

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EVALUATION OF THREE DIFFERENT PCR BASED ASSAYS FOR MALARIA DIAGNOSIS AND SPECIATION

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Rapid and accurate diagnosis of malaria parasites remains problematic despite advances in molecular diagnostic tools. At present, microscopy is the gold standard for malaria detection and species determination in many endemic areas, despite its many challenges. Various PCR diagnostic assays based on the 18S ribosomal DNA gene offer increased sensitivity and specificity. One of the best known and commonly used methods reported by Snounou et al. 1993 involves four separate PCR reactions to detect *Plasmodium falciparum*, *P. vivax*, *P. ovale* and *P. malariae*. Since this method is labor intensive, attempts have been made to develop multiplex PCR methods that are simpler and less expensive. However, the validity of these new methods has not been adequately evaluated. We evaluated the Snounou method and two other PCR-based assays (semi-nested multiplex and one-tube multiplex) for the detection and speciation of the four *Plasmodium* species using serially diluted DNA obtained from cultured parasite isolates and mock mixed infections. All three assays detected single infections with high specificity and sensitivity. However, only the Snounou method consistently detected all four species in the mock mixed infections. The semi-nested multiplex method detected at least three of the four species (*P. falciparum* and *P. vivax* and either *P. malariae* or *P. ovale*) to as low as 40 parasites per microliter. The one tube multiplex assay was the least sensitive, detecting only two species at most in the mock mixed infections. In our hands, the Snounou method was the most expensive followed by the semi-nested multiplex method. Future evaluations of these methods need to be conducted using field samples with known mixed infections to determine the sensitivity and specificity of the assays in the presence of potentially competing artifact contaminants in whole blood. Clearly the semi-nested multiplex assay would be a good alternative to the more cumbersome and expensive Snounou method. However, this method will need to be improved and optimized to enable detection of all four species.

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IMMUNOCHROMATOGRAPHIC DETECTION OF *PLASMODIUM FALCIPARUM* INFECTION USING HUMAN SALIVA AND URINE SAMPLES

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Endemic countries are intensifying effective interventions against malaria, using vector control strategies and artemisinine-based combination therapy. As impact is brought to bear on this resilient disease, the need for accurate and simple diagnosis implementable in its predominantly rural stronghold cannot be overstressed. Current definitive malaria detection is constrained by the necessity to draw blood and requirement for trained personnel that are not readily available at grass-root level. We have previously shown that *Plasmodium falciparum* malaria infection can be detected by PCR on human saliva and urine samples. The current study explores *Pf*HRP2-based immunochromatographic detection of *P. falciparum* malaria infection using human saliva and urine specimens. Of 592 asymptomatic residents from Macha screened by microscopy, 14 (2.4%) were positive for *P. falciparum* malaria infection. A random subset of 74 residents was additionally tested for *P. falciparum* using PCR and three different *Pf* HRP-2 immunochromatographic tests. With all the three immunochromatgraphic tests, the likelihood of detecting antigenaemia was up to 5 times higher with saliva than either urine or blood samples (OR [95% CI]: 4.7 [1.89 - 11.65], p<0.001). Immunochromatographic tests on saliva detected more microscopy or PCR-positive infections than either urine or blood samples. However, specificity was lower with saliva or urine than with blood samples. Implications on possible bloodless testing for malaria infection are discussed.

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LOW QUALITY OF ROUTINE MICROSCOPY FOR MALARIA AT DIFFERENT HEALTH SYSTEM LEVELS IN DAR ES SALAAM: RAPID DIAGNOSTIC TESTS SHOULD ALSO BE IMPLEMENTED IN HOSPITALS AND URBAN SETTINGS

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In malaria-endemic areas WHO recommends Rapid Diagnostic Tests (RDT) in dispensaries but not in health centers and hospitals, where microscopy is supposedly available and of good quality. Conversely, routine microscopy at primary level is known to be of low quality, if available at all. The quality of routine microscopy in better-off urban areas, especially in hospitals, is unknown. The objective of this study was to evaluate the quality of routine microscopy for malaria and the change of positivity rates of malaria tests after RDT implementation at three levels of the health system in an urban setting. 1) We compared the positivity rate of routine microscopy with that of routine RDTs in 3 hospitals, 3 health centers and 3 dispensaries in Dar es Salaam. Initial training of all health workers, as well as quarterly supervision and quality control of RDT performance were done. 2) During 3 consecutive days, we randomly picked routine blood slides in each of the same 9 health facilities plus 3 control health facilities (HF) where RDT were not implemented. We measured sensitivity and specificity of these blood slides using expert microscopy as reference.1) From Apr. to Dec. 2006, the mean positivity rates (PR) using routine microscopy were 41% in hospitals, 52% in health centers and 67% in dispensaries (range 14 to 85%). From Apr. to Dec 2007, the respective mean PR using routine RDTs were 6%, 6% and 7% (range 5 to 10%). Quality of RDT performance was very high. PR of routine microscopy remained the same in the control HF over the two years. 2) A total of 178/335 blood slides (53%) were reported as positive by the HF laboratories while actually only 7 of these slides (2%) were positive by expert microscopy. Sensitivity of routine microscopy versus that of expert microscopy was only 71% and specificity 47%. When the result was positive, the median parasitemia was 3 per 200 white blood cells (WBC) by routine microscopy and 1193 per 200 WBC by expert microscopy. In conclusion, in Dar es Salaam, the quality of routine microscopy was as poor in hospitals and health centers as in dispensaries. Overdiagnosis was massive, with many false positive results reported as very low parasitemia (1 to 5 parasites per 200 WBC). RDTs should replace microscopy as first-line diagnostic tool for malaria in all settings (urban and rural) and at all health facility levels, especially in hospitals where the potential of saving lives thanks to better management of fevers is highest.

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EFFECTIVENESS AND SAFETY OF TRAINING IN FEVER CASE MANAGEMENT AND RDT USE AT HEALTH CENTERS IN UGANDA

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In Africa, malaria is typically diagnosed clinically, though presumptive treatment results in significant overuse of antimalarials. Malaria rapid diagnostic tests (RDTs) may offer a reliable alternative, but effective training for health workers is a key challenge in RDT implementation. We designed a training program in fever case management incorporating RDTs, targeted to staff with limited formal education who work at peripheral health centers in Uganda. We then evaluated its clinical effectiveness and safety as compared with standard-of-care presumptive treatment, for the management of patients who present with suspected malaria at government health centers without microscopy in 3 different endemic zones of Uganda. In each zone, one health center was randomly selected to receive training, while a comparable health center serves as a control. Data collection is on-going; preliminary results from a sample of patients in one zone are presented here. At the health center that received training and RDTs, after the intervention, 93 of 165 patients (56%) were tested with RDTs, and of these, 57 (61%) were positive. At this health center, 127 of 165 (77%) patients were prescribed an antimalarial before RDTs were introduced and 37 of 165 (22%) patients were prescribed an antimalarial after RDTs were introduced. In contrast, at the health center that did not receive RDTs or training, 132 of 210 (63%) patients were prescribed an antimalarial before the intervention and 163 of 250 (65%) patients were prescribed an antimalarial after the intervention. The relative risk (RR) for prescribing an antimalarial after vs before the intervention was 0.29 (95% CI 0.22-0.39) at the health center where RDTs were introduced and 1.04 (95% CI 0.90-1.19) at the health center where RDTs were not introduced ($p<0.0001$ for test of homogeneity of RRs). The proportion of patients with a satisfactory outcome 5 days after presentation was similar at the centers with and without RDTs (91% vs. 90%, $p=1.0$). A basic training program in fever case management incorporating RDTs substantially reduced the proportion of patients prescribed an antimalarial without adversely affecting outcomes.

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DECREASING TRENDS IN COMMUNITY-REPORTED FEVER AND HEALTH FACILITY MALARIA DIAGNOSES IN THE IFAKARA DSS (TANZANIA)

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The ACCESS Programme aims at understanding and improving access to prompt and effective malaria treatment and care in a rural Tanzanian setting with a set of integrated interventions. The objective of this study was to evaluate the programme's impact on reported incidence of fever and severe malaria disease at both the community and health facility levels, and to investigate the value of community-based reporting for routine malaria control programme monitoring.This work was implemented within the Ifakara Demographic Surveillance System (DSS) which comprises 25 villages in southern Tanzania (total population 80,000). The DSS staff collected routinely data on reported incidence of fever (2 week recall) and severe malaria disease in the community. In parallel we collected in-patient and out-patient diagnoses data from the 15 health facilities in the area. Reported fever rates in the community decreased from 47.2 to 41.4/1000 person weeks (IRR=0.94, $p<0.001$) between 2005 and 2007. The rates of malaria diagnoses decreased slightly from 13.5 to 12.6/1000 person-weeks between 2005 and 2007 (IRR=0.96, $p<0.001$). A good temporal and quantitative relationship was found between community- reported fever and health facility malaria diagnoses, suggesting that the former could be used for routine monitoring. In conclusion, the trend of fever cases and malaria cases indicate a reduction in malaria risk. This conclusion is strengthened by the great consistency of the collected data between two independent sources.

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WITHDRAWING ANTIMALARIALS IN FEBRILE CHILDREN WITH A NEGATIVE RAPID DIAGNOSTIC TEST IS SAFE IN A MODERATELY ENDEMIC AREA OF TANZANIA

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In malaria endemic areas, clinicians tend to treat presumptively with antimalarial drugs all febrile patients, regardless of age, availability of microscopy or level of endemicity. The main reason is to be on the safe side, i.e. not miss a malaria case because of an undetected or undetectable parasitemia. Th object of this study was to evaluate in an uncontrolled setting the safety of withdrawing antimalarials in febrile children with a negative Rapid Diagnostic Test (RDT) for malaria in an area with moderate endemicity. We recruited children aged 6 months to 10 years attending a health center for the first time with fever in the past 48 hours. Severe cases who needed to be referred were excluded. An RDT was performed and no antimalarial was prescribed if the result was negative. The child was then treated at the discretion of the clinician. Caretakers were told to bring the child back in case of need. At day 7, all patients were visited at home to assess if they were cured or not. When ill in-between or not cured at day 7, patients were tested again with RDT when that at inclusion was negative or with blood slide when positive. These cases were assessed again at day 14. Among 300 febrile children [median age 2.5 years (IQR: 1.25, 4)] recruited, 41 (14%) had a positive and 259 (86%) had a negative RDT result. 10/300 (3%) children could not be followed up either because they gave a wrong address or they had moved. Among the remaining 290 children, 281 (97%) were completely cured at day 7 and 286 (99%) at day 14. All 4 children not cured at the last visit had negative RDT results, both at inclusion and at follow-up visit. All negative children but one received an oral or injectable antibiotic. 1/41 (2%) of the initially positive RDT children and 31/259 (12%) of those initially negative attended the health facility spontaneously at least one more time (after a median of 3.5 days), 81% of the cases because of persisting fever. All children with an initial negative RDT had again a negative result. In conclusion, not giving antimalarial drugs in febrile children with a negative RDT result is safe in a moderately endemic area. By using RDT as sole test for diagnosis, no malaria case was missed and 99% of the children were cured. The same study will be repeated in a highly endemic area to provide further evidence for a revision of the WHO policy of blanket antimalarial treatment in children under five years.

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MALARIA PARASITEMIA IN BLOOD BANKING IN AN ENDEMIC AREA

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The emergence and wide dissemination of drug resistant malaria parasites underscore the need to prevent transfusion malaria but malaria parasite screening is not done routinely when screening blood donors. We evaluated the prevalence of malaria parasiteamia by microscopy and two rapid diagnostic tests (OptiMAL™: pLDH-based and Clinotech Malaria Cassette™ (*Clinotech)*: *MSP and CSP*-based) in 394 consecutive potential blood donors in southwest Nigeria where malaria is endemic. The prevalence of malaria parasitemia by microscopy was 19.5% (77/394) with a geometric mean parasite density of 119.8/µL (range 34 - 6,289/µL). The prevalence of malaria parasiteamia was significantly higher during the rainy season than dry season; 26.5% (70/264) versus 5.6% (7/126) (ρ=<0.0001, OR 1.47; 95%CI=1.311-1.64). One fifth [20% (59/311)] of those found fit and bled had patent malaria parasitemia. There was no significant association between patent malaria parasitemia and fever (temperature $\geq$ 37.5^{0}C), blood group, gender, history of antimalarial drug use within 30 days of test or anaemia (heamatocrit <30%). Prevalence of patent parasitemia by OptiMal™ and *Clinotech* was 15.6% (49/315) and 39.9% (51/142) respectively. Sensitivity and specificity of OptiMAL® and *Clinotech* were 16.3% and 98.9% versus 68.6% and 46.4% respectively. The sensitivity of OptiMal™ increased to 91% when parasite density was >445 µL. Malaria parasite screening should be done in blood donors who are fit to donate in endemic areas especially during the rainy season to reduce the risk of transfusion malaria in immuno-compromized patients and those already weakened by pre-existing disease, hemorrhage or surgical intervention.

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IMMUNOLOGICAL AND GENETIC FACTORS AFFECTING HUMAN SUSCEPTIBILITY TO ECHINOCOCCOSIS

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Many studies have investigated human immunological and genetic factors controlling susceptibility/resistance to variety of infectious diseases, including two significant echinococcal diseases, cystic/alveolar echinococcosis (AE/CE). Despite epidemiological evidence for putative susceptibility to echinococcosis among endemic communities worldwide, the mechanisms underlying infection and disease have been relatively neglected. There were many reports of early 'death' of the metacestode in echinococcosis cases indicating that outcome of disease may also be determined by host immunological and genetic factors. Disease outcome depends on host's immunological response to parasitic lesions which can be affected by a number of factors including nutrition, co-morbidities (such as HIV, TB and simultaneous AE/CE), immuno-suppression and pregnancy. Further, the host specific cellular response to eliminate the parasite can be modulated by parasite-derived effectors. Such immune suppression has been observed in human and murine studies, respectively, showed to suppress local immune responses. This enables AE/CE infections to persist and evade host cell-mediated responses. Immune compromised cases could be more susceptible to other infectious pathogens. It is clear that human genetic susceptibility to echinococcosis is complex and multifactorial in nature. Many polymorphic variations within genes which are involved in the human immune response to infection may alter the occurrence and course of the disease. Early studies could not well explain by suggestion that the variation in disease distribution seen within villages in China endemic may be influenced by geographical factors despite similar levels of exposure to infection. Given a role of the MHC region in the immune response and high allelic variation within the MHC observed between different geo-populations and racial groups, this paper reviews the evidence that MHC variants may be involved in disease susceptibility that race and of an individual can affect his/her phenotype and subsequent outcome of infection and disease.

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ACCELERATED LARVAL GROWTH OF *ECHINOCOCCUS SPP.* IN THE IMMUNODEFICIENT HOST?

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The infection with *Echinococcus spp.* causes different diseases in humans. Cystic echinococcosis (CE) is due to *E. granulosus*, and alveolar echinococcosis (AE) to *E. multilocularis*. One of the key features is the slow larval growth of both species resulting in prolonged incubation periods of several years to decades. Accelerated growth is generally believed to occur in the immunocompromized host or during and following pregnancy. However, a rapid progression of AE was only seen in a few cases with an HIV co-infection. Therefore, a search was performed in selected treatment centers with the aim (1) to identify cases with AE or CE and coincidence of various modes of immunodeficiency and (2) to evaluate the temporal dynamics of larval growth by imaging techniques. Large cases series were looked at in Romania and in Italy, respectively. 4 HIV-*E. granulosus* co-infections were disclosed. Their clinical courses were not remarkable, and imaging techniques revealed a good evolution, similar to one CE case from Italy who underwent bone marrow transplantation for hematologic malignancy. In contrast, a disseminated CE case with HIV/HCV co-infection was reported from a high HIV prevalence region in South Africa , as reported previously. In this case, the dynamics of clinical symptoms and imaging findings due to CE were rather benign, similar to 5 of 6 AE cases from Germany, all of them were HIV negative. Progressive disease and death due to AE was recorded in an adolescent with Hyper-IgE syndrome. Some accelerated growth of AE could be noted after chemotherapy for cancer, immunosuppressive therapy, and most notably, in the course of anti-TNF treatment for rheumatoid arthritis (3 cases, respectively). AE-lesions appeared to grow fast in 3 women during their pregnancies. This retrospective analysis did not allow to assume that the larvae of *Echinococcus spp*. expanded and spread overwhelmingly during the periods of immunodeficiency. Since assessment of larval proliferation is based on imaging techniques, sharp criteria are lacking which allow to proof an expanding growth of the parasite. Different forms of immunomodulation seem to influence the temporal dynamics of larval growth in various ways. Accelerated disease may clinically more obvious in AE rather than in CE. Thus, a careful monitoring of patients with echinococcosis either co-infected with HIV or receiving immune-modifying drugs is warranted.

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OBSERVATIONS ON THE CYTODIFFERENTIATION OF *ECHINOCOCCUS MULTILOCULARIS IN VITRO*

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Alveolar echinococcosis (AE) caused by the cestode *Echinococcus multilocularis* is a life threatening disease of humans caused by the larval stage of the parasite. Treatment of AE is extremely difficult due to the presence of a germinal layer that is capable of infiltrative growth and metastasis. Currently the only method for *in vitro* cultivation of the metacestode involves passage first through experimental animals followed by isolation of protoscoleces from the metacestode and placing the protoscoleces into culture. Previous attempts to establish a cell line from *Echinococcus* in culture have been hampered by the presence of host cells, especially fibroblasts, which over grow the parasite cells. The present study used secondary metacestode tissue, developed from injected protoscoleces in intermediate hosts. Protoscoleces released from this metacestode tissue were then cultured and allowed to develop *in vitro* for a period of time in order to create cellular material solely of parasite origin and free from host tissue contamination. The resultant culture can be maintained in a simple non-reducing medium and does not require any *Echinococcus* derived factors for growth. The parasite origin of the resultant culture was confirmed using PCR techniques and Fluorescence *In Situ* Hybridisation, and subsequent characterisation undertaken by light, scanning and transmission electron microscopy. The establishment of a continuous cell line of *Echinococcus* will provide a continuing source of material without the need for passage in animals.

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CRITICAL APPRAISAL OF NITAZOXANIDE FOR THE TREATMENT OF ALVEOLAR ECHINOCOCCOSIS

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Nitazoxanide is a FDA approved drug in the United States for the treatment of diarrhea caused by *Giardia intestinalis* and *Cryptosporidium* species. It is used internationally for the treatment of various intestinal parasites. Recent studies in the murine and *in vitro* model revealed marked activity against the larva of *Echinococcus multilocularis*. We report upon ten patients with alveolar echinococcosis (AE) who received nitazoxanide (NTZ) alone or in combination with albendazole (ABZ), amphotericin B (AmB) or liposomal AmB. Reasons for treatment with NTZ were grade III to IV hepatotoxicity due to ABZ or mebendazole (n=7), and relapse while on standard medical treatment with ABZ and/or disseminated disease (n=3). The median time on NTZ was 15 months, varying from 1 month to 36 months. The drug was well tolerated in 5 of 10 cases. Two patients reported on a slight yellowish coloration of the skin, but no other complaints. Three patients exhibited side effects which necessitated treatment interruption (liver enzyme abnormalities, polyarthritis, or neuro- and nephrotoxicity, respectively). Despite the heterogeneity of the study population the efficacy of the drug could be evaluated in 8 of 10 cases treated for at least threee months. One patient died of progressive alveolar echinococcosis despite receiving surgery in combination with prolonged high dose ABZ, AmB and NTZ, or combinations thereof. One ABZ intolerant patient progressed while under NTZ for 11 months, and palliative surgery was performed. In another ABZ intolerant case (17y female) partial liver resection (debulking) was planned. She received NTZ prior to surgery as well as for secondary prophylaxis. A serological relapse was observed 12 months after the last NTZ cycle, imaging studies were inconclusive. Three of the remaining 6 cases were on NTZ monotherapy. Progression was suggested by imaging studies during follow-up at 12 to 24 months, respectively. In contrast, 2 of 3 cases undergoing combination therapy of ABZ and NTZ showed a favorable serological response during follow-up, while imaging studies were less meaningful. In conclusion, the off-label use of NTZ as monotherapy for AE should be discouraged. Our limited observations indicate, that NTZ -given alone - failed to control larval progression, and may even exert side effects. The co-medication with ABZ might offer treatment intensification for advanced disease, but this has to be further studied in clinical trials.

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GEO-ECOLOGICAL AND SOCIO-ECONOMIC ENVIRONMENTS AFFECTING *ECHINOCOCCUS* TRANSMISSION IN NINGXIA HUI AUTONOMOUS REGION OF CHINA

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Ningxia is the smallest provincial Autonomous Region on the Loess Plateau in north central China, covering 66,000 Km^2, with a third of its 5.6 million population being Islamic Hui. Sheep farming is a key component of the economy of Ningxia due to the natural environment and socio-religious situation prevailing there. Home slaughter and throwing offal to dogs is a common practice, particularly during religious (Islamic) festival seasons. Although cystic echinococcosis (CE) occurs throughout Ningxia, alveolar echinococcosis (AE) only occurs in the Liupan mountain area in the south, where conditions are favourable for transmission of both *Echinococcus granulosus* (*Eg*) and *E. multilocularis* (*Em*), with many Microtine rodents, sheep, foxes and dogs present. Predator-prey relationships are facilitated by abundant grassland, shrubs, forest habitats and food. Cloud cover and precipitation are greater in the south, with solar radiation and dry condition being greater in the north. The southern mountainous area is more favourable for *Echinococcus* egg survival. Besides the cold conditions and higher humidity at the higher altitudes, the numerous valley rivulets, gullies, seasonal rivers and resulting reservoirs present in the villages, snow, rain and springs provide essential water sources for humans and animals alike. Echinococcal egg contamination of the various water sources may be a major reason why human AE prevalence is higher in the villages at lower altitudes than those at high altitudes. The monitoring of environmental contamination by serological analysis of school-age children in south Ningxia showed that the seroprevalence distribution was associated with changes in the ecology of wild hosts for *Em* contamination, and with changes in socio-geographic features of the communities for *Eg* contamination. Serological data obtained for children in mass surveys of echinococcosis for disease detection and early treatment purpose appear to be a comprehensive and useful tool to monitor changes of transmission dynamics in humans and provide 'warning signals' to decision-makers for the instigation of specific control measures against the disease.

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HUMAN HYDATIDOSIS IN SUDAN: IS IT A SPORADIC OR ENDEMIC DISEASE?

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Cystic echinococcosis (CE) of animals and humans is conventionally ascribed to infection with *Echinococcus granulosus* metacestodes In Sudan, human CE is particularly acute in rural communities where close contact with dogs (definitive hosts) and various domestic animals acting as intermediate hosts is maintained. The disease is highly prevalent in the southeastern province of Equatoria bordering the hyperendemic focus in northern Kenya. In contrast, human CE seems to occur rather sporadically in other parts of the Sudan despite the high frequency of echinococcosis in animals. The reasons for this are not sufficiently clear, but, as a hypothesis, humans seem to be more resistant to infection with *E. canadensis* ('camel strain') as opposed to *E. granulosus* ('sheep strain'). The rather small number of human CE cases in most of Sudan may therefore be related to the fact that *E. granulosus* is still a rare parasite in these regions. Accordingly, it is of concern that introduction and spread of the pathogenic *E. granulosus* will in future lead to a drastic increase in the number of human cases, because all epidemiological conditions for autochthonous transmission of *E. granulosus* are given: there are large numbers of dogs in rural areas, sheep are frequent, and unsupervised home slaughtering is often the rule. After the end of the civil war in southern Sudan, an increased movement of people and livestock occurs, which increases the danger of the spread of the 'sheep strain' from highly endemic Equatoria to other parts of Sudan. Also, the increasing clash of nomadic and settled populations e.g. in western Sudan causes disruption of traditional migration patterns and uncontrolled movement of refugees. This changing situation calls for a monitoring of this pathogen, whose economic and public health impact is still grossly underestimated. The present study provides preliminary data about the molecular epidemiology of hydatidosis in Sudan.

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TREATMENT OF A LARGE PERITONEAL ECHINOCOCCAL CYST WITH PERCUTANEOUS DRAINAGE AND ALBENDAZOLE

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A 33-year-old Italian male was admitted to our Division because of peritoneal and hepatic echinococcal cysts. The patient had been previously operated on for cystic echinococcosis (CE) of the liver and subsequently re-operated twice because of secondary peritoneal CE. Ultrasound examination showed two CE1 pelvic cysts, the largest being 12 cm in diameter, displacing the bladder without causing symptoms. The larger cyst was immediately under the peritoneum, so percutaneous drainage was thought to be dangerous. Surgery was scheduled, but because of a long waiting list and the patient needing to resume work as soon as possible, it was decided in the meantime to drain the cysts and reduce the risk of rupture that could result from trauma or intense abdominal effort. The cyst was aspirated with ultrasound guidance with a 20 G Chiba needle and 300 cc of clear fluid were aspirated, at the presence of an anesthesiologist. No alcohol or other scolecidal agents were injected to avoid chemical peritonitis. There was no pericyst, the cyst being covered only partially with visceral peritoneum. Light microscopy examination of the fluid showed viable protoscolices. Albendazole (ABZ) had been started weeks before aspiration and was continued for 6 months. At 6 month follow-up, the cyst was 6 cm in diameter and the content was almost completely solid. To the best of our knowledge, this is the first report of a large pelvic cyst successfully treated by percutaneous aspiration and ABZ. Percutaneous aspiration by means of a fine needle is a viable option for large peritoneal cysts when surgery is not immediately available, to avoid or minimize the risk of rupture with ensuing peritonitis, anaphylactic shock and secondary echinococcosis.

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HIGH-THROUGHPUT GENOTYPING AND POPULATION GENOMICS OF *PLASMODIUM FALCIPARUM* MALARIA

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Genetic diversity enables *Plasmodium falciparum* to evolve drug resistance and escape host immunity, resulting in widespread morbidity and mortality. Genome-wide analysis of the extent and distribution of this genetic variation across different geographic regions will enhance our understanding of population structure including allele frequency and linkage disequilibrium (LD). We have designed several tools to assess single nucleotide polymorphisms (SNPs), including molecular barcoding assays, which function as a rapid and robust genotyping tool for selected SNPs, and Affymetrix arrays, which rapidly and simultaneously measure SNPs at up to 75,000 locations within the malaria genome. The molecular barcoding approach leverages large amounts of sequence data to genotype 24 common SNP variants to create a rapid and reliable means of uniquely identifying parasites, providing a useful tool for tracking parasites both in the laboratory and within patients. The Affymetrix Array provides high-throughput genome-wide genotyping tool, and hybridization of a global collection of parasite isolates reveals for the first time population structure on a genomic scale within and between continental populations of parasites. Assayed synonymous and non-coding SNPs exhibit greater diversity than nonsynonymous SNPs (nsSNPs) within malaria populations, but reduced divergence among malaria populations relative to nsSNPs. These observations suggest that population-specific selective pressures and/or variation in the efficacy of selection may be driving the functional divergence of different malaria populations, and that a large fraction of nsSNPs are subject to selection. We find differences in the extent of LD among malaria populations indicative of varying epidemiological conditions, with important implications for genome wide association studies in this organism. This tool is able to identify selective sweeps in drug resistant parasites, and will be applied to whole genome scans for genetic diversity. Analysis of this genome-wide variation dataset informs our understanding of the parasite's demographic history, population size in various locations and disease ecology and epidemiology.

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ANALYSIS OF *PLASMODIUM FALCIPARUM* QUANTITATIVE TRAIT LOCI DETERMINING DIFFERENTIAL INFECTIVITY TO ANOPHELES MOSQUITOES

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Parasite lines differ in their prevalence and intensity of mosquito infection. Our aim is to determine the number of parasite genetic loci that contribute to infectivity differences, and to locate them on a genetic map. We have identified two genetically distinct clones of *Plasmodium*

falciparum (denoted 3D7 and HB3) that differ significantly (P<0.0001) in their ability to establish mature oocyst infections in two different *Anopheles* mosquito species (*A. albimanus* and *A. gambiae*) that are natural vectors of human malaria. Progeny clones have been generated from a genetic cross between 3D7 and HB3, and microsatellite markers have been used to generate a genetic map. Here we present phenotyping data of independent recombinant progeny clones that demonstrate segregation of the parental infection phenotypes (infection prevalence and intensity) in the progeny. This formally demonstrates that the parasite competence trait is a heritable phenotype, that it can be discriminated into two components (prevalence and intensity of infection), and that the determinants of the trait are amenable to positional cloning. The presence of intermediate phenotypes amongst the (haploid) progeny suggests the involvement of several genetic loci and/or incomplete penetrance. We are therefore using a QTL approach to map the contributing loci.

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FIXATION OF MUTATIONS AND A SINGLE ORIGIN OF PFCRT AND PFMDR1 HAPLOTYPES IN *PLASMODIUM FALCIPARUM* FROM VENEZUELA

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Chloroquine resistance has been linked to mutations in the *Plasmodium falciparum* chloroquine resistance transporter (*pfcrt*) and the *P. falciparum* multi drug resistance gene (*pfmdr1*). In Venezuela, chloroquine resistance is almost ubiquitous in *P. falciparum*. We examined 93 samples from Bolivar State, Venezuela collected in 2003 and 2004 to characterize the frequency and evolutionary history of mutations in these two genes in this population. We genotyped mutations involved in drug resistance in *pfcrt* and *pfmdr1* by direct sequencing and pyrosequencing. We also examined 11 microsatellite loci that cover 395 KB around *pfcrt* and 14 microsatellites that cover 538 KB around *pfmdr1*. *Pfcrt* resistant mutations are fixed in these samples as the SVMNT genotype for codon positions 72-76 (n=86). However, there were two forms of the SVMNT genotype. In one, S was coded for by tct (n=78) and in the other it was coded by agt (n=8). Including all markers examined, there are multiple S_{tct}VMNT haplotypes, but there is only one S_{agt}VMNT haplotype. The microsatellite haplotypes of these SVMNT alleles imply they share common origin. For *pfmdr1*, we found two major mutant genotypes Y184F/ N1042D/D1246Y (n=47) and Y184F/S1034C/N1042D/D1246Y (n=28) and no wildtype or sensitive genotypes. While there was more variation in microsatellite haplotypes around these genes, our data supports the conclusion that they also have shared ancestry. We find that mutant *pfcrt* and *pfmdr1* genotypes are fixed even after chloroquine drug pressure was removed. This finding resonates with a previous report of sustained high frequency of SP resistant mutant *dhfr* and *dhps* genotypes even after removal of SP drug pressure in Venezuela. These results may have important implications for the spread and persistence of antimalarial resistance across other regions of low transmission.

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PLASMODIUM VIVAX POPULATION GENETICS IN PERU AND VIETNAM: A COMPARATIVE STUDY USING MICROSATELLITES MARKERS

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In Southeast Asia as well as South America, malaria transmission occurs mainly in forested areas, where *Plasmdium vivax* can be sometimes highly prevalent. Our aim was to analyze and compare the genetic diversity of *P. vivax* in these 2 geographically distinct areas. Human blood samples microscopically positive for *P. vivax* collected during cross sectional surveys carried out in Vietnam (68 samples) and in Peru (70 samples), were analyzed by PCR. The genetic diversity of *P. vivax* in these 2 populations was analyzed and compared using 17 highly polymorphic microsatellites (including 3 new and 14 published). The allelic frequencies for all loci in both populations were calculated together with the expected heterozigosity (He) and multi-clonality. The presence of linkage and populations differentiation (Fst) was investigated using FSTAT. Both populations were highly polymorphic ($He_{vietnam}$ 0.86; He_{peru} 0.71). In Vietnam, 100% of the infections were multi-clonal, compared to 65% in Peru. The average number of alleles per locus per infection was respectively 1.7 and 1.1. As the population was more diverse less linkage was found in Vietnam. A pairwise comparison of the genotype constitution revealed that there was a considerable differences in haplotype constitution between the 2 populations, (Fst 0,161$\pm$0,034, p = 0,05). Microsatellites are highly valuable markers to study population genetics of *P. vivax*. The high diversity and multi-clonality of infections indicates that in Vietnam malaria transmission may be higher than estimated by entomological collections while in Peru the available information on transmission levels matched with the multi-clonality observed. The substantial number of sub-patent mixed infections recently identified in this area in Vietnam may explain the complexity of this parasite population. The hypothesis of a zoonotic (simian) cycle for *P. vivax* in forested areas should be explored.

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SAP1 IS A SELECTIVE MASTER REGULATOR OF MALARIA PARASITE LIVER INFECTION

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Malaria parasite sporozoites prepare for transmission to a mammalian host by upregulation of *UIS* (Upregulated in Infectious Sporozoites) genes. A number of *UIS* gene products are essential for the establishment of the intrahepatocytic niche. However, neither the factors nor the mechanisms that regulate the expression of genes involved in gain of infectivity for the liver are unknown. We have identified a conserved *Plasmodium* sporozoite low complexity asparagine-rich protein, SAP1 (Sporozoite Asparagine-rich Protein 1), that has an essential role in malaria parasite liver infection. SAP1 is localized to the cytoplasm but not to the nucleus of the sporozoites. Targeted deletion of *SAP1* in the rodent malaria models showed transcript depletion for a number of *UIS* genes (e.g.; *UIS3* and *UIS4*) but not of constitutively expressed sporozoite genes (e.g.; *CSP* and *TRAP*). We show that SAP1 employs a selective post-transcriptional mechanism to regulate the expression of *UIS* gene products. Using a global gene expression survey we identified all transcripts that are affected by the absence of SAP1. This subset of genes will be of great value to further investigate regulation of malaria parasite liver infection. Collectively, our data show for the first time a selective post-transcriptional regulatory role of a low complexity protein for gene expression control of a pathogenic organism.

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DETERMINATION OF THE BASIS FOR A LIMITED DIMORPHISM, N417K, IN THE *PLASMODIUM VIVAX* DUFFY-BINDING PROTEIN

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Invasion of human red blood cells by *Plasmodium* merozoites is vital for replication and survival of the parasite and as such, is an attractive target for therapeutic intervention. Merozoite invasion is mediated by specific interactions between parasite ligands and host erythrocyte receptors. An important parasite invasion ligand family is the Duffy-binding like erythrocyte binding protein (DBL-EBP). Members have a cysteine-rich DBL domain(s), also known as region II, for receptor recognition. The *P. vivax* orthologue called the Duffy-binding protein (DBP) is named for its interaction with the human Duffy blood group antigen and is vital for invasion. Interestingly, DBP region II contains nearly all of the DBP's allelic polymorphisms observed in field isolates of *P. vivax*. Successful vaccine development necessitates a deeper understanding of the role of these polymorphisms in both parasite function and evasion of host immune responses. Crystallization of the homologous protein in *Plasmodium knowlesi* predicts the location of a sulfated tyrosine binding site thought to be important in receptor recognition. Immediately adjacent to this site is a surface-exposed dimorphic residue, N417K, which is a residue change that alters DBP sensitivity to inhibitory antibody. In natural isolates only two residues are found at this site, asparagine (N) and lysine (K). We have used a site-directed mutagenesis approach to determine the basis for this limited polymorphism, with respect to both binding ability and response to immune sera. Our results indicate that structural requirements alone cannot explain the restricted nature of the polymorphism of this residue.

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CHARACTERIZATION OF *PLASMODIUM FALCIPARUM* PROTEIN KINASE 2

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Malaria is one of the most important infectious disease problems in humans, particularly in developing countries. The life cycle of *Plasmodium falciparum* consists of sexual and asexual stages. The asexual blood stage is the most severe stages clinically. Thus, it is necessary to investigate the mechanisms of invasion of erythrocytes by merozoites, as well as drugs that target this process. One of the potential drug targets for malaria is the protein kinase of the parasite. Among the signals for eukaryotic protein kinases, a calcium signal is thought to be one of the most important ones. A sustained elevation of free Ca^{2+} is observed on the rupture and release of merozoites of *P. falciparum* from the erythrocytes. The immunoelectron micrographs demonstrate that calmodulin is localized in merozoites. To elucidate the Ca^{2+} signal of *P. falciparum* invasion, we attempted to characterize *P. falciparum* protein kinase 2 (PfPK2), which is homologous to human calcium calmodulin-dependent protein kinase (CaMK). PfPK2 was purified as a fusion protein that was labeled with [γ-^{32}P]ATP; this labeling was then eliminated by phosphatase. This phosphorylation was eliminated when the putative catalytic lysine residue of PfPK2 was replaced with alanine. PfPK2 phosphorylated histone II_{AS} as a representative substrate in a Ca^{2+}- and calmodulin-dependent manner. Calmodulin antagonists and staurosporine inhibited the phosphorylation of PfPK2 *in vitro* and markedly decreased the parasitemia of ring forms in an invasion assay, whereas CaMKII-specific inhibitors had no effect. PfPK2 was localized in the merozoites in the culture of *P. falciparum*. Thus, purified PfPK2 possesses protein kinase activity in a Ca^{2+}- and calmodulin-dependent manner and the catalytic lysine of this protein was determined. These data suggest that PfPK2 is the *Plasmodium* protein kinase expressed in the merozoites during the invasion stage.

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HORIZONTAL GENE TRANSFER OF ANTIBIOTIC RESISTANCE GENES IN COMMENSAL *ESCHERICHIA COLI* FROM REMOTE COMMUNITIES

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The evolution and spread of antibiotic resistance is a major public health concern worldwide. As resistance spreads, and treatment failure increases in frequency, infections require more expensive drugs, and are more likely to be associated with serious morbidity or mortality. Whereas the evolution of antibiotic resistance is associated with exposure to antibiotics, the dissemination of antibiotic resistance genes occur by either clonal expansion or horizontal gene transfer (HGT). Although much is known about these two modes of dissemination, the relative role that HGT plays compared to clonal expansion has not been well characterized in a community setting. In this study, we investigated the presence and dissemination of antibiotic resistance genes in 250 commensal *Escherichia coli* isolates collected from fecal samples in two remote communities in Ecuador. The presence of clonal expansion was evaluated by PFGE, and the presence of HGT was evaluated by both integron identification using RFLP and by β-lactamase sequencing. Eighty (32%) of the isolates collected in the two remote communities carried antibiotic resistance genes. Evidence of integron transference in was found in 2 of the 18 isolates. We also found evidence of β-lactamase cassette transference and in some cases cassette exchange between *E. coli* strains. Although there exists abundant indirect evidence for HGT using phylogenetic analysis, we present a unique data set that provides direct evidence of naturally occurring HGT in a community setting, and suggests active movement of integron cassettes between *E. coli* isolates.

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PROTEOMIC ANALYSIS OF THE PHOP REGULON IN *SALMONELLA ENTERICA* SEROVARS TYPHI AND TYPHIMURIUM

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S. Typhi, a human-restricted *S. enterica* serovar, causes a systemic intracellular infection in humans (typhoid fever). In comparison, *S. Typhimurium* causes only gastroenteritis in humans, but causes a systemic typhoidal illness in mice. The PhoP regulon refers to a coordinately regulated network of proteins required for intracellular survival of *S. enterica*. Differences in the regulatory network of PhoP/PhoQ may contribute to differing pathogenicity of these two organisms in humans. Using high performance mass spectrometry (HPLC-MS/MS), we examined the proteome of three sequenced *S. enterica* strains: *S. Typhimurium* LT2, and *S. Typhi* CT18 and Ty2 in PhoP-inducing and non-inducing concentrations of magnesium. We similarly examined the proteome of *phoP*$^-$/*phoQ*$^-$ mutants derived from *S. Typhimurium* LT2 and *S. Typhi* Ty2. Our analysis identified a total 1071 proteins in *S. Typhimurium* LT2 and 1289 proteins in *S. Typhi,* of which, 54 and 64, respectively, were found to be regulated in PhoP-dependent manner. Many proteins identified in *S. Typhi* demonstrated concordant differential expression with a homologous protein in *S. Typhimurium*. However, three proteins (HlyE, CdtB, and t1476), had no homologs in *S. Typhimurium,* and have not previously been

described as components of the PhoP regulon. HlyE is a pore forming toxin which is regulated by SlyA in *E. coli.* T1476 encodes a stably expressed protein of unknown function transcribed in the same operon as HlyE. CdtB is a cytothelal distending toxin associated with DNA damage and cell-cycle arrest and cellular distension. The potential role of these proteins in virulence during human *S. Typhi* infection (typhoid fever) should be explored.

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MEMORY B CELL RESPONSES IN PATIENTS WITH DEHYDRATING DIARRHEA CAUSED BY *VIBRIO CHOLERAE* O1

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Cholera, caused by *Vibrio cholerae*, is a non invasive enteric disease with a high mortality rate if untreated. Transmission of this disease is fecal-oral, most commonly when a host ingests water that has been contaminated by sewage. Developing countries, such as Bangladesh, suffer from cholera epidemics as a result of inadequate water supplies and access to clean drinking water. A more effective vaccine to prevent cholera is needed. We performed a prospective cohort study of patients with cholera followed for one year. The objective was to quantitate antigen specific memory B-lymphocytes in the peripheral circulation that might be responsible for protective immunity to cholera. We enrolled 38 culture confirmed cholera infected patients and followed them at frequent intervals: time of infection, days 30, 90, 180, 270, and 360. We detected immune responses using a method of polyclonal stimulation of peripheral blood mononuclear cells followed by a double-color ELISPOT procedure for development of cholera toxin B subunit (CTB), lipopolysaccharide (LPS), and toxin-coregulated pilus A (TcpA) specific IgG and IgA memory B-lymphocyte responses. All patients demonstrated CTB, TcpA, and LPS-specific IgG and IgA memory responses by day 90, which persisted up to one year, substantially longer than other immunologic markers following cholera. In conclusion, this study suggests that long term immunity to cholera may be mediated by antigen specific memory B-lymphocytes, and further analysis of these responses in protection from subsequent cholera is ongoing.

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PHYLOGENETIC RELATIONS BETWEEN *BARTONELLA* STRAINS IDENTIFIED IN ANIMALS AND HUMANS FROM THAILAND

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Bartonella species have been detected in animals and humans in Thailand, but little is known about the breadth of infective species. Molecular sequence methods were applied in the analysis of *Bartonella* isolates from human patients, stray dogs, wild carnivores, tree shrews, and rodents from Thailand. Partial sequencing of the citrate synthase gene was performed on 195 amplicons of human (31) and animal (164) origin. Phylogenetic inference analysis against homologous sequences available for recognized members of the genus *Bartonella* delineated our sequences into 19 distinct phylogenetic clades, of which 8 denoted recognized *Bartonella* species or subspecies, whereas 11 were novel. Among the 8 known clades, the most common was related to *B. tribocorum*, originally described in domestic rats in France. Sequences of this clade were found in 5 human patients and 18 rats of the genera *Bandicota* (1) and *Rattus* (17). Strains similar to *B. elizabethae,* associated with a case of human endocarditis in Massachusetts, USA, were detected in 5 human patients and in one rat (*Rattus* sp.). Sequences from a human patient and 13 rats of bandicoot rats were delineated into the clade that includes *B. rattimassiliensis*, originally described in domestic rats in France. Sequences identical to *B. henselae,* the causative agent of cat scratch disease, were found in 3 human patients. Sequences homologous to *B. quintana*, the causative agent of trench fever, were detected in 2 dogs. Sequences similar to *B. vinsonii* subsp. *arupensis*, originally isolated from a sick rancher in Wyoming, USA, were identified in 6 humans and 3 dogs. A sequence related to *B. vinsonii* subsp. *berkhoffii* was detected in a dog; this subspecies is common in dogs globally. Three strains of human origin were recently described under the name of *B. tamiae*, but have not been found among animals. The remaining 11 clades (2 human and 9 rodent) likely represent new *Bartonella* species, but further characterization is required. Through comparative phylogenetic analysis we have better defined the etiology of bartonellosis in Thailand.

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SEROPREVALENCE AND EPIDEMIOLOGY OF *BARTONELLA BACILLIFORMIS* INFECTION IN ECUADOR

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Bartonella bacilliformis is typically associated with biphasic illness characterized by bacteremia and severe hemolytic anemia (Oroya fever) followed by chronic cutaneous lesions (verruga peruana). In Ecuador, severe febrile hemolytic disease has been sporadically reported in the mountainous province of Zamora-Chinchipe bordering Peru. In contrast, atypical illness associated only with chronic verrucous skin lesions has been reported in the coastal lowland provinces of Manabí and Guayas. Recently, a novel strain of *B. bacilliformis* was isolated from a visitor to the geographic region bounded by Quito, Esmeraldas, and Coca who presented with inapparent infection characterized only by chronic splenomegaly and mild anemia. Collectively, this data suggests that the prevalence and epidemiology of Carrion's disease in Ecuador is not well understood and that subclinical infection may result in atypical clinical signs and underdiagnosed or unrecognized infection. Blood was collected by fingerstick onto Whatman filter paper from pediatric and adult populations. Sera and DNA were extracted from duplicate dried blood spots and analyzed by IFA and PCR to detect anti-*B. bacilliformis* antibodies or bacteremia, respectively. In one cohort of healthy adults, 4/9 (44%) from Manabi and 6/6 (100%) from Loja had positive antibody titers (1/64-1/256) to *B. bacilliformis*. In another group of healthy adults from Manabi/Guayas, 22/29 (76%) exhibited seropositivity to *B. bacilliformis* antigens (titers of 1/64-1/512). In Loja, sera from a significant proportion of 317 healthy pediatric subjects exhibited seroreactivity to multiple *Bartonella* species by IFA. This study suggests that past or present subclinical infection with *B. bacilliformis* may be more widespread in Ecuador among adults and children than is presently known. Chronic, subclinical infection with *B. bacilliformis* may have significant consequences on the long-term health and productivity of people, especially coupled with co-infection with other protozoan and viral pathogens such as *Trypanosoma cruzi*, *Plasmodium* sp., and alphaviruses.

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INCIDENCE AND CASE FATALITY RATES OF *BURKHOLDERIA PSEUDOMALLEI* BACTEREMIA IN EASTERN AND NORTHEASTERN THAILAND

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Burkholderia pseudomallei, the causative agent of melioidosis, is endemic in Northeastern Thailand; population-based disease burden estimates are limited and minimal data are available from other regions of the country. We measured the incidence of *B. pseudomallei* bacteremia in the Eastern province, Sa Kaeo and Northeastern province, Nakhon Phanom. During 2005 automated blood culture systems were implemented in both provinces to enhance bacteremia surveillance. Blood cultures were encouraged from patients admitted with suspected pneumonia and children aged <5 years with possible sepsis as well as other patients at clinician discretion. In Nakhon Phanom, we compared the in-hospital case fatality rate (CFR) for *B. pseudomallei* bacteremia before and after implementing automated blood culture systems. From May 2005 (November 2005 in Nakhon Phanom) through June 2007, 1979 pathogen-positive blood cultures were identified; 211 (11%) grew *B. pseudomallei* (42/1039 [4%] in Sa Kaeo and 169/940 [18%] in Nakhon Phanom). The annual incidence of *B. pseudomallei* bacteremia in Sa Kaeo was 4.6/100,000 persons (3.0 in those aged <5 years, 0 in ages 5-29, 11 in ages 30-59, and 35 in those aged ≥60 years). The incidence in Nakhon Phanom was 16/100,000 persons (10 in those aged <5 years, 3.1 in ages 5-14, 2.6 in ages 15-29, 39 in ages 30-59, and 56 in those aged ≥60 years). In 2003, 2004, and 2005, before implementing automated blood cultures in Nakhon Phanom, CFRs for *B. pseudomallei* bacteremia were 60% (30/50), 33% (14/42), and 41% (12/29) respectively, compared to 17% (29/169) after systems were place (p<0.001, before vs. after overall). The incidence of bacteremic melioidosis in Northeastern Thailand is higher than previously reported (4.4 per 100,000 persons, as reported previously) and is also substantial in Eastern Thailand. The CFR for *B. pseudomallei* bacteremia decreased following implementation of automated blood culture systems. Further study is needed to evaluate the contribution of enhanced case detection and improved clinical management to this change.

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PLAGUE IN THE WEST NILE REGION, UGANDA, 1999-2008

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Plague is a zoonosis caused by *Yersinia pestis*. Among ~ 2,500 cases of plague reported annually to the World Health Organization, over 90% occur in Africa. It is postulated that plague foci in North-western Uganda are the oldest on the continent; however, there is limited information on the epidemiology of the disease in this area. To better define the burden of plague illness in Uganda we reviewed clinical register data on all patients with suspect plague as defined by local health care providers from 19 health facilities in Arua and Nebbi districts from 1999 through 2008. In addition we obtained outcome data from district records. We describe demographic and clinical features of cases and disease trends. From January 1999 through April 2008, 1,998 patients diagnosed with suspect plague were identified; 1,081 (55%) were female and the median age was 13 years (range from 2 weeks to 99 years). A total of 1,494 (78%) cases were recorded in the months of September through January and the median number of cases per plague season was 165 (range 41 to 518). Patients resided in Arua district (52%), Nebbi District (46%), and the Democratic Republic of Congo (2%). The form of illness was described in 1,720 records and reported as bubonic in 1,597 (93%), pneumonic in 56 (3%), septicemic in 53 (3%). Of the 1,145 with known outcome of illness, 276 (24%) died. The case-fatality rate differed significantly by clinical form namely 71% in pneumonic plague cases, 54% in septicemic cases, and 22% in bubonic cases. Distributions of age and sex did not differ significantly between those who died and those who survived. The West Nile region of Uganda is endemic for plague and since case-reporting has historically been sporadic, incidence is likely underreported. The case-fatality rate is high despite availability of antibiotic therapy. The seasonality and epidemiological characteristics in this report can be used by Ugandan health providers to aid in the diagnosis, management and control of the disease.

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MOLECULAR DIAGNOSTICS AND SPECIATION GUIDE CHOICE OF ALTERNATIVE, SHORT-COURSE TREATMENT REGIMENS FOR CUTANEOUS LEISHMANIASIS

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Pentavalent antimony compounds are the drugs of choice for most leishmanial infections, but are investigational in the United States, require prolonged IV administration and have a high rate of side effects. Rapid species identification by PCR allowed targeted treatments with alternative regimens that resulted in safer, quicker, more convenient and effective treatments. We relate our experience with 10 consecutive patients treated for various types of cutaneous leishmaniasis at the National Institutes of Health since 2005. Three patients were diagnosed by PCR and isotyping/PCR as *Leishmania brasiliensis panamensis* and treated successfully with oral ketoconazole 600 mg/day for 28 days. One of these developed transient ketoconazole induced hepatitis. Five patients with cutaneous infections with *L. brasiliensis brasiliensis* (n=3), *L. tropica* (n=1 retreatment) and L. *donovani chagasi* (n=1) were treated with and responded completely to intravenous AmBisome (3-5 milligrams/kilogram/day) for 5 to 7 days. One patient developed superinfection with methicillin resistant Staphylococcus aureus infection that delayed healing. Another patient with a long-standing facial lesion due to *L. donovani chagasi* responded to AmBisome (5 mg/kg/day) but had a PCR negative clinical recurrence 6 months later and was successfully re-treated with oral miltefosine (50 mg bid) for 25 days. The last patient who presented with a diffuse infiltrative lesions of the ear due to *L. infantum* responded completely to miltefosine (50 mg/day) for 28 days. The first miltefosine treated patient experienced episodes of dizziness, nausea and vomiting that led to her stopping the medication 3 days early and the last patient experienced only transient gastrointestinal symptoms. No unanticipated adverse events were seen with AmBisome therapy. Our experience demonstrates that regimens of shorter duration, guided by PCR speciation are safe and effective and are alternative treatments to pentavalent antimony based regimens.

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THE EPIDEMIOLOGY OF *LEISHMANIA CHAGASI* INFECTION IN RIO GRANDE DO NORTE, NORTHEAST BRAZIL

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Leishmania chagasi infection presents a wide spectrum of clinical outcomes, ranging from asymptomatic self resolving infection to disease. The determinants that lead the evolution of infection to disease are still unknown, although parasite genetics and host genetically controlled immune response play an important role. We surveyed 268 households in a 16km^2 area in perimetropolitan Natal, Brazil, where human and canine leishmaniasis are endemic. 345 individuals and 346 dogs were studied. *L. chagasi* infection was associated with living in urban and high density areas (X^2, $p < 0.0001$), with lack of sanitation (X^2, $p < 0.0001$), fruitful vegetation (X^2, $p < 0.0001$), mud floors and walls (X^2, $p = 0.0108$), older age (X^2, $p = 0.3123$) and being a male (X^2, $p = 0.0157$). The incidence of positive human DTH responses increased with age. There was an increase in vector density during the rainfall season. We also assessed whether nutritional status influenced the outcome of *Leishmania* infection in humans by comparing children with VL and their relatives. Clinical examination, Montenegro skin test, anti-soluble Leishmania antigen and anti-rk39 antibodies were used. Nutritional status was assessed and the modified-relative-dose-response (MRDR) test was used to evaluate vitamin A status. A decrease in body mass index (ANOVA, $p < 0.0005$) and mid-upper arm circumference for age (ANOVA, $p = 0.022$) z scores for VL children was observed. Vitamin A was lower in VL patients measured by serum retinol (ANOVA, $p = 0.035$) and the MRDR test (ANOVA, $p = 0.009$). Higher birth weight (OR = 0.95, $p = 0.047$) and albumin concentrations (OR = 0.10, $p = 0.040$) protected against disease. Increased breastfeeding time (OR = 1.16, $p = 0.036$) was associated with asymptomatic infection. We hypothesize that high rates of canine and human leishmaniasis in areas of increasing HIV infection and other immunosuppressive states might result in increased cases of opportunistic VL in addition to the endemic rate existing in this resource-poor population.

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MILTEFOSINE FOR BOLIVIAN MUCOSAL LEISHMANIASIS: EFFICACY OF SIX WEEKS OF THERAPY

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We have reported that oral miltefosine (2.5 mg/kg/day x 4 weeks) is as effective as standard parenteral antimonial therapy for Bolivian mucosal leishmaniasis, as reported previously. In the miltefosine group, 51 of 72 patients (71%) cured and 21 patients (29%) failed with 12 months of follow up. In the present report, we address several outstanding issues. 1) Will relapses occur with further follow up? The 51 cured patients were followed for an additional 12 months. 39 remained cured; 2 (5%) failed; 10 could not be located. 2) Would 6 weeks of therapy be more effective than 4 weeks of therapy? 15 of the 21 original failures were retreated with 42 days of therapy. With 12 months of follow up, 9 cured (60%) and 6 failed. Separately, 21 new patients were treated with 6 weeks of therapy. With 12 months of follow up, 15 cured (75%); 5 failed; 1 was lost. For all patients treated with 6 weeks of miltefosine: approximately 1/3 reported no subjective side effects; approximately 1/3 had vomiting for 2-7 days of CTC grade 1 on each day, except for 2 patients who had 9 and 10 days of vomiting of CTC grade 1-2; approximately 1/3 had minor other complaints. At the end of therapy, there were no instances of values below the limit of normal for white blood cells, platelets, or hemoglobin; and no increases above the limit of normal for SGOT, bilirubin, or creatinine. We conclude that in the treatment of Bolivian mucosal leishmaniasis with miltefosine, extending follow up to 24 months or increasing the treatment period to 6 weeks does not markedly alter results. Approximately 70% of patients are cured with acceptable tolerance although gastrointestinal side effects commonly occur.

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CLINICAL CHARACTERISTICS OF THREES PATIENTS WITH ACUTE, ORALLY TRANSMITTED CHAGAS DISEASE: THE PROMINENCE OF GASTROINTESTINAL SYMPTOMS

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Outbreaks of orally transmitted Acute Chagas Disease (ACD) occur regularly in Brazil. We investigated an outbreak of ACD among employees of a municipal government with acute febrile illness. We conducted a retrospective cohort study among the employees who shared meals between August 1- September 14, 2007. A confirmed case was an employee with positive direct parasitologic exam or positive IgM. We conducted entomologic searches at the case-patients' homes, municipal government building, and common dining facility. We conducted retrospective case-finding; a suspect case was a municipality resident who between September 15 - December 31, 2007 presented with fever, headache or edema; a confirmed case was a suspect case with positive direct parasitologic or serologic test. Three confirmed cases occurred in the 31-member cohort (attack rate=9%). Median age was 34 years (34-43). No triatomines were encountered. All patients had fever, weakness, headache, retro-orbital pain, cough, and lower back pain; two had severe dysgustia, nausea, vomit, facial and lower extremity edema. One had normocytic anemia and persistent mild transaminitis; he developed irregular cardiac rhythm and EKGs showed supraventricular extrasystoly and dynamic, diffuse repolarization abnormalities but he had two normal echocardiograms. The other two patients had normal echocardiograms. One underwent endoscopy with findings of moderate antral and corpus gastritis and antral erosions; *H. Pylori* test was negative. All were treated with benzonidazol and discharged. Two patients reported persistent post-prandial epigastric pain, dysgustia and anorexia >8 after discharge. Case finding among 35,306 municipal residents yielded 598 suspect cases of which 155 were tested, one (0.7%) had positive IgG. In conclusion, **i**n a population with low background prevalence, an outbreak of orally-transmitted ACD occurred in a cohort that shared meals and had no vector exposure. Gastrointestinal symptoms predominated acutely, and some persisted for months after treatment.

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MULTICENTER CLINICAL TRIAL OF NIFURTIMOX-EFLORNITHINE COMBINATION THERAPY FOR SECOND-STAGE SLEEPING SICKNESS

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Current therapies for second-stage gambiense human African trypanosomiasis (sleeping sickness) are either toxic or impracticable in field conditions. Most patients are still treated with highly toxic melarsoprol, with failure rates increasing in several foci. Eflornithine, the only alternative, is difficult to implement, and is used in first-line only in a few resource-intensive treatment centers. We compared the efficacy and safety of a simplified nifurtimox-eflornithine drug combination (i.e. 14 intravenous infusions) to the standard eflornithine regimen (i.e. 56 intravenous infusions) in a randomized, non-inferiority, multicenter trial. Participants were parasitologically confirmed, had more than 20 leukocytes/µl of cerebrospinal fluid and were above 14 years old. The investigational treatment was nifurtimox: 15 mg/kg/day, 8-hourly, for 10 days, plus eflornithine: 400 mg/kg/day, 12-hourly for 7 days (N+E). The active comparator was standard eflornithine: 400 mg/kg/day, 6-hourly for 14 days. Safety assessments included clinical adverse events, hematology and biochemistry monitoring of hepatic and renal functions. Patients were followed-up for 18 months for efficacy assessment. Between 2003 and 2006, 287 patients were enrolled in 4 sites (Nkayi, Congo; Isangi, Dipumba and Katanda, Democratic Republic of Congo). There were three deaths with eflornithine and one with N+E. Patients suffering severe adverse events were fewer with N+E (14.0% vs. 28.7%; p=0.002). Outstanding adverse events with N+E were nausea/vomiting and with eflornithine neutropenia, infections, fever, diarrhea, hypertension. Preliminary analysis of safety indicators suggests that N+E is better tolerated than standard eflornithine. Final results and conclusions (efficacy and safety) will be available at the time of the congress.

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SIMILARITIES AND DIFFERENCES BETWEEN PEDIATRIC AND ADULT LEPTOSPIROSIS IN SAO PAULO, BRAZIL

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Pediatric leptospirosis is thought to commonly cause mild symptoms and unspecific presentations. Most data available for clinical pediatric leptospirosis, however, is based on small series. The aim of this study was to compare clinical presentation and outcome of leptospirosis in children (<18 years) and adults (18 years or older), and in pediatric leptospirosis in two different age groups (< and 12 years or older). We report the experience of the Health Municipality Secretariat of Sao Paulo from 2004 to 2006 by a population based study enrolling 42 cases of pediatric and 328 cases of adult leptospirosis. All cases had case confirmation and were defined as severe based on requirement of inpatient. Adult leptospirosis was associated with higher frequency of jaundice (82% vs 68%), oliguria (60% x 39%) and lethal outcome (27% vs 5%) when compared to pediatric disease (p<0.05). Adults had also higher levels of serum creatinine (3.4 ± 2.4 vs 2.4 ± 1.8) (p<0.05). There was no difference on the frequency of thrombocytopenia (42% in adults vs 38% in children) or pulmonary involvement (39% vs 33%)(p>0.05). When comparing the age groups < 12 (n=12) and ≥ 12 years (n=30), older children exhibited lower platelet counts (85,386 ± 61,215 vs 162,000 ± 71,413) (p<0.05) and a trend for higher frequency of thrombocytopenia < 70,000 platelets per mm^3 (48% vs 17%) and jaundice (72% vs 42%)(p<0.10). Atypical presentations were more common in children than in adults: anicteric cases with oliguria comprised 12% and 6% of all cases in children and adults, respectively, while anicteric cases with pulmonary involvement occurred 8% and 4% of children and adults, respectively (p>0.05). Nine cases (23%) of pediatric leptospirosis exhibited the combined syndromes of oliguric renal failure and pulmonary involvement. Case fatality of this condition was 1/9 (11%), which was lower than the adult counterpart (59%) (p<0.05). Thus, pediatric leptospirosis has lower case fatality but life threatening complications do occur and attention should be paid to atypical presentations that may delay the correct diagnosis.

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IDENTIFICATION AND CHARACTERIZATION OF THE ETIOLOGIES OF ACUTE UNDIFFERENTIATED FEBRILE ILLNESS IN CAMBODIA IN 2007

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Emerging and re-emerging diseases throughout Southeast Asia continue to be of great concern in a time of rapid population growth, increasing urbanization, extensive air travel, environmental changes and developing intervention strategies. To determine the epidemiology and etiologies of acute febrile illness of unknown origin among persons seeking medical care in Cambodia, a five year hospital-based passive surveillance study was established. Between December 2006 and May 2008, nasal and throat swabs, serum and stool samples were taken from 1336 acutely ill patients presenting with fever at seven health care centers in Kandal and Oudong provinces. In order to measure rising antibody titers, convalescent blood draws were obtained 14 to 21 days after the initial clinic visit. Patients were tested for influenza, dengue, chikungunya, leptospirosis, Hepatitis A and E and malaria. Blood cultures were drawn for the identification of bacterial pathogens. Additional testing for rickettsial agents and Japanese encephalitis was based on the reported clinical symptoms of the patient. Acute infections were determined by increased serum antibody titers, bacterial isolation or molecular detection. Serology revealed that the most prevalent pathogens in enrolled patients were leptospirosis (160/1107; 14%), dengue (113/1278; 9%) and Hepatitis E (46/1017; 5%). Almost 20% of all enrollees had molecular evidence of influenza infection (279/1336). Influenza A (H1N1 and H3N2) viruses were identified in 169 (60.6%) of the influenza virus positive population, while influenza B was detected in 110 (39.4%). All four serotypes of dengue were detected, although dengue-3 virus accounted for 73% of serotyped specimens. Four patients that presented with fever and persistent joint pain in February 2008 were diagnosed with chikungunya infection. Both *P. falciparum* (24/1350) and *P. vivax* (31/1350) were evident in the study population. S. typhi was detected in 1.3% (15/1166) of blood cultures. These data point to the diversity of pathogens contributing to acute febrile illness in patients reporting for treatment in Cambodia and suggest the value of syndromic surveillance in the diagnosis of rare infections.

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EARLY CHANGES IN GENE EXPRESSION PROFILES IN *BRUGIA PAHANGI* L3 AFTER INFECTION IN JIRDS OR *IN VITRO* CULTURE

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Third stage infective filarial larvae (L3) are the transition stage from the insect vector to the mammalian host. Changes in gene expression that accompany this transition may shed light on parasite molecules and adaptations required for invasion and for survival and development in the mammalian environment. The purpose of this study was compare gene expression profiles for post-infectious *Brugia pahangi* L3 (ipL3 and idL3, each collected three days after injection into jirds) with L3 maintained for three days in culture (cL3) and L3 freshly isolated from mosquitoes (vL3). We used the Version 2 Filarial Microarray with 15, 412, 65-mer *B. malayi* oligonucleotide elements to perform two-way comparisons with these different L3 types. Expression signals were detected for 4980 (32%) elements with one or more L3 types. Eight hybridizations were performed for each element on the array; genes with 2-fold differences in expression signals with $P < 0.01$ were considered to be differentially expressed. 601, 415 and 522 array elements were differentially expressed in ipL3, idL3, and cL3, respectively, relative to vL3. 104 genes had increased expression in all 3 types of post-infective L3 relative to vL3. These included genes that encode muscle and cytoskeletal proteins, immunomodulators, antioxidants, proteins needed for protein synthesis and folding, and many novel genes. These genes may be required for parasite migration, growth, and development. 129 genes were down-regulated in all three post-infectious L3 types relative to vL3. These encode proteases and protease inhibitors (cathepsins, serpin), allergens, ALT's, and novel proteins. These proteins may be involved in invasion, and their rapid down-regulation may be important for evading the host's immune system. It was interesting that while 46 genes were differentially regulated between ipL3 and idL3, only 49 and 4 genes were differentially regulated in ipL3 and idL3 relative to cL3, respectively. These preliminary results suggest that *B. pahangi* vL3 undergo dramatic changes in gene transcription shortly after their transition from mosquitoes into conditions that support growth and molting. Gene expression patterns in idL3 and cL3 were more similar to each other than either was to ipL3; some genes may have site-specific expression. This study has provided interesting clues regarding gene products that might be required for invasion, adaptation, and development of filarial L3.

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CHANGES IN THE *AEDES AEGYPTI* TRANSCRIPTOME IN RESPONSE TO *BRUGIA MALAYI* DEVELOPMENT

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Aedes aegypti is a model vector for laboratory studies of human lymphatic filariasis, which is caused by *Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori*. Although many studies have described susceptible and refractory relationships between mosquito species/strains and these filarial nematodes, the underlying mechanisms of successful or unsuccessful parasite development largely remain unknown. To better understand the intimate mosquito-parasite interactions, we have assayed the response of *Ae. aegypti* (susceptible, black-eyed Liverpool strain) to *B. malayi* infection at the global genome level, with microarrays, at successive time points throughout parasite development. We specifically focused on the times when larvae are penetrating the midgut epithelium and thoracic muscle cells, intracellular L1s, tissue feeding L2s and migrating infective-stage L3s. Changes in transcript abundance in response to the different stages of *B. malayi* infection were diverse. At the early stages of midgut and thoracic muscle cell penetration, a much larger number of genes were repressed compared to those that were induced (80 vs. 14). This difference in transcript abundance could reflect some type of host response suppressive activity exerted by the filarial worms. The non-feeding, intracellular L1s elicited only limited differences of 6 transcripts increased and 15 decreased in their relative abundance to controls. Several antimicrobial gene transcripts increased in abundance when the parasites were at the L2 stage of development, and the L3s elicited the greatest changes in transcript abundance (239 induced, 144 repressed), including a large number (~25% of genes with predicted functions) of putative immunity-related genes. Innate immune responses in mosquitoes are largely regulated by intracellular immune signaling pathways, such as Toll and Imd. We are currently investigating the potential implication of these pathways in the regulation of mosquito permissiveness to *B. malayi* infection.

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WOLBACHIA SEQUENCES IN THE CHROMOSOMAL GENOME OF *ONCHOCERCIA FLEXUOSA* INDICATE PAST *WOLBACHIA* ENDOSYMBIOSIS

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Most species of filarial nematode parasites are dependent on *Wolbachia* α-proteobacterial endosymbionts for proper development and reproduction. Other filarial species are free of *Wolbachia* infection. Phylogenetic studies suggest the absence of the endobacteria in *Wolbachia*-free species could be due to secondary loss. To test this hypothesis, we used oligonucleotide microarray hybridization, low coverage 454 genomic sequencing, and expressed sequence tag (EST) sequencing to study the genome of *Onchocerca flexuosa*, a *Wolbachia*-free filarial parasite of European deer. All of these approaches revealed putative *Wolbachia* derived DNA sequences in the *O. flexuosa* genome despite the fact that previous studies (immunohistochemistry and PCR) have confirmed that this species does not contain the bacteria. First, labeled *O. flexuosa* DNA hybridized to 10 of the 804 *Wolbachia* sequences represented on the Version 2 filarial microarray. Furthermore, at least 47 *Wolbachia*-like DNA sequences were identified by 1-fold genomic sequencing. These sequences have high homology to *Wolbachia* genes involved in protein expression, energy metabolism, heme metabolism, type four secretion, etc., and to *Wolbachia* genes of unknown function. It is likely that some of the transferred genes are transcribed and functionally active, as 10 *Wolbachia*-like sequences were identified as EST's in *O. flexuosa* cDNA. Thus, gene transfer may account for the ability of filarial species like *O. flexuosa* to survive without *Wolbachia*. These results strongly suggest that an *O. flexuosa* ancestor was infected with a *Wolbachia* endosymbiont. We hypothesize that lateral transfer of important *Wolbachia* genes into the *O. flexuosa* nuclear genome freed the parasite from dependence on its endosymbiont, which was subsequently lost. Further analysis of laterally transferred *Wolbachia* genes in *Wolbachia*-free filarial species may help to elucidate the biological role of *Wolbachia* in species that do require these bacteria.

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GLOBOMYCIN: A NEW CLASS OF DRUG WITH EFFICACY AGAINST *WOLBACHIA* AND FILARIAL NEMATODES

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Lymphatic filariasis and onchocerciasis are debilitating diseases caused by parasitic filarial nematodes. In both cases disease pathogenesis is characterised by a host inflammatory response induced by the death of the parasite. Previous studies have demonstrated that this inflammation is in part dependent upon the presence of *Wolbachia* and our laboratory has identified lipoproteins as candidate stimulatory molecules. Lipoproteins are important structural and functional components of bacteria and their biosynthesis is essential for bacterial viability. As such, and given their role in the pathogenesis of filariasis, enzymes involved in *Wolbachia* lipoprotein biosynthesis are potential chemotherapeutic targets. Globomycin, a signal peptidase II (LspA) inhibitor, has previously been demonstrated to have potent anti-bacterial activity against Gram-negative bacteria including *Escherichia coli*. A putative LspA gene has been identified from the *Wolbachia* genome, which bears 25% identity and 49% similarity to its *E. coli* homolog. The amino acids required for function are strictly conserved, suggesting wLspA is functional, which was verified by the complementation test in temperature-sensitive *E. coli* mutant Y815 strain. wLspA-transformed *E. coli* confers significant globomycin resistance. Although the highly-expressed N-terminal GST-fused LspA cannot rescue LspA ts mutant due to mis-localization in the cytoplasm, its overexpression in *E. coli* still confers strong globomycin resistance suggesting an alternative possible strategy for high-throughput drug screening. In terms of screening novel drugs active against *Wolbachia*, a 96-well format cell-based assay has been developed as part of the A-WOL Consortium utilising a *Wolbachia*-containing *Aedes albopictus* cell line (C6/36Wp). We have used this assay to assess the efficacy of globomycin against *Wolbachia* as measured by quantitative PCR (QPCR) and demonstrated, for the first time, that a class of antibiotic other than the tetracyclines or rifampicin can deplete *Wolbachia* numbers. Furthermore, globomycin treatment of *Brugia malayi* adult females *in vitro* resulted in reduced motility and viability of parasites. These experiments validate the use of lipoprotein biosynthesis as a target for anti-wolbachial drugs and offer a potential new anti-filarial drug.

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A-WOL DRUG DISCOVERY - SCREENING OF NOVEL DERIVATIVES OF TETRACYCLINE WITH IMPROVED EFFICACY OVER DOXYCYCLINE IN AN *IN VITRO WOLBACHIA* CELL-LINE ASSAY

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Filarial nematodes are an important group of human pathogens infecting around 150 million people throughout the tropics with more than 1.5 billion at risk of infection. Control of filariasis currently relies on mass drug administration (MDA) programmes using drugs which principally target the microfilarial life-cycle stage. These control programmes are facing major challenges including the absence of a drug with macrofilaricidal or permanent sterilizing activity, and the possibility of the development of drug-resistance against the drugs available. Developing treatments based on the anti-symbiotic targeting of filarial *Wolbachia*, which are essential for worm development, fertility and survival, and are an important component of inflammatory disease pathogenesis, could provide a novel treatment. The Anti-*Wolbachia* Consortium (A-WOL) utilises post-genomic and computational bioinformatics to identify potential drug targets, and *in vitro* screening assays, to screen compound libraries and potential drug candidates for efficacy against *Wolbachia*. *In vitro* screening activities employ both traditional filarial nematode screening systems and a multi-well format *Wolbachia* cell-based assay we have developed for HTS, in which the reduction in numbers of *Wolbachia* are determined using quantitative real-time PCR (qPCR) and expressed as *Wolbachia* 16S:*Aedes* 18S ratios. The log drop in the ratio of 16S:18S gives a quantitative measure of the effect of the drug on *Wolbachia in vitro*. Using this strategy we are currently screening a chemical library of novel tetracyclines and have shown that we can identify compounds, which have improved efficacy over doxycycline against *Wolbachia in vitro*. Hit criteria together with pharmacological indices are currently being defined to select drugs for further screening against nematode screening systems.

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MOLECULAR ANALYSIS OF THE EFFECT OF DIETHYLCARBAMAZINE ON *BRUGIA MALAYI* MICROFILARIAE

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Lymphatic filariasis (LF), caused by the parasitic nematodes *Wuchereria bancrofti*, *Brugia malayi* and *B. timori*, affects over 120 million people worldwide with over a billion individuals at risk for infection. Currently, the global strategy to eliminate LF relies on the use of three main drugs: albendazole, ivermectin and diethylcarbamazine (DEC); of these, DEC is the oldest and most widely distributed. Even after hundreds of millions of doses worldwide over the past six decades, the mechanism of action of DEC is still largely unknown. *In vitro* studies demonstrate that DEC has little or no effect on the viability of microfilariae. We performed a microarray analysis to examine the effects of DEC on microfilariae *in vivo*. *Meriones unguiculatus*, infected intraperitoneally with *B. malayi*, were given 10mg of DEC intraperitoneally for one hour and then microfilariae were isolated. Microarray analyses of the microfilarial RNA revealed over a thousand genes that are significantly ($p < 0.05$) altered by treatment with DEC. After a more stringent statistical analysis to control for false discovery rates, many of the filarial genes that are significantly down-regulated with DEC treatment (such as the 40S ribosomal protein, elongation factor 1α1 and ribosomal proteins L10 and S15A) were shown to be involved in transcription/translation. Other down-regulated genes were involved in a variety of functions including intracellular iron storage and lipid metabolism. Using the more stringent analysis, DEC up-regulated genes largely included hypothetical proteins and unknown transcripts. These data offer initial insights into the parasite pathways affected by DEC.

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MOLECULAR CHARACTERIZATION OF RE-EMERGENT *BRUGIA MALAYI* IN SRI LANKA

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Sri Lanka joined the Global Program to Eliminate Lymphatic Filariasis in 2001. Surveys performed after the 5th round of DEC/albendazole mass drug administration (MDA) documented residual *Wuchereria bancrofti* microfilaremia (Mf) rates below 0.2% in all endemic areas. *Brugia malayi* was last seen in Sri Lanka more than 30 years ago. Therefore, health officials were surprised when post-MDA surveys revealed *Brugia* Mf in blood smears from 7 people in north-western Sri Lanka (Puttlam district). Six were children under 3 years of age and one was a 74 year old woman.

The infected people were asymptomatic. None of them had taken antifilarial drugs, all had negative *W. bancrofti* antigen tests (ICT card test), and two had positive tests for anti-filarial antibodies (Brugia Rapid). Blood from a dog that lived with one of the infected individuals contained two types of Mf. We performed molecular studies to characterize the parasite species in these blood samples. DNA extracted from dried bloods on filter paper or from used ICT cards/ Brugia Rapid cassettes was tested for the *Brugia* HhaI repeat by real-time PCR; all 7 human samples were positive. Ten examples of the 320 bp HhaI repeat were sequenced from two human blood samples, and these sequences were consistent with *B. malayi/B. timori*. Sequences for the 5s rDNA intergenic spacer and the 2nd internal transcribed spacer (ITS-2) had 96% and 97% identity with the TRS-strain of *B. malayi*, respectively, and 94% and 92% identity with *B. timori.* These results suggest that the parasites in the human blood samples are *B. malayi*. The dog sample was weakly positive for *Brugia* DNA by HhaI PCR. However, 5s rDNA and ITS-2 sequences revealed only *Dirofilaria repens*. *B. malayi* has not been previously reported in animals in Sri Lanka. It is most likely that the human *B. malayi* cases represent an unrecognized, persistent focus of infection, since they occurred in a formerly endemic area. It is also possible that the parasite has been reintroduced into this area by migrants from other countries. We conclude that *B. malayi* is present in Sri Lanka; infections in young children indicate recent (if not ongoing) transmission events. Additional studies are needed to determine the extent of this problem and to determine whether there is an animal reservoir for *B. malayi* in Sri Lanka.

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PRIMARY CEREBRAL HYDATID CYST: REPORT OF A CASE AND REVIEW OF THE LITERATURE

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Hydatid disease, a serious human cestode infection, is worldwide zoonoses. The incidence of hydatidosis ranges from 3 to 50 cases per 100,000 inhabitants in endemic areas. The hydatid cysts tend to develop in the liver and lung but may be found in any organ of the body, including brain, heart, kidney and bones due to hematogenous dissemination. Primary intracranial cysts are very rare (1.7 %). In the present study, we aim to present a case with primary cerebral hydatid cyst and review of the literature. A 20-year-old man was admitted to neurosurgery department with a one-month history of progressive headache and then nausea, vomiting, weakness and dizziness. On admission, the patient was awake, oriented, and cooperative. His vital signs were normal. On neurological examination, the patient did not show any decreased motor, sensorial and reflex responses in his all extremities. No pupil edema was noted. All laboratory results were within normal limits. Cranial computed tomography (CT) revealed a solitary intracranial hydatid cyst, with the size of approximately 6 cm in diameter. The cyst was surgically removed without rupture. Alive protoscoleces were seen in the cyst fluid. The patient's postoperative period was uneventfully. Pathologic examination revealed hydatid cyst. No primary focus was found in other organ. Primary intracranial hydatid cysts are solitary, as in our case, while secondary cysts are multiple. In the present study, thorax CT and abdominal ultrasonography detected no other primary focus. Headache and vomiting are the most frequent symptoms of cerebral hydatid cysts due to increased intracranial pressure. Our patient presented with progressive headache and than vomiting, weakness. Neurological examination may reveal loss of strength, pupil edema, and pathological reflexes. Radiological imaging, especially CT and MRI is the basis of diagnosis. Cerebral hydatid cysts should be treated with both surgically and medically. As a result, the possibility of primary cerebral hydatid cyst should be considered in the differential diagnosis of nonspecific neurological symptoms such as headache, progressive vomiting in patients from the endemic areas.

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UPDATE ON HUMAN POLYCYSTIC ECHINOCOCCOSIS IN NORTH OF BRAZIL

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Polycystic echinococcosis (PE) is a parasitic zoonosis of increasing concern in South America. The ethiological agent (*Echinoccus vogeli*) is a minute, neotropical tapeworm inhabiting the small intestine of carnivores and viscera of rodents. The natural cycle of *E. vogeli* has bush dogs as only known natural final hosts and pacas (*Cuniculus paca*) as its natural intermediate host. PE also has a domiciliary transmission that involves domestic dogs and pacas. Intermediate hosts become infected after ingesting eggs released with feces of carnivores. Dogs are infected when they feed on the viscera of infected pacas. Humans are infected with metacestodes when they accidentally ingest eggs from domestic dogs. A number of epidemiological information accounts for diagnosis of PE: geographical origin of the patients because all humans cases came from neotropical forests; hunter pacas, dogs feeding viscera of pacas and close contact with these dogs. It is estimated that over 160 human cases have been reported in Brazil. This study aims to release 21 new cases of PE in patients from north of Brazil. All individuals attended to a public hospital in Rio Branco city. Abdominal pain was the main clinical presentation, although mesentery and intestine were affected in three patients. Sera from 21 patients who underwent abdominal computed tomography (CT) and/or abdominal surgery were analyzed for the detection of antibodies to *E. vogeli* by Immunoblot (IB) using the fluid from metacestodes of *E. granulosus* as antigen. Serum samples from patients with PE confirmed by imaging exams and serologic assay were used as positive control, while sera from non-endemic area of PE were used as negative control. IB was positive for 20 of 21 patients. The only negative case had calcified hepatic cysts. Reactions with Mr of 21 to 31 kDa proteins were considered specific. Our results highlight the importance of PE for patients from tropical forests with abdominal masses. Based on serology, surgery and imaging more cases can be detected, thus clarifying the true prevalence of human PE in Brazil.

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EVALUATION OF *TAENIA SOLIUM* CALRETICULIN AS AN ORAL VACCINE IN EXPERIMENTAL TAPEWORM INFECTION

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Epidemiological studies show that the presence of a human carrier of the intestinal *Taenia solium* is the main risk factor for acquiring cysticercosis. Rodent models can be used to study taeniosis. *T.solium* calreticulin (TsCRT) was identified in a cDNA library from the adult tapeworm; the recombinant protein is functional, located in the sub-tegument and expressed during spermiogenesis, ovogenesis and embryogenesis. These data suggest that TsCRT might be a good vaccine candidate against taeniosis. Two experiments were performed in hamsters, using weekly oral immunization with 100 µg of rTsCRT alone or with cholera toxin (CT). Control groups were immunized with CT, non-transfected bacteria or the diluent. Fifteen days later each hamster was orally challenged with 4 cysticerci obtained from naturally infected pigs. After 3 weeks, hamsters were humanely euthanized to search for intestinal tapeworms. In the first experiment, cysticerci were very small and whitish; during necropsy all control hamsters were infected but only 50% of cysticerci transformed

into tapeworms. In the second experiment, big and reddish cysticerci were used, and there was 100% recovery of tapeworms. Animals immunized with rTsCRT and TC did not harbor any tapeworm in the first experiment and 48% in the second experiment. Also, tapeworms in vaccinated hamsters were found anchored farther in the duodenum. Significant differences between vaccinated and control groups in the number of tapeworms and in their location were found. These results suggest that rTsCRT is a good vaccine candidate since 52% reduction in the recovery of tapeworms was obtained

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A COMPREHENSIVE APPROACH TO UNDERSTANDING *TAENIA SOLIUM* CYSTICERCOSIS IN EASTERN AND SOUTHERN AFRICA: THE CESA PROJECT

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Cysticercosis is emerging as a serious public health and agricultural problem in countries of eastern and southern Africa (ESA). The disease remains neglected in ESA due to lack of information and awareness about the extent of the problem, suitable diagnostic and management capacity, and appropriate prevention and control strategies. In 2006 a three year project "Cross-disciplinary Risk Assessment of *Taenia solium* Cysticercosis in Eastern and Southern Africa (CESA)" was initiated by a multidisciplinary coalition of researchers from veterinary, agricultural, human medical, and social sciences in Tanzania, Mozambique, Kenya and Denmark to address these issues. CESA is addressing major obstacles for understanding and combating cysticercosis: a) characterizing the disease and its social impact by documenting the epidemiology and societal costs in both humans and pigs; b) identifying appropriate and sustainable "best bet" prevention and control strategies by estimating health risks associated with sanitation and hygiene, consumer habits, pig management and marketing, and pork inspection and control practices in addition to assessing perceptions, attitudes, and practices concerning the disease; and c) promoting collaboration among the various relevant stakeholder groups through generation of awareness concerning *T. solium* infection risks. These activities are primarily being accomplished through investigations conducted by local post-doctoral researchers, Masters and PhD students in the study sites of Mbeya Region in the southern highlands of Tanzania and Tete Province in northwestern Mozambique. Results thus far indicate that the prevalence of porcine cysticercosis is greater than 30% by Antigen-ELISA and porcine cysticercosis is a major constraint for smallholder famers, especially women, to market their pigs. Stakeholder meetings have been held in both countries to inform about the project and initial findings. At its conclusion, CESA will have raised awareness about the true extent of the cysticercosis problem and appropriate responses to it hopefully resulting in a sustainable coordinated control strategy that leads to reduced incidence of cysticercosis and improved lives and livelihoods of poor farming communities and consumers in the ESA region.

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CYSTICERCOSIS AND TAENIASIS IN PAPUA, INDONESIA

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Cysticercosis and taeniasis is known to be present in Papua, Indonesia. Several small studies in the past found a high prevalence of cysticercosis (23.5 - 56.9%) among people in the central highlands of Papua. In an effort to begin a control program for cysticercosis, valid baseline data on the prevalence of cysticercosis and taeniasis are needed. A seroepidemiological survey was carried out in 4 districts, Jayawijaya, Paniai, Pegunungan Bintang, and Puncak Jaya, in the central highland region of Papua. Anti-cysticercosis and taeniasis antibodies were measured in 2931 people, selected using Probability Proportional to Size cluster sampling technique, using recombinant T24H as a measure of cysticercosis exposure and recombinant TSES38 as a measure of taeniasis exposure. We found that the prevalence of cysticercosis- taeniasis was very high in Jayawijaya and Paniai districts (20.8 and 29.2% for cysticercosis and 7 and 9.6% for taeniasis, respectively) and lowest in the other 2 districts (Pegunungan Bintang and Puncak Jaya) (2 and 2% for cysticercosis and 1.7 and 10.7% for taeniasis, respectively). Our data show that the prevalence of cysticercosis and taeniasis are unchanged from that reported nearly 35 years ago, at the beginning of cysticercosis- taeniasis epidemics in Papua. Based on this survey, a concerted effort is needed to control cysticercosis- taeniasis in Papua. Control strategies should be initiated as soon as possible to begin to reduce the burden of disease in this region.

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ASSAY DEVELOPMENT AND OPTIMIZATION FOR CYSTICERCOSIS USING RECOMBINANT AND SYNTHETIC DIAGNOSTIC PROTEINS

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One of the most well characterized tests for diagnosis of neurocysticercosis is the enzyme-linked immunoelectrotransfer blot (EITB) developed at CDC that uses lentil-lectin purified glycoproteins (LLGP) extracted from *T. solium* cysticerci. Although this method is considered by many the gold standard for laboratory diagnosis of neurocysticercosis, purification of the LLGP antigens has been difficult to standardize and the polyacrylamide gel system used for the immunoblot assay is not easily transferable to other laboratories. Therefore, over the last 10 years we systematically purified and cloned the diagnostic glycoproteins in the LLGP fraction. We found that the seven diagnostic proteins are members of 3 antigenic protein families: gp50, gp24, and the 8-kD family. In this study we generated synthetic peptides (TSRS1) or recombinant proteins (T24 and gp50) from each of the 3 protein families and evaluated these separately or in combination in a recombinant antigen EITB assay. A panel of 249 confirmed positive sera and 401 negative sera were used to compare the sensitivity and specificity of the recombinant antigen EITB. Using this battery of sera, the sensitivity of the recombinant antigen EITB was 99% and the specificity was 99%. The recombinant antigen EITB demonstrated slightly improved detection of neurocysticercosis cases with single cysts when compared to the LLGP EITB, 56% versus 52%. Overall our data show that the recombinant antigen EITB is comparable or slightly better than the 'gold standard' LLGP assay and through the use of recombinant proteins, these assays are sustainable and easily transferable to laboratories within the U.S. and throughout the world.

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POLICY IMPLICATIONS OF THE RESULTS FROM THE RANDOMIZED DOUBLE BLIND PLACEBO CONTROLLED TRIAL OF SP, LAPDAP OR MEFLOQUINE FOR PREVENTION OF MALARIA IN INFANTS STUDY IN NORTH-EASTERN TANZANIA

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The authors will discuss the policy implications of the results from the randomized double blind placebo controlled trial of IPTi using SP, chlorproguanil/ dapsone and mefloquine study which took place in an area of high resistance to SP in north-eastern Tanzania in 2 different transmission settings. They will address antimalarial drugs for IPTi including alternative drug options where SP no longer works in prevention of malaria, how policymakers may decide whether SP will be effective for prevention, and safety issues surrounding drug administration in asymptomatic children. The authors will highlight applicability of the intervention regarding changing transmission of malaria in sub Saharan Africa, heterogeneity of malaria risk and access to health care.

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FIRST AUTOCHTHONES OF *LEISHMANIA TROPICA* IN A REMOTE BORDER AREA OF NORTH-SINAI, EGYPT

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Leishmaniasis is one of eight priority targets of the WHO with almost 1,500,000 new cases occuring annually. Cutaneous leishmaniasis (CL) is a health problem in many parts of the world especially in Mediterranean and Middle Eastern countries. CL is prevalent in the Sinai Peninsula and previous research has consistently documented the etiological agent to be *Leishmania major*. Here we report the first autochthones cases of CL caused by *L. tropica* in an Eastern Sinai community bordering Palestine. Using parasite culturing, real time PCR, gene sequencing and RFLP analysis, we found CL cases in this community to be caused by either *L. major* or *L. tropica*. We implicate *Gerbillus pyramidum floweri* as the sylvan reservoir for *L. tropica. W*e also documented *P. papatasi* sand flies infected with *L. major*, however only non-infected individuals of *P. sergenti,* the suspected sand fly vector for *L. tropica*, were recorded. This scenario is consistent with an incursion of *L. topica* from bordering countries and raises concerns about further expansion of this parasite into Egypt.

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NEAR-FATAL ANAPHYLACTIC SHOCK FROM PERCUTANEOUS ASPIRATION OF AN ECHINOCOCCAL CYSTS IN A PATIENT WHO UNDERWENT FOUR PREVIOUS UNEVENTFUL INTERVENTIONS FOR ABDOMINAL ECHINOCOCCOSIS

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A 33-year-old Italian male was admitted to our Division for abdominal cystic echinococcosis (CE). The patient had been operated previously three times for CE and had recently been treated with percutaneous aspiration and albendazole (ABZ) for a peritoneal CE1 cyst in the pelvis. An echo-free cavity, 10 cm in diameter, with clear-cut borders was seen in the liver. As there was no obvious double line sign, it was unclear whether the finding should be interpreted as a residual cavity after surgery or a CE1, active cyst. For this reason, we decided to aspirate the cyst with a 20 G Chiba needle and look for protoscoleces, under ABZ coverage and with anesthesiological assistance. Two minutes into the aspiration, the detachment of the endocyst was noted, revealing that the cavity was actually a CE1 cysts, but the patient suddenly became pale, cyanotic, and severe cardiovascular shock developed. As no respiratory component was present, the patient was not put on a respirator. Adrenaline was immediately administered and the patient was transferred to the Intensive Care Unit. Fortunately the patient recovered and was transferred back to our Division two days later. Absence of history of anaphylactic shock on previous interventions for CE does not make this complication less likely when a new echinococcal cyst is drained, and having cardiopulmonary resuscitation available remains crucial even in these cases.

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LEPTOSPIROSIS IN SAO PAULO, BRAZIL: EVEN MORE FULMINANT, EVEN MORE A PULMONARY DISEASE

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Leptospirosis is globally distributed zoonosis with major impact related to the high case fatality of its severe forms: 10-20% in classical Weil´s triad of jaundice, acute renal failure and hemorrhages; and 50-74% in severe pulmonary hemorrhagic syndrome (SPHS). Increasing severity of leptospirosis can be measured by higher rates of case fatality and shortening intervals between the onset of symptoms and death. Leptospirosis is the sixth cause of death among Infectious Diseases in Sao Paulo. The aim of this study was to compare annual variations of demographic and clinical features of lethal cases of leptospirosis in Sao Paulo Metropolitan area, from 2004 to 2007. An active surveillance system identified 144 lethal cases of leptospirosis and 75 cases in which necropsy was performed. Numeric variables are reported as median (quartile 1-quartile 3). An active surveillance system detected a trend for shorter intervals between onset of symptoms and death (in days): 8 (7-15) in 2004, 12 (7-17) in 2005, 8 (5-14) in 2006 and 8 (5-11) (p=0.06). The detection of pulmonary involvement prior to death was progressively more common in lethal cases from 2004 to 2007 (58%, 78%, 91% and 95%), Chi-square test for trend p=0.0007, suggesting that lethal leptospirosis is becoming an even more fulminant disease, and is probably explained by a higher frequency of severe pulmonary involvement. In the period, the case fatality of each year was: 14.7% in 2004, 11.4% in 2005, 18.4% in 2006 and 19.5% in 2007. The interval between the onset of symptoms and death (in days) was: 6 (4-8) in patients presenting pure pulmonary forms (n=19), 13 (8.5-19.5) in patients with pure renal forms (n=20) and 8 (6-12) in patients presenting both syndromes combined (n=96), Kruskal-Wallis test p=0.002. A higher frequency of necropsy-proven pulmonary hemorrhage (from 2004 to 2007: 64%, 86%, 73% and 78%) could not explain the increased frequency of fulminant and clinical pulmonary involvement prior to death. In conclusion, our data alert for new challenges such as the occurrence of more fulminant forms in Sao Paulo. Pulmonary forms, especially when unassociated with renal failure, have a markedly more fulminant evolution.

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CLINICAL SPECTRUM OF PATIENTS PRESENTING WITH TROPICAL PARASITIC LUNG DISEASES IN NEPAL

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Tropical Parasitic Lung diseases are common cause of respiratory symptoms in patients attending health care facilities in Nepal yet little is known about their clinical spectrum. Within this background we conducted a hospital based descriptive study over the period of 3 years (Jan 2003 to Dec 2006) to find out clinical spectrum of patients presenting with Tropical Parasitic Lung diseases at referral clinic for the respiratory diseases at B.P Koirala Institute of Health Sciences, a university teaching hospital in eastern Nepal. 70 patients presented with Tropical Parasitic Lung diseases. Among them 49 (70 %) were males; 21(30%) females (average age 48.3 years;). Paroxysmal Productive Cough was most common symptom, 49 (70%) had wheeze and dyspnoea. 35 (50%) had fever. Urticaria, chest pain, hemoptysis and abdominal pain were seen in 28(40%), 14(20%), 10 (15%) and 7(10%) respectively. Chest X-ray was abnormal in 56 (80%).Diffuse reticulonodular infiltrates (40%), lobar consolidations (20%), pulmonary opacities (20%) pulmonary oedema (15%) and pleural effusion (5%) were most common abnormalities. Parasites were isolated in > 70 % of patients from examination of appropriate and relevant specimens. The most common etiological diagnoses were Tropical pulmonary eosinophilia 25(36%), Pulmonary involvement due to Malaria 15 (21.5%), Pulmonary ascariasis 14(20%), Pulmonary hydatid disease 6(8.5%), Pulmonary amoebiasis 6(8.5%) and visceral leishmaniasis in 4 (5.5.%). 65% of patients were treated on outpatient basis, while 35% required hospitalisation Parasitic diseases are common cause of respiratory morbidity in Tropics and substantial improvement in lung health can be achieved by focusing on intervention aimed at controlling them.

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INTERRELATIONSHIP BETWEEN THROMBOCYTOPENIA, ACUTE RENAL FAILURE AND PULMONARY INVOLVEMENT IN SEVERE LEPTOSPIROSIS

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Thrombocytopenia, acute renal failure (ARF) and pulmonary involvement are common life threatening complications of leptospirosis but all three have a poorly understood pathogenesis and it is unknown in which extent they are related to each other. The aim of this study was to evaluate risk factors for each one of these complications and, furthermore, risk factors for necropsy-proven lethal pulmonary hemorrhage. An active surveillance system of the Health Municipality Secretariat enrolled all patients with severe (requiring inpatient) confirmed cases of leptospirosis, with at least two laboratory tests available at admission, from the 2004-2006 period in Sao Paulo, Brazil. Relationship between clinical (jaundice, oliguria and pulmonary involvement) and laboratory (platelet count, serum bilirrubin, serum creatinine) features were evaluated using logistic regression, with the dependent variable being thrombocytopenia, acute renal failure, pulmonary involvement or death due to pulmonary hemorrhage. A total of 370 patients were analyzed. Oliguria was the only independent risk factor associated with both thrombocytopenia (OR=1.9 95%CI: 1.1-3.2) and pulmonary involvement (OR=4.0 95%CI: 2.2-7.4). As expected, age > 40 years (OR 3.8, 95%CI: 2.3-6.2) and jaundice (OR 2.2, 95%CI: 1.1-4.2) were independent risk factors for oliguric ARF. Serum creatinine levels > 3 mg/dl were an independent risk factor for lethal outcome due to (necropsy-proven) pulmonary hemorrhage (OR= 5.4 95%CI: 1.1-25.7) and there was a trend observed for thrombocytopenia < 70,000 mm^3 (OR= 3.6 95%CI: 0.9-14.8, p=0.071). In conclusion, renal failure is an independent risk factor for both thrombocytopenia and pulmonary involvement in severe cases of leptospirosis in Sao Paulo. Uremia could not explain all cases of pulmonary disease suggesting that such complication may represent a heterogeneous group of lung lesions with diverse triggering factors.

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FACTORS RELATED WITH POOR OUTCOMES IN CHILDREN HOSPITALIZED WITH SEVERE MALARIA IN PEDIATRIC INTENSIVE CARE UNIT (PICU) IN NEPAL

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Significant proportions of children hospitalized due to severe malaria in Nepal require transfer to intensive care units during course of their illness, yet studies that provide information regarding their outcomes are lacking. Within this background, we conducted a hospital based descriptive study to evaluate the factors related with poor outcome in children with severe malaria transferred to pediatric intensive care unit (PICU) of department of pediatrics at B.P Koirala Institute of Health Sciences, Dharan,Nepal over the period of 5 yrs (Jan 2002 to Dec 2007).During this period, 94 children with severe malaria were hospitalized, among them 32 (34%) required transfer to PICU; 18 were males (56%) and 14 were females (46%) with (age range 12 months - 15 yrs, mean age 7.5 year.) Indication for PICU transfer were altered sensorium in 26 (82%), Seizure in 9(60%), severe anemia in 8(26%) and acute renal failure in 5 (15%). 25(78%) children were discharged. Neurological status was normal in 18(72%) and impaired in 7(28%). Outcome in children undergoing exchange transfusion was good (100 % Survival).7(22%) children had died. Worst outcome was seen with septicemia and MODS (mortality 100%), altered sensorium with GCS < 8 and seizure (mortality 80%), Decerebrate posturing and shock, prolonged ventilatory support, meningitis,thrombocytopenia, dyselectrolytemia hypoglycemia and acidosis were associated poor outcome. Certain specific clinical parameters are associated with poor prognosis in children admitted with severe malaria in PICU in tropical countries; their early identification followed by appropriate care significantly helps in improving the outcome.

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NOVEL EXO-ANTIGEN BASED ELISAS FOR DIAGNOSIS OF VISCERAL AND CUTANEOUS *LEISHMANIA* INFECTIONS

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Immunological detection of leishmania infection has been reported to be more sensitive than parasitological and clinical diagnosis. Previous studies have shown that promastigote stages of *Leishmania* parasites secrete genus-specific exoantigens when maintained in serum-free and protein-free medium *in vitro*. Following appropriate modifications in our laboratory, exoantigens derived from *L. donovani* and *L. infantum* species were used for construction of an antibody detection ELISA for Visceral Leishmaniasis (VL). The exoantigens from *L. mexicana, L. tropica* and *L. panamensis* were used in a similar type of ELISA for diagnosis of different types of Cutaneous Leishmania (CL) infection. The level of antibody seen in human serum samples tested from different endemic regions has indicated that exo-antigen based serology may prove to be a valuable tool for specific diagnosis of both VL and CL infections. An exo-antigen-based ELISA was also found to be useful for identifying asymptomatic dogs exposed to leishmania infections in the endemic regions. These ELISA tests are potentially useful for assessing the exposure to leishmania infections in human beings visiting the endemic areas as tourists or deployed as military personnel.

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PHARMACOKINETICS AND BIOEQUIVALENCE EVALUATION OF TWO FIXED TABLET FORMULATIONS OF DIHYDROARTEMISININ AND PIPERAQUINE IN VIETNAMESE SUBJECTS

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The fixed-dose combination of dihydroartemisinin and piperaquine, marketed as Artekin®, is highly effective against uncomplicated falciparum malaria. This study was conducted to compare the pharmacokinetics of the components of Artekin® and a new formulation marketed as Arterakine® and to assess the bioequivalence between the two formulations. In an open-label, randomized two-way crossover study, 24 healthy Vietnamese subjects received a single oral dose of 120-mg dihydroartemisinin and 960-mg piperaquine in the form of 3 Arterakine tablets or 3 Artekin tablets. Serial blood samples were collected up to 4 weeks after drug administration, with a wash-out period of 10 weeks. The two formulations of dihydroartemisinin-piperaquine were well tolerated. The maximum plasma concentrations (C_{max}) and area under the plasma concentration-time curve ($AUC_{0\text{-last}}$) of dihydroartemisinin where higher after Arterakine than Artekin administration, with geometric mean values of 198 vs 159 ng/ml and 442 vs 366 ng·h/ml, respectively. Marginally higher C_{max} (232 vs 204 ng/ml) and $AUC_{0\text{-last}}$ (13,431 vs 11,988 ng·h/ml) values of piperaquine were measured following Arterakine administration. The elimination half-lives of dihydroartemisinin (0.98 vs 1.01 h) and piperaquine (613 vs 589 h) were comparable between the two formulations. Bioequivalence between Arterakine and Artekin could not be demonstrated for mean log-transformed data of C_{max} and $AUC_{0\text{-}\infty}$ which were outside the accepted range of 80-125% using a 90% confidence interval. Because the rate and extent of dihydroartemisinin and piperaquine absorption was only marginally higher after Arterakine than Artekin administration, the two formulations are expected to result in similar therapeutic efficacy in the treatment of malaria infections.

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EVALUATION OF ARTEMISONE COMBINATIONS IN MALARIA-INFECTED AOTUS MONKEYS

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Artemisone is an artemisinin derivative with very low neurotoxicity and greater *in vitro* and *ex vivo* activity against *Plasmodium falciparum* than artesunate. In studies with Aotus monkeys infected with *P. falciparum* FVO, parasite clearance was faster after artemisone (10 mg/kg/day x 3 days) than the same dosage of artesunate, and parasite recrudescence was delayed (days 20-29 for artemisone; days 9-20 for artesunate). One-day treatment with artemisone (30 mg/kg) resulted in rapid clearance of parasitemia but did not achieve radical cure of infections in non-immune or partially-immune monkeys. The addition of amodiaquine (20 mg/kg) to the single dose regimen also failed to cure the infections. In contrast, a 3-day course of amodiaquine (20 mg/kg daily) and artemisone (10 mg/kg daily) cured 3 out of 3 monkeys. A 2-day course of amodiaquine and artemisone (30 mg/kg daily) cured 2 out of 3 monkeys (recrudescence on day 16). All 3 monkeys treated with a daily dose of amodiaquine alone (20 mg/kg) for 3 days had a recrudescence between days 13 and 20. The findings indicate that a 3-day course of artemisone, given in combination with amodiaquine is very effective in curing infections that cannot be cured by artemisone or amodiaquine alone. Falciparum infections also were cured by 3 days of daily clindamycin (100 mg/kg) and artemisone (30 mg/kg) and by single doses of mefloquine (5 mg/kg) and artemisone (10 mg/kg). When given in combination with an extremely low dosage of mefloquine (2.5 mg/kg), recrudescence was observed on day 24. These results demonstrate the utility of the Aotus - *P. falciparum* model for assessing antimalarial drug combinations. In this model artemisone cleared parasitemia faster than artesunate and recrudescence was delayed. These data suggest combinations of artemisone with amodiaquine, mefloquine, and clindamycin would be at least as effective as current ACTs containing artesunate.

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MALARIA AMONG ASYMPTOMATIC SCHOOL CHILDREN IN EZINIHITTE LOCAL GOVERNMENT AREA OF IMO STATE, NIGERIA

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This study was designed to investigate malaria signs and rates of parasiteamia among asymptomatic school children in Ezinihitte Local Government Area, Imo State, Nigeria. Tests for malaria parasites were made using the thick and thin bloods smear method while anaemia level was determined using the cyanmethaemoglobin method in 469 randomly selected primary school pupils aged six to eleven years who had no clinical signs and symptons of malaria. Clinical examination was done to determine spleen size. Parents of selected pupils were also interviewed to study sociodemographic factors. 12.8% of the pupils were positive for malaria parasites, 48.6% were anaemic and 11.3% had spleen enlargement. 4.9% of the study pupils had all three of the symptoms. Chidren with malaria parasiteamia were more often absent from school. Malaria parasiteamia was higher in chidren living in communities where an artificial or man made community pond known as *iyi ala* was located compared to children living in communities where *iyi ala* was not located (X^2=10.6, p<0.001). This study established a significant association between malaria infections, anaemia, and splenomegally and identified the study area as a high risk area for malaria.The role of the presence of artificial ponds in perpetuating the vectors of malaria in the area should further be investigated. This will enhance the effectiveness of any intervention strategies implemented in the area.

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PATIENTS WHO HAVE RECOVERED FROM LEPTOSPIROSIS WITH NO DEMONSTRABLE *IN VITRO* MEMORY T-CELL RESPONSES TO *LEPTOSPIRA* OR LEPTOSPIRAL PROTEIN ANTIGENS

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Leptospirosis, caused by diverse species and serological varieties of *Leptospira*, is the most common zoonotic disease in the world, and has variable clinical outcomes, ranging from self-resolving infection to fulminant disease and death. There is no effective vaccine against human leptospirosis. Detailed molecular mechanisms of cell-mediated immunity and the development of T cell memory in leptospirosis are essentially unknown. In this study, we tested the hypothesis that recovered leptospirosis patients have peripheral blood memory T cells specific for *Leptospira* whose function could be demonstrated by *in vitro* recall responses to *Leptospira* or leptospiral proteins. Peripheral blood mononuclear cells (PBMC) were obtained from patients (N=23) living in Iquitos, in the Amazon rainforest of Perú, who had recovered from

leptospirosis. As controls, PBMC were obtained from individuals (N=18) with no documented history of leptospirosis. PBMC were assessed for *in vitro* proliferation, phenotyping and cytokine production in response to stimulation by homologous (obtained from patient) and heterologous (obtained from other humans) isolates of *Leptospira* and by different overlapping peptides of outer membrane proteins of *Leptospira.* Unexpectedly, control subjects' PBMC proliferated more in response to stimulation by both homologous and heterologous *Leptospira* strains than PBMC from self-cured leptospirosis patients. ($p<0.05$). The major proliferating cell population induced by many *Leptospira* strains was the $TCR\alpha\beta^+$ T cell. However, $TCR\gamma\delta^+$ T cell expansion was significant in PBMC stimulated with a newly discovered intermediately pathogenic strain *L. licerasiae* serovar Var10 or when stimulated with heterologous isolates representing the most pathogenic strain, *L. interrogans* serovar Icterohaemorrhagiae ($p<0.05$). Finally, control PBMCs showed high production of IL-10 compared to recovered patients' PBMC when stimulated with heterologous isolates. These results suggest that *Leptospira* induces T cell tolerance in recovered patients via anergy, suppression and/or deletion.

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ETHNOMEDICAL SURVEY OF ANTIMALARIAL HERBS AND ANTIMALARIAL ACTIVITY OF *MOMORDICA CHARANTIA* LINN

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Malaria continues to cause morbidity and mortality on large scale in tropical countries. In an attempt to search for new antimalarial drugs, a survey of plants used in the treatment of Malaria was carried out amongst the Yoruba traditional healers in the South-Western region of Nigeria. Methanolic extract was obtained from *Momordica charantia L.*, a prominenet herb, and was investigated against early malarial infections *in-vivo* using swiss albino mice at dose range 50mg/kg, 100mg/kg, 200mg/mg and 400mg/kg per day infected with malaria strain P*lasmodium berghei* var *Anka I.* Chloroquine at 5mg/kg per day was used as positive control for the early malarial infections while distilled water was administered as negative control. Chemosuppression was high at the various concentrations. The most effective dose of *M. charantia L.* methanolic extract (86%) however, was at 50mg/kg per day. Higher doses were less active i.e. 50mg/kg >100mg/kg >200mg/kg >400 mg/kg.

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EXTRALESIONAL PRESENCE OF *LEISHMANIA VIANNIA* IN ACTIVE AMERICAN CUTANEOUS LEISHMANIASIS

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American Cutaneous Leishmaniasis (ACL) has been typically characterized as a localized disease in which clinical manifestations are the expression of the host response to the presence of parasites. Although considered a systemic infection, the extent of dissemination of parasites from the inoculation site following infection in localized cutaneous leishmaniasis is unknown. The objective of this study was to explore the feasibility of detecting *Leishmania* in diverse extralesional sites. Swabs developed for forensic purposes were used to obtain samples from lesions and conjunctival, nasal, and tonsil mucosa. Molecular detection methods (PCR of kDNA and Southern blot) were used to evaluate swab samples as well as blood monocytes and lesion aspirates. Eighteen adult patients with parasitological diagnosis of ACL were included in the study. *Leishmania* species were identified using monoclonal antibodies. Overall, 55.6% of patients (10/18) presented evidence of *Leishmania* DNA in extralesional sites. 4/16 tonsils, 1/12 conjunctival swabs and 6/17 blood samples were positive for parasitic DNA. 11/12 lesion swabs and 15/18 lesion aspirates were positive. All nasal swabs were negative. The location of lesions in patients with positive tonsil swabs were upper (2/4) and lower (2/4) limbs. The patient with the positive conjunctival swab had a single lesion on the right leg. *Leishmania* parasites were isolated and identified in 15 patients: *L. panamensis* (10), *L. brazileinsis* (3), and *L. guyanensis* (2). Parasite DNA can be detected in multiple and diverse extralesional sites in patients with American Cutaneous Leishmaniasis. The presence of *Leishmania* DNA in tonsil and conjunctiva tissues demonstrates subclinical mucosal involvement and widespread dissemination of *Leishmania* during cutaneous leishmaniasis.

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A SURVEY OF THE CARE SEEKING BEHAVIOUR OF MOTHERS OF SICK INFANTS IN AJEROMI/IFELODUN LOCAL GOVERNMENT AREA OF LAGOS STATE, NIGERIA

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Infectious diseases, malnutrition and poor maternal health account for one third of global disease burden. In the poorer countries, the disease burden is as high as 50%.In Nigeria, infant mortality rate is 99deaths per 1000 live births. To improve child survival, the existing practices of mothers in the management of infant illnesses in a densely populated community in Lagos was assessed. With due written consent, 742 mothers whose infants had been sick in the preceding two weeks in were interviewed at home and in the hospitals to obtain demographic details and their care seeking behaviour. From the results, 97% received anti natal care though only 14.2% from the government hospitals. Likewise, 57%, 43.7% 10% 9.7% were delivered of their infants in private hospitals, Traditional birth attendant's compound, home and government hospitals respectively. Signs, symptoms presented included fever(65.2%), diarrhoea (41.4%), cough/catarrh (74.5%), fast breathing (21.6%). First line of treatment within 24hrs was home management 41%, private hospitals 22.2%, government hospitals 11.1% and patent medicine vendors 7%. Other s (36.7%) sought treatment 1-3days later, 9% after 3 days. Reasons ranged from high hospital bills, prompt attention received in private hospitals and frequent lack of drugs and vaccines at the government health facilities. The study showed that 8.4% of the care givers lost their infants of which 51.6% died in the hospital, 35.5% at home and 12.9% at unspecified places due to malaria, (22.6%) acute respiratory infections (14.5%), diarrhoea, (12.9%).Statistical tests showed survival to significantly depend on delivery at full term, birth weight, duration of breast- feeding and source of treatment ($p< 0.05$). This study reveals that reducing infant mortality requires health education for mothers on antenatal care, the need to be delivered of their babies in hospitals with adequate facilities and appropriate care seeking behaviour at different fora, not only at health posts, Also to improve on the facilities in the government hospitals..

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USEFULNESS OF TELEDIAGNOSIS IN THE IDENTIFICATION OF TISSUE PARASITES: AN EVALUATION BASED ON TWO YEARS (FROM 2006 TO 2008) OF TELEDIAGNOSIS SUBMISSIONS TO THE CDC DPDX PROJECT

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DPDx is an Internet-based diagnostic consultation service aimed at strengthening the diagnosis of parasitic diseases. Telediagnostic assistance through DPDx costs about 80% less than the traditional way of submitting specimens. By using telediagnosis, laboratorians that request diagnostic assistance receive a near real-time response. Our previous evaluations showed that requests for telediagnostic assistance make up roughly 21% of the inquiries received by DPDx. In this study, we evaluated the usefulness of telediagnosis for the identification of tissue parasites. Most images of suspect tissue parasites that are submitted to DPDx are captured from hematoxylin and eosin (HandE) stained tissue sections. Sections are often from tissues obtained from chronic infections and therefore the morphologic diagnostic features of the parasites may be affected due to degeneration. In addition, the tissue parasites might be difficult to identify depending on anatomic location and specimen quality. Therefore, it is not unusual that laboratorians, pathologists, and other health professionals contact CDC basis to obtain diagnostic assistance for cases of suspected disseminated parasitic infections. A total of 89 telediagnosis requests for confirmatory identification of parasites in tissue sections were received from May 15, 2006 through May 15, 2008. In 71% of the cases (63 out of 89) we were able to identify the organisms present in the tissue sections. Identification was not possible in 29% of the cases (26 out of 89) based on review of the submitted images alone and evaluation of additional slides, tissue blocks, or clinical specimens was necessary to make a final identification. Based on these data, telediagnosis is useful for reliable and rapid identification of parasites in tissue sections. Also, this evaluation demonstrates the value of telediagnosis for screening and accelerating requests for additional laboratory testing for diagnosis of such infections.

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POPULATION PHARMACOKINETICS OF ARTESUNATE AND DIHYDROARTEMISININ IN HEALTHY VOLUNTEERS

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A novel artesunate-pyronaridine combination (Pyramax®) is currently under development for the treatment of uncomplicated falciparum and vivax malaria. The aim of this study is to characterize the population pharmacokinetics of artesunate and its metabolite dihydroartemisinin in healthy Korean subjects. Concentration-time data of artesunate and dihydroartemisinin were obtained from four previously conducted Phase I clinical studies in healthy Korean volunteers, i.e. single dose, multiple dose, food interaction and drug interaction studies. Subjects received 2, 3, 4 or 5 mg/kg of artesunate orally. A total of 663 and 1012 observations from 90 subjects were used for the analysis of artesunate and dihydroartemisinin, respectively. Nonlinear mixed-effect modeling (NONMEM) with first-order conditional estimation with interaction was used. Covariates were evaluated using stepwise forward selection and backward elimination approach, and their significance was determined by the difference in objective functions between hierarchical models. Final model selection was based on physiological plausibility of the estimates, minimum objective function, diagnostic plots and variability of the estimates. Bootstrap analysis was done to evaluate the model. A one-compartmental model with first order elimination best fit the concentration-time data. Inter-patient variability on V/F, CL/F and absorption rate constant was best fit using an exponential error model. Residual variability was best described by a proportional and additive model. Body weight was identified as the significant covariate for CL/F of dihydroartemisinin. All estimated parameters were within the bootstrap 95% confidence interval, suggesting the parameters were reliably estimated. A one-compartmental model with first-order absorption and elimination best described the population pharmacokinetics of artesunate and dihydroartemisinin in healthy Korean subjects. Body weight was identified as a significant covariate for CL/F of dihydroartemisinin.

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THE USE OF ANTI-MSP1 ELISA TO IDENTIFY NON-IMMUNE INDIVIDUALS FOR INCLUSION IN MALARIA PROPHYLAXIS TRIALS

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Traditionally, efficacy of prophylactic drugs for malaria was established in placebo controlled trials utilizing semi-immune individuals. Currently, this approach is called into question by a requirement that study populations have potential to benefit from the results of the research. In settings with mixed populations of semi-immune and non-immune individuals, the ability to identify non-immune individuals would allow identification of a population that stands to benefit from prophylaxis. We hypothesize that baseline anti-MSP1 ELISA will accurately identify non-immune individuals from a population with varying exposure status. In 2005, 237 first-year students at Maseno University in western Kenya were classified at enrollment as exposed or non-exposed based on their home of origin, travel history and absence of baseline parasitemia. They were followed prospectively for 9 months. Blood was collected every 3 months and following the identification of malaria parasitemia. Incident cases of malaria were identified by microscopy. Baseline and end of study serum samples will be tested for anti-MSP1 by ELISA. In the 100 exposed subjects, there were 40 incident cases of malaria in 34 (34%) individuals during the study period. Among the 137 non-exposed subjects, there were 23 incident cases in 19 (14%) individuals. The majority (69% and 92% respectively) of incident cases in both groups were symptomatic. Serologic testing results will be available at the time of presentation. Populations of mixed exposure status represent an underutilized setting for chemoprophylaxis drug trials. We anticipate that baseline negative MSP1 ELISA accurately identifies individuals who are non-immune and could be enrolled in such trials. Further, we have found that the incidence of symptomatic malaria requiring treatment was greater than anticipated among previously exposed individuals, raising the possibility that they too might derive a benefit from inclusion in trials of prophylactic drugs.

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ACCELERATED LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP) OF *ENTEROCYTOZOON BIENEUSI* AND *ENCEPHALITOZOON INTESTINALIS* (PHYLUM MICROSPORIDIA)

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Microsporidia are obligately intracellular, single-celled fungal parasites that infect invertebrate and vertebrate hosts. Infections due to microsporidia occur worldwide in both immune-deficient and immune-competent

humans of all age groups and are associated with diarrhea and systemic disease. Molecular-based PCR methods have improved sensitivity and specificity for diagnosing microsporidiosis but require costly equipment (eg. thermocylcer) and take hours to perform. In this study, an accelerated LAMP method was applied to improve the efficiency for molecular detection of the two most prevalent microsporidia species infecting humans, *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis*. Primer Explorer LAMP software (http://primerexplorer.jp/e/v3_manual/index.html) was used to select primers from the ribosomal RNA gene targets of *Ent. bieneusi* (Genbank L07123) and *Enc. intestinalis* (Genbank L19567). Control *Ent. bieneusi* and *Enc. intestinalis* spores were obtained from rhesus macaque bile and tissue culture supernatants, respectively, and reacted with 0.8 uM FIP and BIP primers, 0.2 uM F3 and B3 primers, 0.4 uM LF and LB loop primers, 1.5 M betaine, 16 units *Bst* polymerase, and 0.5 M dNTPs in *Bst* buffer at a final volume of 50 ul for 1 hour at 63° C followed by 10 min enzyme deactivation at 80° C. Lower concentrations of betaine or higher concentrations of dNTPs typically generated negative results. The advantages of the accelerated LAMP over standard PCR include lower cost, shorter time, higher specificity, and no requirement for special equipment. These results support continued work to assess the application of the accelerated LAMP to more efficiently detect microsporidia-positive specimens in clinical laboratory and field studies.

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CLINICAL PRESENTATIONS OF *STRONGYLOIDES*

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Once a person is infected with *Strongyloides stercoralis* a repeated autoinfection cycle maintains a lifetime infection. Most human infections are asymptomatic or have minimal symptoms. Eosinophilia is often the indicator that leads to a diagnosis. Infected humans who become immunosuppressived may develop a more serious "hyperinfection syndrome". The GeoSentinel data base of 83,303 diagnoses includes 701 *Strongyloides* patients of whom 20 had hyperinfection syndrome. Fifteen patients seen at the UofU Travel Clinic were diagnosed with *Strongyloides* of whom 5 had evidence of serious disease. The following patients are representative of more serious infections. A 20 yo expatriate living in Mozambique developed diarrhea, weight loss, chronic cough and a 35% eosinophilia. Schistosoma serology was negative. *Strongyloides* serology was positive and symptoms/eosinophilia resolved with treatment. Workup for immune deficiency, including HTLV-I, was negative. A 50 yo tourist spent 2 weeks in Peru and Bolivia. She was given a steroid injection of her rt. knee followed in 2 weeks by an "aseptic meningitis" that resolved without treatment. Two weeks following a second injection of steroids she developed aseptic meningitis. A *Strongyloides* serology was positive and symptoms resolved with therapy. A Pacific Islander was diagnosed with Kaposi's sarcoma (HIV negative) and started on chemotherapy. He developed cough, SOB and asthma with repeated "pneumonias." In 1/08 he was treated for culture negative bacterial meningitis. In 3/08 he had VRE bacteremia and meningitis. He responded rapidly to antibiotic therapy. A *Strongyloides* serology was positive and his respiratory symptoms resolved with treatment. *Strongyloides* infection should be considered in patients who have traveled or lived in tropical areas and present with eosinophilia, diarrhea, respiratory symptoms, sepsis and/or meningitis especially if symptoms develop following treatment with steroids or they have recently become immunocompromised.

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TRAVEL HEALTH ADVICE-SEEKING BEHAVIOR OF US TRAVELERS TO YELLOW FEVER- AND JAPANESE ENCEPHALITIS-ENDEMIC COUNTRIES: FINDINGS FROM THE 2007 HEALTHSTYLES SURVEY

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International travel continues to grow each year, including travel to yellow fever (YF)- and Japanese encephalitis (JE)-endemic countries. Obtaining adequate information about the trip destination and possible health risks would better prepare travelers for safer travel. The aim of this study was to assess the health knowledge of US travelers visiting countries where they may face unaccustomed health risks. Data were analyzed from the 2007 Porter Novelli HealthStyles survey, an annual national mail-in survey that gathers demographic information and health-related knowledge, attitude and practices of the US population. We compared the source of health knowledge of travelers who answered "yes" to the question about their travel during the last 12 months to any of the 27 listed YF/JE-endemic countries with that of travelers who answered "no." Of 4,398 respondents, 117 (3%) had traveled to a YF/JE-endemic country, of which the top three countries visited were Japan (n=31), Brazil (n=25), and China (n=24). YF/JE travelers were more likely than non-travelers to seek health information on the Internet (OR=1.51 CI=1.03-2.23). Travelers were twice (OR=2.3, CI=1.5-3.3) as likely to remember hearing about health/disease information from the CDC than non-travelers, with telephone calls being the most popular mode of communication (OR=10.5, CI=2.7-37.3). During the last 12 months, travelers were three times more likely to get health/disease information from CDC (OR=3.3, CI=1.9-5.7) than were non-travelers. Travelers and non-travelers did not differ significantly in the number of visits to a primary health-care physician or specialist during the last 12 months. In conclusion, travelers were more likely than non-travelers to seek health information and to seek health information from CDC. Details about the purpose of the physician visit were unavailable; thus, further study will determine whether CDC's recommendation to seek pre-travel advice from a provider familiar with travel medicine is being heeded by travelers to higher-risk destinations.

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AN OPEN LABEL, RANDOMISED TRIAL OF ARTESUNATE + AMODIAQUINE, ARTESUNATE + CHLORPROGUANIL-DAPSONE AND ARTEMETHER-LUMEFANTRINE FOR THE TREATMENT OF UNCOMPLICATED MALARIA

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Artesunate + amodiaquine (AS+AQ) and artemether-lumefantrine (AL) are now the most frequently recommended first line treatments for uncomplicated malaria in Africa. Artesunate + chlorproguanil-dapsone (AS+CD) is a potential alternative for treatment of uncomplicated malaria. A comparison of the efficacy and safety of these three drug combinations is necessary to make evidence based drug treatment policies. Five hundred and thirty-four glucose-6-phosphate dehydrogenase (G6PD) normal children were randomised in blocks of 15 to the AS+AQ, AL or AS+CD groups. Administration of study drugs was supervised by project staff and the children were followed up at their homes on days 1,2,3,7,14 and 28 post treatment. Parasitological and clinical failures and adverse events were compared between the study groups. In a per-protocol analysis, the

parasitological and clinical failure rate at day 28 post treatment (PCF28) was lower in the AS+AQ group compared to the AL or AS+CD groups (corrected for re-infections: 6.6% vs 13.8% and 13.8% respectively, p=0.08; uncorrected: 14.6% vs 27.6% and 28.1% respectively, p= 0.005). In the intention to treat analysis, the rate of early treatment failure was high in all three groups (AS+AQ 13.3%; AL 15.2%; and AS+CD 9.3%, p=0.2) primarily due to vomiting. However, the PCF28 corrected for re-infection was lower, though not significantly, in the AS+AQ group compared to the AL or the AS+CD groups (AS+AQ 18.3%; AL 24.2%; AS+CD 20.8%, p=0.4) The incidence of adverse events was comparable between the groups. In conclusion, AS+AQ is an appropriate first line treatment for uncomplicated malaria in Ghana and possibly in the neighbouring countries in West Africa. The efficacy and safety of AL and AS+CD need to be studied further in West Africa.

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FACILITATING PROGRAMMING OF QUESTIONNAIRES FOR PERSONAL DIGITAL ASSISTANTS BY NON-PROGRAMMERS

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Personal digital assistants (PDAs) are small, mobile computers that can facilitate health surveys and surveillance by integrating data collection and entry. The ability to add programming to questionnaires improves data quality by enforcing skip patterns, conducting range checks, permitting double data entry for key variables and performing logic checks while data is being entered. Commercial applications exist for non-programmers, but may not be appropriate for use in developing countries for health research as they may be expensive, oriented towards business applications or have cumbersome interfaces. Although it is possible to develop custom applications, advanced programming skills are required, which are often not available. The objective of this study is to develop an inexpensive tool to permit non-programmers to create and modify questionnaires for use on PDAs. We have created a user interface for a table wherein specifications for each question are detailed (including the text of the question, permitted answers, type of response, help notes, range or logic checks, actions resulting from answer selection, etc.). A program has been written that translates the question specifications into individual data entry screens on a PDA. The user interface is simple to use and provides an intuitive structure for the design of a questionnaire. Among other features, the interface uses check nodes to allow previous information (such as age, sex, meeting case definition requirements) to determine whether the question is asked of a particular respondent, permitting complex skip patterns. Modifications to the design of the questionnaire only require changes to be made to the table containing the question specifications followed by updating the PDAs. In conclusion, global PDA use for data collection is currently limited by the expertise needed for programming. This inexpensive tool with a user-friendly interface could permit the wider implementation of PDA data collection systems and the improvement of data quality in surveillance and research in developing countries.

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TESTING VALIDITY OF REPORTED DRUG COVERAGE RATES OF THE NEGLECTED TROPICAL DISEASE CONTROL PROGRAM IN FOUR COUNTRIES

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Evaluation of reported coverage rates in national health programs is of key importance. With the recent development of national programs for the co-implementation of multiple drugs for the control of neglected tropical diseases (NTDs) tools to validate reported coverage rates are also required. We present results of a cluster survey designed to validate the reported coverage of mass drug distribution conducted during the first-year of the NTD Control Program in four African countries: Burkina Faso, Ghana, Niger and Uganda. The results compare reported rates versus survey-weighted coverage rates, by age and gender. We will discuss the precision of the survey design for estimating coverage rates. Lessons learned during data collection and analysis will be highlighted and recommendations for the design of future NTD coverage validation surveys will be made.

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AMERICAN VISCERAL LEISHMANIASIS: FEATURES EPIDEMIOLOGIC, CLINIC AND THERAPEUTIC RESPONSE. TRUJILLO STATE, VENEZUELA

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A retrospective study was conduced trough review of clinical records of patients with American Visceral Leishmaniasis in October 1975 at December 2007 period. Trough periods, 82 cases of AVL were admitted at the most important reference centers health in Trujillo state in the Cordillera of Andes in Venezuela. The analyses on data revealed an occurrence of 2.6 cases/year with an overall case-fatality rate of 5%. The clinical recovery rate was 78%. The media of hospitalization was 31±14 days. The incidence was highest in 0.1- 3.9 years (51.2%), 24% were under nutrition. The 20-50 years age group had 39% of cases. The male/female ratio was 1:1 in children and 4:1 in adults. The predominant signs were prolonged irregular fever, skin-mucosal pallor, esplenomegaly and hepatomegaly, abdominal distention, cough, weigh loss, asthenia, anemia, leukopenia, thrombocytopenia, hypergammaglobulinaemia and hypoalbuminemia. So, this picture clinic is consistent whit the typical presentation in the world. Secondary infections disease**s** were found in 32% patients (respiratory, cutaneous, urinary tract and parasitic infections). The bone marrow was helpful for diagnosis. The antimonial pentavalent are at the presently the treatment of choice in this zone, however, the relapse 17% were successfully treated with gabromicine, ketoconazole, allopurinol or anfotericin B. In Trujillo state there is not yet any experience with miltefosine in the practice therapeutics.

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IGG AS A RISK FACTOR FOR NON-HEALING DERMAL LEISHMANIASIS CAUSED BY *LEISHMANIA (VIANNIA) PANAMENSIS*

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Evidence of different studies suggests that immunoglobulin G not only fails as a defense mechanism, but could also act as a virulence factor in leishmaniasis. This study examined the role of IgG in the immune response of patients with cutaneous leishmaniasis caused by *Leishmania Viannia panamensis*. We conducted two studies: i) an observational study of cross-sectional analysis comparing the prevalence of total and individual subclasses of specific IgG in the serum of patients with chronic and recurrent cutaneous leishmaniasis, and individuals with asymptomatic infection and ii) a pilot *in vitro* study using human macrophages to determine the influence of IgG on the intracellular viability, persistence of the parasite and TNFα, IL-12 and IL-10 secretion. The results showed a direct association ($p < 0.05$) between the prevalence of IgG_1 with pathogenicity of infection caused by *L. (V) panamensis*. Infection rates with promastigotes or amastigotes of *L. (V) panamensis* were significantly higher ($p < 0.05$) when the parasites are opsonized with specific IgG. The entry of promastigotes and amastigotes of *L. (V) panamensis* through of Fcγ receptor, induced an inverse pattern of production of IL-12 and IL-10, and increased the production of TNFα. Presence of antibodies of IgG_1 subclass in serum was associated with the pathogenicity of the infection and IgG_3 with infection. In conclusion, the results suggest the involvement of IgG as a mechanism of persistent infection and pathogenicity for *L. (V) panamensis.*

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EFFICACY OF DIHYDROARTEMISININ-PIPERAQUINE *VS*. ARTESUNATE-AMODIAQUINE FOR THE TREATMENT OF UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA IN CENTRAL VIETNAM

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In Vietnam, the fixed combination of dihydroartemisinin-piperaquine (DHA-PQ, Arterakine®) was introduced in 2007 as a potential replacement to CV8 (dihydroartemisinin-piperaquine-trimethoprim-primaquine) for the treatment of uncomplicated *Plasmodium falciparum* malaria. Artesunate-amodiaquine (AAQ) has been shown to be efficacious in Africa, but there is little data available on the efficacy of AAQ in Southeast Asia. We carried out an open, randomized clinical trial of DHA-PQ *vs.* AAQ for the treatment of falciparum malaria at Phuoc Chien Commune, in Ninh Thuan Province, Vietnam. One hundred and sixteen patients (children aged 6-14 years, n=36, adults aged 15-60 years, n=80) satisfied the selection criteria and where allocated a 3-day course of either DHA-PQ (~2.3 mg/kg dihydroartemisinin plus ~18.5 mg/kg of piperaquine per day) or AAQ (~4.4 mg/kg of artesunate plus ~10.6 mg/kg of amodiaquine per day). Of the patients who completed 42 days of follow-up after treatment as per protocol, 49 were on DHA-PQ (15 children, 34 adults) and 49 were on AAQ (14 children, 35 adults). The two drug combinations were well tolerated by all age groups with no obvious drug associated adverse events. The 42 day cure rates adjusted for reinfection identified by PCR genotyping for the two groups were similar [100% (49/49) and 98% (48/49) for DHA-PQ and AAQ, respectively]. Because the number of patients evaluated in this study was small, further studies are required to determine whether AAQ, which is an inexpensive artemisinin-based combination, could be an additional option to DHA-PQ for the treatment of multidrug-resistant falciparum malaria in Vietnam.

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OPPORTUNISTIC COINFECTIONS ASSOCIATED TO ACUTE CARRION'S DISEASE IN A REFERENCIAL NATIONAL HOSPITAL IN LIMA, PERU

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Carrion's disease is an important disease in Peru. Complicated cases from the East area of Lima are being transferred to National Hospital Hipolito Unanue. During hospitalization some of these cases develop severe opportunistic coinfections and, subsequently, death as a important clinical manifestation of inmunosupression of Acute Carrion's Disease (ACD.) However, these conditions has not been previously reported and researched in Peruvian hospitals. The aim of our study was to describe the associated opportunistic coinfections of ACD patients in a National Hospital in an area of the East of Lima. A retrospective and prospective study was performed in the National Hospital Hipolito Unanue, Lima -Peru located near an endemic area of CD and in the East area of Lima-Peru, between January 1982 and January 2007. All hospitalized patients with clinical manifestations of ACD were enrolled. A clinical-epidemiological questionnaire was administered and blood samples were collected for thin smear and culture. Sera were tested for evidence of toxoplasmosis and *Salmonella*. Blood culture, bone marrow culture, stool cultures, stool examination for parasites, BAAR in sputum and thick smear for malaria were also performed. Those who had thin smear or culture positive for Bb and clinical-epidemiological data for ACD were considered as confirmed cases of ACD. A total of 170 subjects were enrolled; of them 29 were confirmed cases of ACD. The median age was 17 years old (03 years-80 years) and 83% were male. The main opportunistic infections were bacterial: *Salmonella typhi* (04 cultures positive), *Staphylococcus aureus* (01 culture), *Escherichia coli* and *Shigella* (02 stool cultures); *Mycobacterium tuberculosis* (01 culture). The main parasite was: *Strongyloides stercolaris* (01 case) and *Toxoplasma gondii*. No cases of *Plasmodium vivax* were observed. The lethality rates of the ACD infections was 0 (0/36). In conclusion, the main associated infections to ACD were bacterial Infections, Strongiloidiasis and toxoplasmosis. Regional endemic infectious diseases such as malaria and Tuberculosis should be also considered in the diferencial diagnosis.

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THE DOMESTIC DOG IS A POTENTIAL RESERVOIR OF CUTANEOUS LEISHMANIASIS IN COLOMBIA

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Although the domestic dog (*Canis familiaris*) is recognized as a zoonotic reservoir for visceral leishmaniasis, evidence for a role as a reservoir of American Cutaneous Leishmaniasis (ACL) is only circumstantial. We conducted a cross-sectional study in 2007 to determine the presence of *Leishmania* infection in dogs from Chaparral, Tolima, Colombia, during a recent outbreak of human ACL, mainly caused by *Leishmania guyanensis.* Two of 280 dogs had leishmaniasis-like cutaneous lesions in the scrotum area (0.7%). Serology (ELISA) detected infection in 10 of 280 dogs (3.6%), of which 8 dogs were asymptomatic and 2 symptomatic. Risk factors for infection will be analyzed using owner questionnaire data. kDNA-PCR was also used to detect parasites in healthy ear skin and lesion biopsies from ELISA-positive dogs. PCR detected *Leishmania (Viannia)* infection in lesions from both symptomatic dogs, and in an ear skin biopsy from an asymptomatic dog, indicating possible metastasis of parasites to

healthy skin in this animal. These findings confirm that *L. guyanensis* was transmitted to dogs in the region and that both symptomatic and asymptomatic dogs may be able to transmit parasites to the sandfly vector.

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EFFECTS OF APPLYING NEW MALARIA TREATMENT POLICIES IN A RURAL DISTRICT OF CASAMANCE, SOUTHERN SENEGAL

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In Senegal, national malaria treatment policy changed in 2004 from chloroquine or quinine on clinical grounds to amodiaquine+sulfadoxine-pyrimethamine and then in 2006 to artesunate+amodiaquine for parasitologically-confirmed cases. Oussouye (Casamance, Southern Senegal) is a rural district of 39,000 inhabitants with four peripheral health centres (PHCs) and a referral hospital. Malaria is mesoendemic with a peak during the rainy season during July-October and 25 infected mosquito bites per person-year. We piloted a staggered implementation of the policy since 2000 in Mlomp, one of the PHCs while the others adopted artesunate+amodiaquine systematically in 2006. From inspection of the clinic registries we documented 239,325 consultations and 89,012 malaria treatments over 12 years (1996-2007) at the four PHCs. Both indicators have been decreasing in Mlomp since 2003 and remained stable elsewhere. P.falciparum was confirmed in 11,515/30,159 smears (38.2%). 87% of the 89,012 treatments were on clinical and 13% after parasitological confirmation; of these, 5198 treatments (45%) were artesunate+amodiaquine. In Mlomp, since 2003 artesunate+amodiaquine accounts for 20-30% of all malaria treatments and is given to 75-100% of smear-positive cases. The proportion of fevers with a positive malaria smear decreased from 39-54% before 2000 to 12% in 2007 in Mlomp, but remains stable in the other PHCs. Other factors that could influence the decreased burden of malaria observed in Mlomp such as rainfalls and bednets deployment are discussed.

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DOSING ACCURACY OF ARTESUNATE AND AMODIAQUINE AS TREATMENT FOR FALCIPARUM MALARIA IN CASAMANCE, SENEGAL

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Several products of artesunate plus amodiaquine (AS+AQ) are being deployed in malaria-endemic countries for treating uncomplicated falciparum malaria but dosing accuracy and effects on efficacy and tolerability have not hitherto been examined. During 2000-2006, 3277 patients with parasitologically-confirmed, uncomplicated falciparum malaria were treated and followed by either a research team (n=966) or local staff (n=2311) at health centres in Oussouye District, Senegal. AS+AQ was given as: (i) a loose combination of individual products (AS 50mg + AQ 200mg, n=1972), dosed on body weight, (ii) a co-blistered product (AS 50mg + AQ 153mg, n=1305) dosed by weight (n=343) or age (manufacturer's instructions, n=962). Target doses (therapeutic dose ranges) were: (i) AS 4 (2-10) mg/kg/d and (ii) AQ 10 (7.5-15) mg/kg/d. Patients receiving therapeutic doses defined dosing accuracy. Treatment emergent signs and symptoms (TESS) were recorded. AS was dosed correctly in >99% with all regimens. The loose AQ by weight was 98% correct. The co-blister AQ overdosed 18% of patients when dosed by age and underdosed 13% by weight. A lower weight was an independent risk factor for overdosing. The co-blister had significantly more TESS than the loose product [117/1305 (9%) vs. 41/1972 (2%), relative risk = 4.3 (95% CI 3.0-6.1, p<0.0001). TESS occurred mostly within one day (72%) and were mild or moderate (75%). The long-term implications of over and under-dosing warrant more research.

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DEVELOPMENT AND IMPLEMENTATION OF A LIFE CYCLE-DEPENDENT, PHENOTYPIC HIGH THROUGHPUT *LEISHMANIA MAJOR* PROMASTIGOTE DRUG SUSCEPTIBILITY ASSAYS

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We hypothesize that effective novel anti-leishmanials can be identified using robust whole parasite high throughput screening (HTS) assays. Our overall objective is (1) to characterize differences in drug sensitivity profiles of the *Leishmania major* (*L. major*) life cycle forms and (2) to identify novel chemotypes that will serve as the next generation of anti-leishmanial therapeutics. Therefore, we developed, validated and implemented a Cell TiterBlue™-based *L. major* promastigote drug susceptibility assay in a 384-well format. The robustness of our HTS assay was documented by screening 1280 compounds in a Library of Pharmacologically Active Compounds (LOPAC) set. We successfully identified several previously reported anti-leishmanials including tamoxifen, ketoconazole and pentamide (Z-factor=0.7±0.1, signal-to-background=20.1±0.7). Subsequently, we interrogated 198,185 unique compounds with an overall throughput of ~150 assay plates (48,000 compounds) per 48 hour screening cycle. Compounds were tested at a single concentration (10 μM) with the average Z-factors and signal-to-background being 0.9 ± 0.1 and 26.1 ± 1.0, respectively. After secondary confirmation response assays, we identified 95 compounds with IC_{50}s<1 μM against *L. major* promastigotes. Significantly, 70 of these compounds showed no cytotoxicity in our A549-based drug susceptibility assay with compound concentrations ≤5 μM, suggesting *L. major* promastigote specific cytotoxicity. A Leadscope analysis of all confirmed inhibitors identified 16 compound structural clusters with a majority being singletons. Significantly, we identified a class of substituted methylquinolines that exhibit a specific growth inhibitory effect on *L. major* promastigotes. This HTS assay represents the first of three *L. major*-based HTS formats we are developing with the other two assays focusing on the axenic amastigote and cell-based amastigote life cycle forms. These initial results with the *L. major* promastigotes suggest novel anti-leishmanials can be identified using robust whole parasite HTS assays.

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MALARIA CLINICAL TRIALS CAPACITY DEVELOPMENT IN AFRICA: CHALLENGES AND EXPERIENCES FROM THE KINTAMPO HEALTH RESEARCH CENTRE, GHANA

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Human resource capacity and infrastructure needs to be developed in well defined populations in sub-Saharan Africa where clinical trials to control infectious diseases can be conducted. In pursuance of its objectives, the

Kintampo Health Research Centre (KHRC) developed the middle belt of Ghana to conduct internationally recognized clinical trials between 2002 - 2007. Key strategies employed in achieving our goals were as follows: Malaria epidemiology: In 2003, baseline malaria epidemiology required in evaluating new malaria drugs and vaccines were determined. These included Entomological Inoculation Rate -269 ib/per/yr; prevalence of malaria parasite among young children ~60%. Human resource capacity: This has been boosted with a wide range of expertise relevant to clinical trials -patient care; public health specialist, microbiologist, microscopist, project managers, data managers and clinical trial ethicist. Clinical trial resources: Computer laboratory, cold chain management, clinical laboratory and a 40-bed pediatric facility are established. Ethics review: Independent institutional scientific and ethics review committees were set up to ensure adherence to scientific and ethical guidelines. Collaborations: Existing collaborations with academic institutions such as The London School of Hygiene and Tropical Medicine were strengthened while new ones were established. Main outcomes: Human resource capacity and infrastructure for clinical trial is developed in KHRC. These have been used to successfully conduct high quality operational clinical trials and investigational new drug trials under GCP/ GCLP standards. In conclusion, development of clinical trial site to conduct internationally acceptable clinical trials is achievable within 5 years in Africa. However, challenges such as availability of reliable electricity, maintenance of cold chain for investigational products, internet connectivity and core funding to maintain developed infrastructure need to be strategically considered in Africa.

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WHEN MULTIPLE REGRESSION IS JUST NOT ENOUGH

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In tropical medicine, just as in other branches of medicine, risk factors for the onset or outcome of diseases are usually sought using conventional statistical regression methods. These methods assume that the risk factors (predictor variables) do not influence each other (i.e. are statistically independent). This assumption is frequently invalid, in which case the conclusions drawn may be incomplete, or even invalid. Deeper insight can sometimes be achieved by adding risk factors in blocks, using hierarchical regression methods; adjustments can be made for the effects of potential confounder variables in the same way. However, these methods still assume (usually incorrectly) that the risk factors are independent. Conventional regression analyses essentially fit a simple mathematical model to data. In recent years, structural equation modelling (SEM) methods have been developed that hugely increase the potential complexity of these mathematical models. Importantly, they allow for relationships to exist between risk factors. SEM methods have been widely used in psychology for many years, but are only slowly starting to be used to address important medical research questions. The potential, and very considerable, gains possible with SEM methods will be demonstrated using data from a case-control study of 381 pre-school children with severe anemia (haemoglobin concentration <5g/dl) and 757 pre-school children without severe anemia in urban and rural settings in Malawi. The possible multiple causes of severe anemia in Malawian pre-school children will be evaluated using (a) univariate statistical methods only, (b) simple multiple regression, (c) hierarchical multiple regression, and (d) SEM methods. SEM methods will be shown to provide a considerably greater insight into the causes of anemia in this population. In particular, clinically important inter-relationships between six known risk factors (iron deficiency, hookworm, HIV, vitamin A deficiency, malaria and bacteremia), and the influence of these inter-relationships on their association with severe anemia, will be described that were identifiable only using SEM methods.

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MALARIA DECISION SUPPORT SYSTEMS - LESSONS LEARNT

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There are a large number of human pathogens in nature that are transmitted by arthropods. These vector borne pathogens typically infect, replicate and develop in both the vector and human host. There are over 100 countries at risk from malaria encompassing almost 50% of the worlds population. There are up to 300 million episodes and 1 to 3 million deaths a year from malaria. This disease has major economic and health impacts for a disease endemic country. A public health surveillance system, based on the systematic collection of relevant information and the analysis and timely dissemination of data, to those responsible for controlling the disease, is essential. For a vector borne disease a good surveillance system should include: Disease detection via passive (patient data from health facilities) or active surveillance (visiting the community and testing individuals); Entomological surveillance through monitoring of species density, infectivity (sporozoite rate) and insecticide resistance; and Environmental surveillance including climate and geographical data. Successful malaria control currently relies on effective drug treatment and vector control. Vector control is insecticide-based mainly through indoor residual spraying (IRS) or deployment of insecticide treated bednets (ITN). This paper focuses on the development of a Malaria Decision Support System that has cross cutting implications to other arthropod vector borne diseases.

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AN ASSESSMENT OF BLOOD VOLUMES IN RELATION TO SYMPTOM RESOLUTION IN SEVERELY ANEMIC MALAWIAN CHILDREN

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Evidence based guidelines for the in-hospital management of children with severe anemia requiring life-saving blood transfusion is lacking. The WHO currently recommends that 10mls/kg of packed cells be given to all children with hemoglobin levels of < 4 g/dl and those with hemoglobin of 4 - 6 g/dl and clinical signs heart failure over 3 - 4 hours. The rate at which this is given and how much blood is required especially in the early stages of transfusion is controversial. The aim of this study was to assess the clinical responses to blood volumes transfused in children with severe anemia. Children with severe anemia requiring a blood transfusion presenting to the Pediatric A and E Department of the Queen Elizabeth Central Hospital, Blantyre Malawi were recruited into the study. Demographic and clinical parameters were recorded on standard observation sheets. Detailed clinical assessments were carried out every 15 minutes for the 1st 2 hours, then every 30 minutes for the last hour. Post-transfusion hemoglobin was measured after 24 hours. A total of 132 children (48.5% males) whose ages ranged from 3 - 64 months were recruited over a period of 8 weeks. The in-hospital mortality was 4.5% (6/132). Mean pre-transfusion Hb was 4.07 ± 1.00 g/dl, and mean post-transfusion Hb was 6.41 ±1.91 g/dl. 96 children (73.8%) had malaria parasitaemia. The average time it took from admission to receive a transfusion was 69.4 minutes, and transfusions took an average of 175.7 minutes to be completed. No child required administration of frusemide for fluid overload. Significant drop in heart rates (157.6 to 148.8 beats/min; mean difference 8.8 ± 10.9; P = 0.00; 95% CI 6.9 - 10.7) were observed within 30 minutes of blood transfusion, but mean heart rate did not fall below 140 beats/min (WHO criterium) till 105 minutes into transfusion. A drop in mean respiratory rate (50.9 to 48.5 cycles/min; mean difference 2.5 ± 10.2; P = 0.01; 95% CI 0.7 - 4.4) was observed at 45 minutes of transfusion. In conclusion, significant changes in heart rate

and respiratory rates can be seen early in transfusion without signs of fluid overload. A more aggressive approach to the speed of correcting severe anemia may be be considered in order to prevent early death without risking fluid overload. There is need to review the WHO recommendations for blood transfusion in severe anemia, to ensure limited morbidity and mortality during the post-discharge period.

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IMPLICATIONS OF A CHANGE IN THE CASE DEFINITION OF LYME DISEASE SURVEILLANCE - MAINE, 2007

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Lyme disease (LD) is a tickborne infection caused by the spirochete *Borrelia burgdorferi*. Within 1 month of infection, erythema migrans (EM) occurs in 70%-80% of cases and is considered the most reliable early clinical sign of LD. Late manifestations include rheumatologic, neurologic, and cardiac complications. In 2008, the Council of State and Territorial Epidemiologists implemented a new LD surveillance case definition for reporting in the United States. We evaluated the impact of the new definition by reviewing LD reports in Maine in 2007 using both old and new case definitions. The 1996 confirmed LD case definition included EM, or at least one late manifestation, and laboratory confirmation (two-tier testing: ELISA followed by IgM and IgG immunoblots). The 2008 case definition included EM with known tick exposure, EM with laboratory confirmation and without known tick exposure, or at least one late manifestation with laboratory confirmation. Laboratory confirmation included two-tier testing or a single seropositive IgG immunoblot. The new case definition also included probable and suspect case categories. Among 1,103 reported cases in 2007, on the basis of the 1996 definition, 529 (39.7 cases / 100,000 population) confirmed cases were identified. Using the 2008 definition, 568 (43.0 cases / 100,000 population) confirmed cases were identified. Use of the new case definition also resulted in 141 probable cases and 60 suspect cases. Noncases decreased by 42% from 574 noncases that used the 1996 case definition to 334 noncases that used the 2008 case definition. Applying the 2008 case definition resulted in a 7% (n=39) increase in the number of laboratory-confirmed cases of LD. All involved late manifestations (90% [35] arthritis and 10% [4] Bell's palsy) with a single seropositive IgG immunoblot. Implementation of the 2008 LD case definition resulted in a small increase in confirmed cases. A decrease in the number of noncases resulted from creation of probable and suspect case categories. These findings should be considered when examining trends in reported LD cases.

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IDENTIFICATION OF *RICKETTSIA* FROM TICK SPECIES COLLECTED IN TENNESSEE

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Rocky Mountain Spotted Fever (RMSF), caused by *Rickettsia rickettsii*, is an important tick-borne disease of the southeastern United States. Since 1990, human RMSF incidence rates in Tennessee have rapidly increased and follow a gradient with low incidence, 0-1.4 cases per 100,000, in the eastern third of the state and high incidence, 2-2.9 cases per 100,000, in the western third. This pattern is poorly understood due to limited data on Tennessee's tick species diversity, host diversity, and pathogen prevalence. We present here an extensive *Rickettsia* spp. survey conducted in Tennessee. Ticks used in this study were collected by the USDA, Animal and Plant Health Inspection Service, Wildlife Services program and Tennessee Department of Health (TDH) both from animal hosts and by dragging. To date, 1,242 ticks from twenty-seven counties have been sent to the TDH. All ticks were speciated and identified to life stage. DNA was extracted and tested for *Rickettsia* spp. using real-time and conventional PCR. Bacterial species were determined via sequencing or restriction fragment length polymorphisms. Of 1,242 collected ticks, 1,031 (83%) were American dog ticks (*Dermacentor variabilis*), 159 (13%) lone star ticks (*Amblyomma americanum*), 29 (2.3%) *Ixodes texanus,* 19 (1.5%) *Ixodes cookei,* and 4 (0.3%) *Ixodes scapularis.* Of 454 ticks tested, 113 (24.8%) were positive for *Rickettsia*, though 18 are equivocal. Of 323 *D. variabilis* tested, 37 (11.4%) were positive for *R. montana,* a non-pathogenic strain of *Rickettsia*, and 3 (1%) for *R. amblyommii*, a putative agent of Southern Tick Associated Rash Illness. Of 97 *A. americanum* tested, 51 (53%) were positive for *R. amblyommii,* 1 (1%) for *R. montana,* and 1 (1%) for *R. parkeri,* a pathogenic Spotted Fever Group rickettsia. Of 4 *I. scapularis* tested, 2 (50%) were positive for an unidentified rickettsia. This is the first report of *R. parkeri* isolated from a lone star tick in Tennessee. We did not identify any ticks positive for *R. rickettsii.* Rickettsial species other than *R. rickettsii* may play a role in tick-borne diseases in Tennessee, including cases serologically classified as RMSF.

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UTILIZATION OF QPCR FOR RAPID CHARACTERIZATION AND AUTHENTICATION OF LARGE AND DIVERSE RICKETTSIA COLLECTIONS

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In support of *Rickettsia* research and product development efforts, the ATCC and BEI Resources have begun to characterize our extensive *Rickettsia* collections which contain representatives from a broad range of clinical and animal samples of *Rickettsia* and near neighbors. To screen our samples we designed and optimized three unique qPCR assays. The first qPCR assay is a pan-*Rickettsial* assay that was used to confirm the molecular identity of the deposited samples as *Rickettsia*. The second and third assays are species specific tests designed to detect either *R. prowazekii* or *R. rickettsii*. Each assay has been developed with a plasmid-based quantitation standard and has a detection limit of approximately 10 copies per reaction. Specificity has been established for all three assays using both a *Rickettsia* organism control panel and a broad exclusivity panel that contained representatives from near neighbors and unrelated organisms. The pan-*Rickettsial*, *R. rickettsii,* and the *R. prowazekii* assays specifically identified all of the *Rickettsia, R. rickettsii* and *R. prowazekii* samples respectively and did not detect organisms in the exclusivity panel. Using the pan-*Rickettsial* qPCR assay, we were able to evaluate and verify the *Rickettsia* samples in our collection. Using the *R. prowazekii* and *R. rickettsii* specific assays we confirmed the identities of these two species where expected and were able to identify and segregate mixed populations. This ensured that samples destined for customers did not contain trace amounts of either *R. prowazekii* or *R. rickettsii* which have been included on the HHS Select Agent List and NIAID Priority Pathogen List. As a result of this testing, numerous *Rickettsia* organisms and their associated nucleic acids are being made available through the BEI Resources and/or ATCC programs for research and development purposes.

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DETECTION OF SPOTTED FEVER GROUP *RICKETTSIA* IN IXODID TICKS COLLECTED IN LOS ANGELES COUNTY, CALIFORNIA

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Dogs are commonly attacked by the same species of ixodid ticks that bite humans and can become infected with spotted fever group rickettsiae (SFG), so they can serve as sentinels for human rickettsial diseases. Several cases of canine Rocky Mountain spotted fever (RMSF) were identified in Los Angeles County, CA during 2006-2007 based on clinical diagnosis and serological testing. Detection and identification of SFG in ticks was conducted to improve our understanding of the epidemiology of rickettsial diseases in the county. 445 questing ticks, including 285 *Dermacentor occidentalis* and 160 *Ixodes pacificus*, were collected from vegetation along seven hiking trails visited by sick dogs and their owners. DNA was extracted from individual ticks and rickettsial screening was conducted using a SYBR-Green PCR assay detecting a fragment of the OmpA gene of the SFG. Semi-nested PCR was performed for positive specimens to amplify a 70-602 nt fragment of *omp*A which was used for species identification of rickettsiae detected by DNA sequencing. In total, 83% of *I. pacificus* and 37% of *D. occidentalis* were found positive for SFG rickettsiae using SYBR-Green PCR. All of the *I. pacificus* tested were from the same site and were positive for DNA of a SFG rickettsia whose *omp*A sequence is closest to that of the rickettsial endosymbiont of *I. scapularis*, which is believed to be non-pathogenic. *Dermacentor occidentalis* positive for SFG rickettsiae were found at 6 of 7 collection sites, with a detection rate ranging from 16% to 80%. DNA of *Rickettsia rhipicephali* was detected most frequently in *D. occidentalis* and it was found in 4 locations. SFG genotype 364D was found in 5 ticks collected at 3 of the 4 locations which also had *R. rhipicephali*. In conclusion, our study provides the first molecular data on the prevalence and species identification of SFG rickettsiae in *Ixodes pacificus* and corroborates our previous findings with *Dermacentor occidentalis* from other sites in Los Angeles County of California. Since *R. rickettsii* was not found in these ticks, either the unique genotypes of *R. rhipicephali* found in *D. occidentalis* (as reported previously) cause canine disease or 364D genotype may be the cause of spotted fever infections in dogs and possibly humans.

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CYTOKINE-RELATED GENE EXPRESSION IN THE PERIPHERAL BLOOD AND DENGUE INFECTION SEVERITY

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Dengue is the most prevalent mosquito-borne viral disease and has become one of the most important public health problems worldwide. Infection by any of the serotypes of dengue viruses (DEN-1, -2, -3 and -4) can produce a wide spectrum of clinical manifestations ranging from a simple febrile illness, dengue fever (DF) to a severe form with plasma leakage, bleeding and shock. The pathogenesis of dengue hemorrhagic fever and dengue shock syndrome (DHF/DSS) is thought to be mediated by various host factors. However, the mechanism underlying disease severity is not fully understood. Previous studies have suggested an involvement of immune response mediators in the severity of dengue infection. The aim of this study was to elucidate the cellular gene responses to dengue viral infection at the transcriptional level and to correlate expression levels with disease activity and/or clinical manifestation. Whole blood mRNA from children with dengue infection was analyzed on the day of defervescence. Expression levels of IL-8, IL-1β, MMP-9 and IL-10 in peripheral blood leukocytes were assayed in 30 children with DF, 19 children with DHF and 10 healthy controls by real-time reverse transcription quantitative polymerase chain reaction (RT-PCR). Compared to controls, the expression levels of IL-8, IL-1β, MMP-9 and IL10 were higher in children with dengue infection. IL-8 mRNA levels were also elevated in patients with DHF compared to those of DF. However, there was no statistically significant difference between patients with DF and DHF in the expression levels of IL-1β, MMP-9 and IL10. In conclusion, the expression levels of IL-8, IL-1β, MMP-9 and IL10 were higher in children with dengue infection suggesting that these mediators may be involved in the disease pathogenesis. The mRNA expression levels of IL-8 were elevated in DHF compared to those of DF while the others were not. The expression pattern of these genes in peripheral blood leukocytes might serve as a predictor of dengue infection as well as disease activity.

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CLIMATIC FACTORS, ENTOMOLOGIC ATTRIBUTES AND EPIDEMICS OF DENGUE IN TAIWAN, 1998 - 2006

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Dengue has seasonal trends like most of other mosquito-borne diseases. Meteorological factors affect on not only the biology of dengue viruses and mosquito vectors, but also the viral transmission between mosquitoes and humans. The mechanism of climate influencing the occurrence of dengue is very complicated and controversial. This study intended to simplify the correlations among meteorological factors, mosquito measurements and biweekly dengue data in southern Taiwan from 1998 to 2006 using regression model. We hope to make the meteorological knowledge more informative and helpful in predicting the epidemics of dengue for making best public health decision. The preliminary results found that previous biweekly number of dengue cases, temperatures and El Nino southern oscillation index were very good predictors for subsequent dengue cases. Other meteorological factors like wind, sunshine were also found explainable for the followed-up trends of dengue and they were worthy for further investigation. The uniqueness of this study is to consider meteorological factors in a more comprehensive way integrating with the statistical predication models that well fit assumptions of the distribution of variables. Future efforts include simulating the occurrence of disease with climatic and entomologic data to better predict the impact of global warming on the epidemics of dengue with more international perspectives.

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DETECTION AND IDENTIFICATION OF BIOMARKERS FOR DENGUE FEVER (DF) AND DENGUE HEMORRHAGIC FEVER (DHF) USING PLASMA SAMPLES FROM THAI CHILDREN AND SELDI-TOF-MS TECHNOLOGY

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Surface-Enhanced, Laser-Desorption and Ionization, Time-Of-Flight Mass Spectrometry **(**SELDI-TOF MS) permits the study of the protein/peptide content of diverse biological fluids such as serum, plasma, urine, cell lysates and tissue extracts. This high throughput proteomic platform has been used to identify biomarkers for a wide range of inflammatory, infectious and neoplastic conditions. The promising results obtained in

these varied conditions raised the possibility that SELDI could be applied to dengue virus (DENV) infection to develop urgently needed diagnostic tests. Plasma from 21 pediatric Thai children with either confirmed primary dengue fever (DF) or dengue hemorrhagic fever (DHF) and 15 samples from Thai children admitted to hospital with other febrile illnesses (OFI) were analyzed and their proteomic profiles obtained by SELDI-TOF MS. Biomarkers that could discriminate between patients with DENV infection and those with OFI were sought. Seventy-two candidate biomarkers with p-values ≤ 0.01 and ROC values ≥ 0.85 were detected. Eleven prognostic biomarkers that discriminated between DF and DHF were identified; three of which were present early in infection (at time of hospital admission). The most promising candidate biomarkers were identified using SDS-PAGE gels and MS microsequencing. Twenty-eight distinct proteins/peptides were identified, several of which may plausibly be involved DENV pathology. Finally, proteomic profiles were obtained from 51 plasma samples from children with confirmed secondary DF or DHF. These profiles were strikingly different between primary and secondary DENV infection (>100 candidate biomarkers) despite the apparent clinical similarities between these two conditions. This study demonstrates the potential for high-throughput proteomics to develop useful diagnostic and prognostic tests for DF/DHF. Moreover, these biomarkers may give unique insight into the mechanisms that underly the varied manifestations of DENV infection.

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CLASSIFICATION AND REGRESSION TREE (CART) ANALYSIS USING CLINICAL LABORATORY VARIABLES KNOWN TO BE ASSOCIATED WITH DENGUE TO ESTABLISH EARLY DISEASE CLASSIFICATION

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Establishing an early diagnostic tool to effectively distinguish patients with dengue from patients with other febrile illness (OFI) based on routine laboratory measures would have a substantial impact on clinical practice in dengue-endemic, resource-poor countries. We analyzed data from the Dengue Hemorrhagic Fever (DHF) Project, a prospective study of Thai children aged 6 months to 14 years who presented to Bangkok Children's Hospital or Kamphaeng Phet Provincial Hospital with fever onset <72 hours prior to study entry and temperature >=38°C. Clinical laboratory data were collected daily until hospital discharge or 5 days, whichever occurred first. Variables of interest for this analysis were those measured at day of study enrollment and included: platelets, AST, ALT, albumin, WBC, neutrophils, lymphocytes, monocytes, and hematocrit. Classification and regression tree analysis (CART) was used to create a classification tree to distinguish patients with dengue from patients with OFI. Minimum risk was used to establish the final tree, where any child node that did not have a significant risk at α=.05 was "pruned." Stopping criteria was defined as a child node with at least 5% of the original sample. From 1994-1997 and 1999-2001, 390 patients had dengue (153 DHF and 237 dengue fever) and 202 had OFI. A classification tree using WBC and then neutrophil count was found to best distinguish patients with dengue from patients with OFI. This classification tree yielded an overall sensitivity and specificity of 86% and 75%, respectively. In conclusion, a classification tree using only WBC and neutrophil counts provides a moderate overall sensitivity and specificity in distinguishing patients with dengue from patients with OFI presenting within the first 72 hours of illness. Platelet count, AST, ALT, albumin, and hematocrit were not found to improve the classification tree at this early stage of illness. CART has advantages over logistic regression models such as: 1) it is a non-parametric method and is thus not bound to the assumptions of logistic regression, 2) it does not require complex calculations, as in some logistic regression models, and 3) the resulting algorithm mimics a clinician's way of thinking. Application of a simple diagnostic algorithm such as this could improve the utilization of limited resources in poorer countries, especially during dengue outbreaks.

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PRIMARY AND SECONDARY INFECTIONS TO DENGUE VIRUS IN PERU - 2007

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The infection by dengue fever (DF) and hemorrhagic dengue fever (HDF) represents a problem of public health in Peru, with epidemic outbreaks every year by different serotypes in diverse areas of the country. The objective was to identify primary and secondary dengue infections in acute samples diagnosed like DF in 2007. We included 255 acute samples diagnosed by viral isolation in C6/36 and/or RT-PCR assay, in different outbreaks in new or endemic areas from the North coast, Eastern and Central forest during the 2007. The samples including were: 106 for DEN1, 1 for DEN2, 92 for DEN3 and 56 for DEN 4, respectively, these samples were analyzed by Inhibition ELISA (IE), MAC-ELISA and GAC-ELISA. We finding 6 (2,3%) positives samples for total antibodies for IE that correspond to secondary infections and 249 (97.7%) positive samples that correspond primary infections. The high percentage of primary infections in new populations by DEN1, DEN3 and DEN4 were associated to DF; on the other hand, populations with secondary infections by DEN3 were associated to DF with hemorrhagic manifestations. In this year we reported HDF associated to primary infections by DEN3.

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NOVEL SUBUNIT VACCINES FOR PREVENTION OF DISEASES CAUSED BY DENGUE AND OTHER FLAVIVIRAL PATHOGENS

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Dengue viruses (DV) cause significant morbidity and mortality annually worldwide. Although primary DV infection with any of four serotypes usually results in asymptomatic or mild febrile illness, secondary infection with a different serotype can lead to more severe disease (i.e., dengue hemorrhagic fever (DHF) and/or dengue shock syndrome (DSS)) via a phenomenon known as "antibody-dependent enhancement" (ADE). ADE is thought to be mediated by antigenically cross-reactive antibodies that are generated during primary infection but are unable to neutralize newly infecting different serotypes. Instead, in this instance such antibodies act to facilitate viral entry into antibody Fc? receptor-bearing cells. To minimize ADE activity while maximizing the induction of serotype-specific neutralizing antibody responses, vaccine formulations now being evaluated are tetravalent (either killed or live attenuated), and all involve the administration of all or most of the viral E glycoprotein, the major coat protein of the virus. E protein consists of three domains (domains I, II and III). Antigenically cross-reactive but mostly non-neutralizing epitopes are located within domains I and II, whereas serotype-specific neutralizing epitopes have been found to reside primarily within domain III (DIII). In this study we examine the possibility that vaccination with DIII alone can elicit serotype-specific neutralizing antibody responses in the absence of ADE activity. Toward that end we have generated recombinant DIII immunogens of all four DV serotypes and also Yellow Fever Virus (YFV), to be tested in mice. We have found that administration of these immunogens, either alone or in combination, causes the induction of serotype-specific neutralizing antibody responses that exhibit minimal antigenic cross-reactivity by ELISA with other flavivirus serotypes. Thus, this appears to be a feasible strategy for induction of broadly protective immunity against dengue and other flaviviral pathogens.

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IMMUNOGENICITY OF A PSORALEN-INACTIVATED DENGUE-1 VIRUS VACCINE CANDIDATE IN MICE

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Dengue viruses are geographically widespread flaviviruses transmitted to humans by the *Aedes aegypti* mosquito. Up to 100 million cases of dengue virus infections occur every year in tropical and subtropical regions. There is no commercially-available vaccine to prevent either primary dengue fever or the more severe dengue hemorrhagic fever. Psoralens are photoreactive compounds that cross-link pyrimidine residues after exposure to UV-A radiation. In this study, we evaluate the immunogenicity of a novel psoralen-inactivated dengue-1 virus (DENV-1) vaccine candidate in *Mus musculus*. We tested three psoralen compounds with varying doses of UV radiation to find the optimum combination for viral inactivation. A concentration of 10 µg/ml of 4-aminomethyltroxsalen (AMT) in combination with 5 minutes of exposure to UV-A radiation at a dose of 1000 µW/cm2 led to inactivation of DENV-1, WestPac 74 strain, 5 ml at a concentration of 3.4 x 10^5 PFU/ml. No plaque-forming units were identified when the treated specimen was titered in Vero cells, as compared with 5.63x10^5 PFU/ml noted when the DENV-1 specimen was exposed to an equivalent amount of UV-A in the absence of a psoralen. Three groups of 7 mice each received either 5 ng per dose of purified, psoralen-inactivated DENV-1, plus alum (1.3 mg/ml aluminum hydroxide gel) adjuvant; 10 ng per dose of psoralen-inactivated dengue virus, plus alum; or alum alone (controls). All doses were injected intradermally into the mouse's tail on study days 0, 14, and 28. Sera was collected from the mice on study days 0, 14, and 28 days and assayed for anti-DENV-1 IgM and IgG by enzyme-linked immunosorbent assay (EIA) and PRNT. Anti-DENV-1 IgG EIA, IgM EIA, and PRNT were negative in all mice at baseline. At 28 days, the 5 ng dose mice showed detectable IgG in 7/7 (range 0.707-3.000) but neutralizing antibody by PRNT in only 3/7 (1:70-1:166). All 10 ng group mice (7/7) had detectable IgG (1.600-3.000) and positive PRNTs (1:42-1:293). Anti-DENV1 IgG, IgM, and PRNT were negative in all control mice at 28 days. IgM was detected only in 2/7 5 ng group mice and 1/7 10 ng group mice at 28 days. In conclusion, Psoralen-inactivated DENV-1 is immunogenic in mice and leads to the production of *in vitro* neutralizing antibody, with greater potency seen in the 10 ng group than in the 5 ng group or controls at 28 days. Further testing in non-human primates is needed as part of development of this agent as a vaccine candidate.

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HANDHELD TECHNOLOGY FOR EFFICIENT INTERVIEWS TO ESTIMATE THE BURDEN AND ECONOMIC COST OF SYMPTOMATIC DENGUE: PILOT IN PUERTO RICO

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Quantifying the disease burden and economic cost of illness are important steps towards developing effective policies for prevention and control. As the systematic collection of required data through patient interviews is time consuming and expensive, few comprehensive studies exist for dengue or other neglected tropical diseases (NTDs). To address this challenge, we are piloting and evaluating a handheld device to interview dengue patients. We developed a bilingual (English or Spanish) electronic questionnaire which adapts the wording to the sequence number of the interview (first or second) and the interviewee (proxy or patient). It incorporates branch logic depending on treatment patterns and household size. The questionnaire also incorporates reminders for interviewers and prompts to respondents to facilitate complete recall of illness duration, quality of life, use of health services, time and income lost, and expenditures incurred by each household member. With automatic cross references in the second interview for names and dates, the procedure minimizes the risk of omitting or duplicating relevant information. We programmed this questionnaire in software for a handheld device (Questionnaire Development System) allowing its use at the patient's bedside, clinic, or home. We are applying this questionnaire to estimate the full economic costs of suspected dengue for patients enrolled at two health care facilities in Puerto Rico (Auxilio Mutuo Hospital in San Juan and Centro Servicios de Salud in Patillas). We are merging the resulting costs per case with official case reporting and expansion factors based on active surveillance to adjust for underreporting. This technology is proving to be efficient and accurate. The automatic cross references and prompts reduce the interview time. In addition, automatic range checks and forced responses minimize invalid and missing data. Furthermore, as the data are collected directly into the device and easily transferred to a computerized database, the time and expense for coding, double entry, and cleaning are avoided, and preliminary findings can be reported rapidly. In conclusion, adaptive wording, branching, and multiple languages, electronic questionnaires in handheld devices are an efficient and promising tool for estimating the burden and cost of one or several NTDs, especially in multi-facility and multi-country studies.

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INFECTION OF *AEDES AEGYPTI* BY DENGUE VIRUS TYPE 2 STRAIN 16681

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The four serotypes of dengue virus are the most medically important arthropod-borne viruses infecting humans today. Approximately one third of the world's population is at risk of becoming infected by dengue due to the distribution of its primary vector, *Aedes aegypti*, and the need for a dengue vaccine has never been greater. Currently, the leading vaccine candidates are live attenuated formulations that must be evaluated for safety and efficacy. An important immunogen in these vaccines is envelope (E) glycoprotein, which is involved in virus-host cell attachment and virus mediated cell membrane fusion, and is the primary target of neutralizing antibodies. A desirable characteristic of a live attenuated dengue vaccine is its inability to infect *Ae. aegypti* in order to prevent the potential spread of virus from vaccinees. Dengue virus type 2 strain 16681 (DENV2, 16681) is the parental virus for the vaccine candidate PDK-53. We have introduced selected mutations into the E protein gene of an infectious cDNA clone of DENV2, 16681 (D2/IC 30P-NBX), and assessed the mutant phenotypes to determine the role of the E protein in mosquito infection. While parental D2/IC 30P-NBX infects and replicates efficiently in most tissues of *Ae. aegypti* RexvilleD mosquitoes after intrathoracic inoculation, infection of the midgut epithelium after introduction of virus by infectious bloodmeal is less efficient, suggesting that midgut attachment and entry is a limiting step in transmission of this strain of DENV2. Seven recombinant virus genomes were engineered to result in single amino acid mutations in the fusion loop of the E protein. Infectious fusion-loop mutant viruses were recovered and replicated as well as the parent strain in cultured C6/36 mosquito cells. With the exception of one mutant virus, all viruses infected a lower proportion of RexvilleD mosquitoes than the parent after intrathoracic inoculation. The D2/IC 30P-NBX infectious clone can be used as a powerful tool to investigate the determinants of the E protein that are required for mosquito infection.

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AN AUTOMATED DENGUE VIRUS MICRONEUTRALIZATION PLAQUE ASSAY PERFORMED IN VERO CELLS AND IN HUMAN FCγ RECEPTOR-EXPRESSING CV-1 CELLS

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We describe microneutralization assays that employed automated ELISPOT readout instrumentation to measure dengue virus (DENV) antibodies in conventional Vero cells, and in CV-1 cells that were engineered to constitutively express human FcγRIIA (CD32). The assays were performed in 96-well plates and used a DENV serogroup-reactive anti-NS1 monoclonal antibody to immunostain virus plaques. Classical 50% plaque reduction neutralization end-point titers (PRNT50) were determined against all four DENV serotypes in Vero cells and with DENV-2 in empty vector control- and CD32-expressing CV-1 cell lines. Neutralization results in Vero and control CV-1 cells were similar. Strikingly lower PRNT titers against virulent strain 16681 DENV-2, however, were observed in CV-1 /CD32 transfectants compared to those observed in control CV-1 or Vero cells. Since DENVs may preferentially replicate in CD32-expressing monocytes/macrophages and dendritic cells, *in vivo*, it is possible that CD32 introduced into a conventional dengue virus neutralization assay might provide results that will better correlate with protection.

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EPIDEMIOLOGICAL BURDEN OF DENGUE OF OFFICIALLY REPORTED DENGUE CASES IN EIGHT COUNTRIES IN THE AMERICAS AND ASIA

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Dengue disease burden has not been carefuly studied in most of the dengue endemic countries. Using a common protocol, we present national estimates of the dengue disease burden in QALYs (Quality Adjusted Life Years) in eight American and Asian countries. We conducted prospective studies of the cost of dengue in Brazil, Cambodia, El Salvador, Guatemala, Malaysia, Panama, Thailand and Venezuela. All studies followed the same core protocol with interviews and medical record reviews. The study populations were patients treated in ambulatory and hospital settings with a clinical diagnosis of dengue. Most studies were performed in 2005. For each patient, we documented the number of days of fever, of feeling bad or very bad, and of illness. In addition, using the Euro-Qol thermometer scale, we obtained information about the self- or proxy- perceived quality of life during the illness episode. As quality of life was measured in a visual scale, we explored various transformations into a utility scale for QALYs estimation. For each country, we obtained the number 2001-2005 officially reported dengue cases by setting (ambulatory and hospitalized) and age group (children and adults). Finally, we estimated dengue burden by country with and without expansion factors for underreporting (based on literature review). We studied 1,695 patients (48% pediatric and 52% adult); none died. The average number of days of fever was 4.9 (SD 2.9) days among ambulatory patients and 5.9 (SD 2.8) days among hospitalized patients. The average illness lasted 11.9(SD 6.9) days for ambulatory patients and 10.9 (SD 4.6) days for hospitalized patients. The average days in which patients felt either "bad" or "very bad" were 7.3 days and 6.3 among ambulatory and hospitalized patients, respectively. The lowest quality of life reported averaged 30.4 in ambulatory and 32.6 in hospitalized patients, corresponding to losses of 69.6% and 67.4%, respectively, from the best imaginable health status. Annually, the number of dengue cases and deaths officially reported averaged 574,000 and 399, respectively. Reported expansion factors for cases ranged from 1.5 to 27. For global QALY estimations, linear transformation of the visual scale into utility provides an initial step. In conclusion, reported dengue is associated with a large disease burden; after adjustment for underreporting, the burden is substantially greater.

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THE SEROEPIDEMIOLOGIC INVESTIGATION OF DENGUE ILLNESS VERSUS DENGUE VIRUS INFECTION AFTER THE 2007 OUTBREAK IN TAINAN CITY, TAIWAN

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Tainan City experienced the most severe outbreak of dengue from June 2007 to January 2008 with nearly 1500 cases caused by serotype 1 (DENV-1) since 1943. The outbreak spread the Central Districts of the city with higher population density. Since Tainan had outbreaks of DENV-1 (1987-88), DENV-2 (1997) and DENV-3 (1998), it is very crucial to conduct a community- based seroepidemiological study. The aims of this study are to measure the seroprevalence of DENV infection, to identify the high risk populations using our newly developed temporal indices [occurrence (wks of dengue cases/year), duration (mean duration/wave), and transmission intensity (incidence of cases with consecutive weeks per wave)] , as reported previously, and spatial statistics, to find out the relationships between the infection and dengue illness, and to search for their possible risk/prevention factors. Both dengue-IgM and IgG antibodies were screened by ELISA tests and only seropositive ones were serotyped by plaque reduction neutralization test (PRNT) Past history of dengue and environmental factors were collected from questionnaire. Based on the high vs low clusters of the above three indices, we chose the three Li units (the smallest administrative unit in Taiwan) located near downtown Tainan for further study. In high cluster group, Yu-Nong Li with occurrence index: 2.01, duration index: 0.95 wks/wave and intensity index: 2.69 dengue cases/10,000 population/wave), and Liou-Jia Li (occurrence index: 6.62, duration index: 2.47 wks /wave and intensity index: 4.64 dengue cases/10,000 population/wave) were selected. In the low cluster group, Tong-An Li (occurrence index: 0.68, duration index: 0.33 wks /wave and intensity index: 0.29 dengue cases/10,000 population/wave) was targeted. In addition, most dengue cases were 19-64 year-old adults (70.58%, 854/1210), strikingly different from epidemiological characteristics of dengue in South East Asia. In fact, pre-epidemic seroprevalence of DENV-IgG in 2006 was 6.10 % (5/82) in one elementary school children. However, 6-12 year old children accounted for 5.37% (65/1210) of the total dengue cases in this epidemic. This study used an innovative approach to select study sites considering epidemiological characteristics. We believe our approaches can apply to dengue endemic areas such as Thailand and South America countries.

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CHARACTERIZATION AND GROWTH OF A DEN-2 PDK-53-BASED CHIMERIC TETRAVALENT VACCINE

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Dengue fever is a worldwide public health threat caused by infection with one of four different RNA viruses: DEN-1, DEN-2, DEN-3 or DEN-4. No dengue vaccine is currently available nor is there an antiviral therapy for dengue virus infection. Invivagen's DENVax tetravalent vaccine is based on the live-attenuated candidate dengue type 2 (D2) PDK-53 vaccine which has been shown to be safe and immunogenic, generating long-lasting neutralizing antibodies in human clinical trials. Using D2 PDK-53 as the genetic backbone, we have engineered candidate chimeric vaccine viruses that express the structural genes of D1, D3 and D4. To complete development and clinical testing of these vaccine viruses, we have formed an international consortium consisting of scientists at Invivagen, the Centers for Disease Control and Prevention, the University of Wisconsin and Shantha Biotechnics. GMP-quality seed stocks for each of the four vaccine viruses were rederived via transfection of certified Vero cells with viral genomic RNA transcribed from the original infectious cDNA clones. Following amplification of these seeds, all four re-derived viruses contained the expected genomic sequences. The seed viruses were sequentially plaque-purified, and 24 isolates were spot sequenced to ensure retention of the three D2 PDK-53-specific attenuating mutations. A single premaster seed virus for each dengue serotype was chosen based on genome sequence analysis. Here we present data on the characterization of our vaccine master seed viruses including genome sequencing, temperature sensitivity in mammalian cells, reduced replication in insect cells, neurovirulence testing in newborn ICR mice, and TaqMAMA analysis. Additionally, we have optimized conditions for large scale flavivirus vaccine manufacture, including growth in a novel growth medium containing a polyoxyethylene polyoxypropylene block copolymer. This media accelerates virus growth while contributing to stability of the final formulated vaccine. These studies are in preparation for human clinical testing of the tetravalent DENVax vaccine. Development of an affordable, safe, and effective dengue vaccine will protect those most at risk of dengue, DSS, and DHF.

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RAPID MOLECULAR TYPING OF DENGUE VIRUSES

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Dengue fever is the most important arbovirus infection in the world. There are four serotypes of dengue viruses. Exact subtyping is important for epidemiological investigations and for understanding the global distribion and the emergence of dengue viruses in new regions. We compared an oligonucleotide array, a lateral flow dipstick assay and partial genome sequencing for their ability to subtype dengue viruses from clinical material and from our virus strain collection. Both, the oligonucleotide array and the lateral flow dipstick assay proved to reliably distinguish and subtype all dengue virus strains from patients and from cultured material tested. While sequencing gave the most detailled information expensive equipment and high experience are necessary to use this technique. The oligonucleotide array and the lateral flow dipstick assay proved to be rapid and simple and therefore can be conducted with low laboratory equipment and also und field conditions.

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USE OF A HIGH THROUGHPUT DENGUE REPORTER VIRUS NEUTRALIZATION ASSAY TO SCREEN PATIENT AND VACCINEE SERA

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Despite the critical need for a safe and effective tetravalent dengue vaccine, development has been hindered by the lack of reliable, high-throughput tools for measuring protective humoral immunity. The gold standard for detecting neutralizing antibodies in human serum is the plaque reduction neutralization test (PRNT), but PRNT is a manual technique poorly suited to large scale, high-throughput assays. Here we use a tool, the Dengue Reporter Virus Particle (DRVP), to rapidly monitor serum for neutralizing anti-dengue virus (DENV) antibodies. DRVPs have been developed by combining a subgenomic replicon encoding an optical reporter (GFP or luciferase) with structural components from defined strains of DENV. Infection is monitored by expression of the reporter gene, providing an objective output that can be quantified using standard optical detection platforms. DRVPs retain the antigenic determinants of wild-type virions, and so can be used to rapidly assess humoral protection against all four DENV serotypes. However, they lack the viral machinery required to propagate infection, allowing their use under BSL2 conditions. Importantly, the DRVP assay can be fully automated for high throughput screening of sera from vaccine trials or epidemiological surveys. We are using DRVPs generated from the DENV-1 WestPac and DENV-2 S16803 PDK50 strains to quantify the neutralizing antibody titers in sera from individuals vaccinated with a live attenuated DENV vaccine and in sera from natural DENV infections. Neutralizing anti-DENV antibody titers derived using DRVPs will be compared with those derived using conventional assays. We are also using DRVPs to determine immune-mediated enhancement of DENV infection in some of these same sera.

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IMPORTANT STRATEGIES TO PREVENT SEVERE EPIDEMICS OF DENGUE HEMORRHAGIC FEVER: TAIWAN'S EPIDEMIOLOGIC FINDINGS TO HELP GLOBAL CONTROL

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Facing the challenging of increasing cases of dengue hemorrhagic fever (DHF) in recent decades in many parts of the world, to prevent and control global epidemics of DHF using epidemiological characteristics is the most important public health concern. In Taiwan where dengue is not endemic, we have had an unprecedented opportunity to identify risk factors of DHF in southern Taiwan that can be used to help minimize the severity of DHF epidemics globally. Studying the past dengue epidemics, we found that DHF cases emerged after a failure to control and the spread of DF clusters. Using geographical information system (GIS), two diffusion patterns (neighborhood spread and relocation diffusion) were identified and that once relocation diffusion occurred, containment became further complicated. In areas with control failure, increasing percentages of DHF cases were observed in later periods of the past three epidemics regardless of serotypes of dengue viruses (DENV) or different geographical locations. While DHF cases emerge, DHF patients have higher viral load than patients with dengue fever (DF), which made it difficult to contain the virus transmission in areas with more DHF cases. The longer the mean duration

per epidemic wave and/or the greater the intensity of transmission at local sites, the higher percentages of DHF cases at the epidemic foci. Once there is an occurrence of DHF or a large-scale DF outbreak, it is crucial to monitor high risk populations in high risk areas through virologic and serologic surveillance. In addition, growing diversity of the quasispecies of DENV has been detected from patients, indicating that the dynamic changes in the population of dengue viruses and natural selection can occur through a series of transmission chains between human and mosquito hosts, resulting in increasing number of DHF late in an epidemic. Our recent research found that daily monitoring of meteorological factors can help predict future outbreaks makes it possible for mostly responsive dengue surveillance system in Asia to become more proactive. Therefore, we believe that improving ecologic/virologic/serologic/clinical/epidemiological surveillance, implementing more comprehensive and integrated community-based prevention and control programs, and using GIS to closely monitor tempo-spatial trends of dengue clusters all together would make preventing or minimizing global severe epidemics of DHF to be possible.

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VECTORIAL COMPETENCE IN *AEDES AEGYPTI* OF TWO DIFFERENT DENGUE TYPE 2 VIRUSES ISOLATED FROM THE SAME GEOGRAPHIC AREA IN MEDELLIN, COLOMBIA

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Dengue viruses (DENV) serotypes 1-4 cause dengue fever (DF), dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS); the most important arthropod-borne viral infection of humans with about 100 million cases and 25,000 deaths annually. In tropical and subtropical regions, DF and DHF are considered one of the most serious health problems. In Colombia, all four DENV serotypes are actively circulating in many parts of the country year round, causing significant epidemics and resulting in an alarming number of cases of DF and DHF/DSS. There has also been a significant increase in the population of the vector *Aedes aegypti* which has expanded into new geographic areas increasing the population at risk of DENV infection. Dengue transmission depends mainly on the vectorial competence of *Ae. aegypti* mosquitoes-defined as the ability of infected mosquitoes to transmit the virus to a new susceptible host. This competence could be affected by phenotypic/genotypic changes in the virus, and due to high mutational rate of DENV, mosquito populations could increase fitness of some viral genotypes circulating in the area. Previous phylogenetic studies have documented the genetic variations occurring in DENV-2 strains during the last decade. These variations could result in phenotypic changes and increase their epidemic potential. We are evaluating in *Ae. aegypti* the vectorial competence of DENV-2 isolated from the same geographic area (Medellin, Colombia), but twelve years apart (1995 and 2007). To test this, DENV-2 isolates (469/1995 and 3986/2007) and a reference strain (S16803) were amplified in C6/36 HT cell cultures and then used to infect *Ae. aegypti* by an infectious bloodmeal via water-jacket membrane feeding apparatus. The viral antigen presence and viral genome quantity were evaluated by IF of midgut and qRT-PCR from heads, respectively, during 5, 8, 12 and 14 days post-blood meal. Our results suggest that there is a difference between infection levels in *Ae. aegypti* with the field isolates and reference strain, showing better infection efficiency in virus proceeding from clinical isolates.

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POTENTIAL USE OF STATINS IN PREVENTION AND TREATMENT OF DENGUE VIRUS INFECTION: *IN VITRO* STUDY

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Statins affect cellular process like cholesterol synthesis, lipid metabolism and protein prenylation; and some of these processes are involved in the replication of several viruses (Hepatitis C, Human Immunodeficiency, Epstein Barr Viruses). Dengue virus (DENV) replication needs the presence of cholesterol and the isoprenylate protein Rab 5. Moreover, in DENV-infected cells the actin is reorganized, and this process is regulated by the Rho GTPases, another group of prenylated proteins. We determined whether Lovastatin (LOV), had inhibitory effect on the DENV infection in VERO (Monkey Fibroblasts) and HMEC (Human Endothelial cells), using three experimental strategies: Before infection (PRE-treatment), during infection (TRANS-Treatment) and after withdrawing the viral inoculum (POST-Treatment). The infections were done with DENV 2, New Guinea Strain at different MOI (10, 1, 0.1). At 24 hours post-inoculation, the supernatants were collected in order to do virus titration and Real Time RT-PCR, and the monolayers were fixed and processed by Cell-ELISA technique. Our results showed that in PRE-Treatment LOV inhibits the amount of viral protein (ANOVA $p<0.05$) approximately 30% in comparison to the controls. In contrast, in POST-Treatment the amount of viral protein increased in presence of LOV approximately 50%. In addition, the amount of viral infectious particles and viral RNA was reduced in PRE and POST-Treatment, approximately a 90% and 50% compared with the control without LOV. TRANS-treatment does not inhibit in significantly manner the protein, RNA or infectious particles viral (ANOVA $p>0.05$). Based in our results, we propose several mechanisms to explain LOV inhibition of DENV infection: When the LOV is added before infection, the initial steps of the viral cycle are being affected, which could involve cholesterol and isoprenylated proteins. When the LOV is added after infection, the final steps of the viral cycle (assembly and release) are being affected. Our results permit postulate that LOV could be used both as prevention and as treatment of the disease. Additional studies are being conducted in our laboratory to determine the mechanism involved in this inhibition.

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ROLE OF INTERFERON IN RESPONSE TO WEST NILE VACCINATION

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Type I-interferons (IFN) control viral infections through establishing a non-specific anti-microbial state (a well defined aspect of the innate immune response), which is thought to directly combat infection, as well as to modulate the adaptive immune response. This latter activity has been used to suggest that the IFN system is important for the development of protective immunity after vaccination, especially by live-attenuated vaccines. Recently we developed a single-round infectious flavivirus vaccine candidate to prevent West Nile (WN) encephalitis named RepliVAX WN. Here, we investigated the importance of an intact IFN system in response to RepliVAX WN vaccination and subsequent lethal WN virus (WNV) challenge. AG129 mice lacking the IFN-α/β and the IFN-γ receptors, A129 mice lacking the IFN-α/β receptor and wild type S129 mice were vaccinated with $2x10^5$ infectious units of RepliVAX WN. Interestingly, similar IgG responses against WNV E and NS1 proteins (measured by ELISA) were observed in all three genotypes. Furthermore, all three genotypes developed identical 90% neutralizing antibody titers (1:80) at 21 days post vaccination. All animals were challenged with $10LD_{50}$ of WNV at 28 days post vaccination. Naïve AG129 and A129 mice died by 4 days post challenge, whereas naïve S129 mice died by 10 days post challenge.

Interestingly, all vaccinated A129 and S129 mice survived for 21 days, while 86% of vaccinated AG129 mice died by 14 days post challenge. These results indicate that an intact IFN system is not required in the development of antibody response against WNV in response to RepliVAX WN vaccination. In addition, these data indicate that IFN-γ related immune responses (but not IFN-α/β responses) are as important as vaccine-induced antibodies in protection from lethal WNV challenge. Ongoing studies examining other cytokine/chemokine responses to RepliVAX vaccination should provide additional information on vaccination-associated responses important for the adaptive immune response to this new type of vaccine.

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BIOTINYLATION OF ANTIBODIES IN SERUM SAMPLES ALLEVIATES THE NEED FOR SPECIES-SPECIFIC DETECTION CONJUGATES WHEN ASSAYED FOR IN A MICROSPHERE-BASED SYSTEM

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Limited methods exist for rapid identification of antigen-specific antibodies in animal species for which commercial secondary antibody conjugates are unavailable. The available assays are often of a complex nature, involving the use of live pathogens. This makes surveillance for antigen-specific antibodies in (for instance) wild avian species or zoo animals, cumbersome. A serum antibody biotinylation system was developed to alleviate the need for species-specific conjugates in a rapid assay (2-3 hrs). The proof-of-principal method used commercially-available reagents and supplies, and was easy to perform. The free amines in sera were biotinylated, then size-filtered in a 96-well format to remove excess biotin and small molecular weight components. The biotinylated antibodies were captured by West Nile and St. Louis encephalitis viral antigens in a microsphere assay, and detected with streptavidin-phycoerythrin using a BioPlex system (Bio-Rad Laboratories). The result reflected total antigen-specific antibody content. Serum samples collected from multiple wild and domestic species, including wild-caught birds, mammals, reptiles and humans, were assayed for antibodies to these viruses. Results were compared to those obtained using confirmatory tests. The infecting virus could reliably be identified in most but not all species using this method. Horses were a notable exception, for which results were uninterpretable. A possible testing regimen would be to screen serum samples for antibody content using this method, and follow up a positive or uninterpretable result with a confirmatory test, thus reducing the volume of confirmatory testing. While the biotinylation method proved useful for some domestic species and humans, it might not be the method of choice where species-specific conjugates are available. By using this method for wild or exotic species, large numbers of samples could be screened, using technology that is increasingly common. It is expected that this format could potentially be adapted for use with other etiologic agents of veterinary importance.

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ANTIBODIES TO WEST NILE VIRUS DETECTED IN WILD MAMMALS IN IOWA: 2005 - 2007

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Surveillance for evidence of West Nile virus (WNV) infection in small- and medium-sized wild mammals was conducted in Iowa from May 2005 to June 2007. Sera were collected from 330 mammals (247 mice, 33 raccoons, 32 chipmunks, 14 opossums, 1 muskrat, 1 beaver, 1 tree squirrel and 1 groundhog). Sera were screened by epitope-blocking enzyme-linked immunosorbent assay (ELISA) using the flavivirus-specific monoclonal antibody (MAb) 6B6C-1 and the WNV-specific MAb 3.1112G. Thirty-five mammals had antibodies to flaviviruses, and were as follows: raccoons (n = 16), mice (n = 10), opossums (n = 5), chipmunks (n = 3) and a tree squirrel (n = 1). The highest seroprevalence for flaviviruses was observed with raccoons (48%) and opossums (36%). Eleven mammals had antibodies to WNV, and were as follows: raccoons (n = 6), opossums (n = 2), a chipmunk (n = 1), a tree squirrel (n = 1) and a mouse (n = 1). The highest seroprevalence for WNV was observed with raccoons (18%) and opossums (14%). Plaque reduction neutralization tests are in progress. The high WNV antibody prevalence rates among select mammal species in this study suggests that additional research is needed to evaluate the significance of mammals as reservoirs for WNV.

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GENETIC AND BIOLOGICAL CHARACTERIZATION OF THE FIRST CARIBBEAN WEST NILE VIRUS ISOLATES

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While considerable serological evidence exists to support the circulation of West Nile virus (WNV) in the Caribbean, past attempts to isolate the virus in the region have been unsuccessful. In June of 2007, sentinel chicken surveillance in northeastern Puerto Rico revealed evidence of the establishment of epizootic transmission of WNV on the island. Following the confirmation of seroconversion in sentinel chickens, WNV isolates were obtained from one sentinel chicken, two pools of field-collected *Culex* spp. mosquitoes, the brain of a dead resident falcon, and the serum of an asymptomatic human blood donor identified by the American Red Cross. Maximum likelihood and Bayesian Markov Chain Monte Carlo analyses (conducted using PAUP and BEAST programs, respectively) were executed on a large dataset containing the complete coding regions and individual genes of WNV isolates from North America (WNV-NA) and Puerto Rico (WNV-PR) to characterize the molecular phylogeny and southern spread of WNV. Additionally, the infection, dissemination, and transmission rates of WNV-PR in field-collected *Culex* spp. mosquitoes were compared to other contemporary WNV isolates in order to characterize the WNV transmission dynamics in Puerto Rico. The relative sequence stability of lineage I WNV isolates makes source identification difficult. However, WNV-PR contain the Val to Ala substitution at position 159 of the viral envelope characteristic of the dominant North American clade, while sequence differences in the non-structural protein genes place WNV-PR in a distinct subclade. The effects of observed sequence differences between North American WNV isolates including NY99 and our Puerto Rican WNV isolates on viral phenotype in field-collected mosquitoes will be discussed.

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A RARE PRESENTATION OF NEUROINVASIVE WEST NILE VIRUS INFECTION

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West Nile virus (WNV) is one of the most widely distributed arboviruses. The index case in the United States was reported from New York in 1999. Since then, cases have been reported from all over the country [1]. 80% of WNV infections are asymptomatic. The remaining present with a self-limiting febrile illness [2] and less than 1% develop West Nile Neuroinvasive Disease (WNND) [3,4]. Our case demonstrates an unusual presentation of WNND. A 34 year old, African American female presents with headache, intermittent fever, unsteady gait, nausea, vomiting and photophobia for 3 weeks. Physical exam revealed bilateral horizontal

nystagmus, III and VI cranial nerve palsies. Neck rigidity and a positive Kernigs' sign were present. Magnetic resonance imaging (MRI) of the brain showed diffuse areas of signal intensity on T2 sequences. Cerebrospinal fluid (CSF) showed WBC 447 mm^3, IgG = 25.5mg/dl (Normal 0.48 - 6.24 mg/dl). Initial empirical therapy for meningo-encephalitis was then changed to supportive management with fluids and close observation. The patient showed clinical improvement and at discharge serology was positive for serum WNV IgG = 2.22 mg/dl (Positive > 1.49 mg/dl) and equivocal for IgM = 0.12 mg/dl (Negative < 0.9 mg/dl). WNV infection follows a seasonal trend with peak incidence during late summer or early fall [5]. Post infection immunity persists lifelong and recurrent infection is rare. Diagnosis is by serology or viral isolation. Detection of viral antigen, IgM or a four-fold rise in IgG in serum or CSF is diagnostic. In our patient CSF antigen and WNV specific IgG and IgM were not sent, but a CSF IgG of 25.5 mg/dl was highly indicative of recent infection. WNND includes meningitis, encephalitis, and acute flaccid paralysis [6]. Neuroimaging with MRI may show enhancement of leptomeninges or periventricular areas [2,7]. With appropriate clinical, radiological and serological findings, a diagnosis of WNND can be established. Our case demonstrates how appropriate clinical suspicion along with a judicious work-up can establish a diagnosis in an uncommon disease with an atypical presentation.

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ACUTE WEST NILE DISEASE IN NEW MEXICO: THE QUEST FOR NUCLEIC ACID

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West Nile virus (WNV) was first detected in New Mexico in 2002, with the first human cases appearing in 2003. Since that time it has become endemic in the region, and as of year-end 2005, 330 New Mexicans had been diagnosed with West Nile Fever or the more severe neuroinvasive disease as reported by the New Mexico Department of Health. An ongoing study at the University of New Mexico has collected interview and physical exam data for these individuals as well as collecting CSF and serum from their period of acute illness. The study has also collected convalescent samples from many patients recruited into the study. While all of these samples have been tested to determine WNV IgM seropositivity, none of them have been characterized by the use of nucleic acid amplification test (NAT). In our study, we have begun to characterize this sample set using Real-Time reverse transcriptase polymerase chain-reaction (RT-PCR), an extremely sensitive NAT. With 140 samples, the blood and cerebral spinal fluid collection at UNM represents a well-characterized, comprehensive specimen archive. Preliminary data on acute samples shows no qPCR amplification of 29 acute (26 serum, 2 CSF) samples (in duplicate). If any samples are found to be RT-PCR positive, gene segments will be sequenced for entry into a national database, and we will correlate both presence of virus, quantitation of virus and sequence data with the clinical parameters and outcomes that are available from the comprehensive existing database. Final results will be presented.

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TRENDS IN WEST NILE VIRUS TRANSMISSION IN SUBURBAN COOK COUNTY, ILLINOIS

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In 2002, the United States experienced the largest outbreak of West Nile virus worldwide and the greatest epidemic of mosquito-borne encephalitis. The center for this outbreak was in the north-central states with the greatest number of cases and deaths in Cook County, Illinois. In the following two years, transmission to humans in Illinois declined despite continued evidence of epizootic transmission. A rebound in human cases occurred in 2005 and 2006, in conjunction with high densities of infected mosquitoes (abundance of vectors caught in gravid traps times estimated proportion of infected mosquitoes). In the following year (2007), the number of human cases and positive mosquito pools was intermediate between the low (2003 and 2004) and high (2002, 2005, and 2006) outbreak years. Several trends were evident. First, the timing of meteorological conditions, especially temperature and rainfall, appeared to have a major impact on the magnitude of the outbreak and the shape of the outbreak curve. Second, infection rates were abnormally high across broad suburban areas in Cook County manifested by an impact on the abundance of birds, particularly sentinel crows. Third, the abundance of *Culex pipiens* seemed to rise faster in Cook County than in a southern site about 130 miles south in Champaign County possibly due to the vast underground stormwater-sewage infrastructure in the suburbs, which may serve as hibernacula for this vector species. Fourth, bloodfeeding behavior of *Culex pipiens* and presence of antibodies in bird species indicates a limited portion of the available resident bird species is responsible for the majority of bloodfeeding. The primary species involved are American Robins, Northern Cardinals, Mourning Doves, and House Sparrows. The pattern of bloodfeeding was related to the availability of hosts and possibly degree of antimosquito feeding behaviors. Fifth, the timing of the peak infection rates and human cases has been earlier since 2002. The decline in transmission may involve vector diapause, increasing immunity in hosts, avian dispersal from transmission foci, and trends in temperature and rainfall. In general, the density of infected *Culex* precedes human cases by 2-3 weeks and explains the temporal pattern of cases.

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ANALYSIS OF THE TRANSCRIPTOMIC RESPONSE TO WEST NILE VIRUS INFECTION IN THE EQUINE HOST

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West Nile virus is one of the leading causes of arboviral encephalitis in the United States and can lead to grave neurological disease and death. Yet despite the impact of WNV and other encephalitic viruses on human and animal health, little is known about viral pathogenesis and the host response to central nervous system infections. The long term goal of this project is to gather and use host expression data from tissues of horses experimentally infected with WNV at the level of brain, spinal cord, and spleen to develop interventional strategies for viral encephalitis. It is hypothesized that there are gene pathways whose expression changes in a consistent manner during WNV infection, disease, and recovery. This hypothesis will be investigated through the following specific aims: 1.) Create a tissue specific expression library from CNS tissues and spleen from normal horses and those infected with WNV (naïve and non-naïve), 2.) Create and validate a custom high density equine brain WNV microarray, 3.) Utilize the WNV microarray to analyze mRNA expression in the central nervous system and correlate this response to peripheral blood mononuclear cell (PBMC) gene expression during WNV infection, and 4.) Develop a model of functional gene expression that predicts survival to WNV infection. Initial titration runs on the library from SA 1 (approximately 5% of the library) have revealed that 24% of the library consists of genes predicted by EST collections other than the equine demonstrating enrichment of predicted sequences, 31% of the genes are novel for the horse genome, and the identification of three small RNA sequences. This library will be used to create an equine specific microarray to analyze overall changes in gene expression due to WNV infection in the CNS and peripheral circulation. Pathway analysis will be performed on the data to analyze relationships between gene expression and WNV survival. This information can then be used to provide new interventional strategies, predict survival, and develop better diagnostic tests for WNV and other viral encephalitides.