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LOW TEMPERATURE INDUCES TROPHOZOITES OF *ACANTHAMOEBA* SPP. TO PHAGOCYTOSE *FRANCISELLA TULARENSIS*, EXPLAINING A POSSIBLE MECHANISM OF SURVIVAL DURING WINTER

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Free-living Acanthamoebae are commonly found in aquatic systems as part of natural biofilms. Acanthamoebae exist in both the trophozoite feeding stage and the highly resistant and dormant cyst stage. Acanthamoebae and pathogenic bacteria are closely involved in complex symbiotic relationships. *Francisella tularensis* is the causative agent of tularemia in humans and is classified by the CDC as a Category-A biological weapons agent due to ease of dispersion through drinking water and inhalation of aerosols. An infecting dose of as few as ten *F. tularensis* can induce severe and often fatal pulmonary tularemia. It is known that *F. tularensis* survives and develops within *Acanthamoeba castellanii*. The process begins when trophozoites of *A. castellanii* engulf *F. tularensis*, which may reproduce and grow within vacuoles. This process resembles what takes place in macrophages during human infection with *F. tularensis.* Because of its high virulence, *F. tularensis* is an exceptionally dangerous pathogen. How *F. tularensis* exists in its silent stage in nature and its relationship to naturally occurring Acanthamoebae in soil and aquatic environments remains unknown. Acanthamoebae under cold conditions may exist in the cyst stage for up to 24 years. However, the mechanism of survival of *F. tularensis,* a bacterium that does not form protective spores or cysts in the environment at winter temperatures, remains unknown. In this study we examined the effects of cold temperature on the ability of *A. astronyxis* (non-pathogenic), *A. castellanii* (semi-pathogenic), and *A. culbertsoni* (highly pathogenic) to phagocytose *F. tularensis*. We found that *Acanthamoeba* spp. excyst and go into a state of frenzy feeding as temperature approaches freezing. During this frenzy, trophozoites actively phagocytose, feed, and cannibalize each other before encysting as temperatures approach freezing. During this feeding frenzy we also found that trophozoites of *Acanthamoeba* spp. engulf *F. tularensis*. This finding may explain how *F. tularensis* exists in its silent-cycle and elucidate its relationship with *Acanthamoeba* spp. by using them as a vehicle for surviving in the environment. This relationship represents one of the most scientifically intriguing questions to be answered concerning the epidemiology and natural transmission of tularemia.

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MOLECULAR SIGNALING DURING THE MIRACIDIUM TO SPOROCYST TRANSFORMATION IN *SCHISTOSOMA MANSONI*

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In vitro Schistosoma mansoni miracidial transformation is associated with many transcriptomic, biochemical and morphological changes. We performed a high-throughput small molecule screen of 1250 compounds to identify chemicals interfering with this dynamic process. Forty-seven chemicals were identified that block or delay larval transformation including dopamine D2-type antagonists, serotonin reuptake inhibitors and forskolin, an adenylyl cyclase (AC) activator. The majority of these compounds are thought to interfere with cAMP homeostasis by directly affecting adenylyl cyclase (AC) activation or indirectly, by activating or inactivating g-protein coupled receptors (GPCRs) linked to adenylyl cyclase. Forskolin, an AC activator, has been shown to inhibit larval transformation *in vitro*. D2-type dopamine receptors are G-inhibitory proteins, which upon inhibition, stimulates AC activity and increases cAMP levels. Similarly, serotonin reuptake inhibitors increase extracellular levels of serotonin by inhibiting its uptake back into the presynaptic cell leading to an increase in cAMP through activation of AC by the serotonin receptor. These results suggest that miracidial transformation is linked to a decrease in cAMP levels. Using a polyclonal-cAMP antibody and confocal microscopy we demonstrate that these specific compounds inhibiting transformation increase cAMP and do so in a tissue/cell specific manner. cAMP may be signaling through the cAMP responsive element binding (CREB) pathway. CREB is a transcription factor activated by cAMP-dependent protein kinase (PKA) and may function by regulating stage-specific transcription of genes involved in the transformation process. We have found that transcript levels of 2 CREB-binding proteins are higher in miracidia than early primary sporocysts suggesting a linkage of this pathway to the transformation process.

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DIFFERENTIAL GENE EXPRESSION IN *BIOMPHALARIA GLABRATA* NEONATE SNAILS IN RESPONSE TO *SCHISTOSOMA MANSONI* INFECTION

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A differential screening strategy was devised as an alternative to using microarrays to assess modulation in gene expression between resistant and susceptible *Biomphalaria glabrata* snails in response to *Schistosoma mansoni* infection. We used neonate snails since even at a young age resistant snails are capable of withstanding parasite infection and this methodology also has an added advantage of not processing transcripts from tissues that are not defense related. We constructed four different unidirectional lambda ZAP cDNA expression libraries using polyA+ RNA from freshly hatched neonate snails (0.5-0.75 mm in diameter) treated as follows; 1- unexposed resistant BS-90 snails, 2- resistant BS-90 snails exposed for 24 h to *S. mansoni* miracidia, 3- unexposed susceptible NMRI snails, and 4- susceptible NMRI snails exposed for 24 h to *S. mansoni* miracidia. Antibodies were raised in mice against the 4 different treatment groups mentioned above and *S. mansoni* miracidia alone as control. Differential antibody screening of the libraries was performed to identify functional protein corresponding to differentially expressed transcripts, thus making data interpretation more informative. Preliminary analysis identified clones from the NMRI unexposed library encoding QM-like protein that protects cells against oxidative stress, zinc carboxypeptidase an exopeptidase involved in protein assimilation and regulation of peptide hormones and temptin, a water-borne protein pheromone that stimulates attraction and mating behavior in mollusks. From the BS90 unexposed library we identified protein phosphatase 2A regulatory subunit that plays a role in cell cycle regulation and cell fate determination and type 2 cystatin that comprises a class of cysteine peptidase inhibitor presumed to mediate protective functions while from the BS90 exposed library we identified nucleoside diphosphate kinase and a small cardioactive peptide (SCP) that has been shown to command neurons of defensive behavior. The complete results from this study will be discussed.

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CHARACTERIZATION OF THE TRANSCRIPTIONAL PROFILE OF *BIOMPHALARIA GLABRATA* AFTER BACTERIAL AND PARASITE CHALLENGE

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Biomphalaria glabrata is a freshwater snail that serves as an intermediate host for the medically important trematode, *Schistosoma mansoni.* Using an oligo-based microarray consisting of 1152 features emphasizing immune/stress responses, we monitored the transcriptional profiles of *B. glabrata* after exposure to bacterial and trematode pathogens. It has been demonstrated that *B. glabrata's* ability to resist infection by a parasite is determined by the immunological strategies employed by the host, as well as the evasion/immuno-suppression strategies used by the parasite. With this in mind, we targeted array studies to address questions relating to: the specificity of the *B. glabrata* immune response;

the long-term relationship between the trematode parasites *S. mansoni* and *Echinostoma paraensei* in both susceptible and resistant snail strains; and the transcriptional profile associated with *B. glabrata* acquiring resistance to infection by prior sensitization to *E. paraensei.* From these array experiments we demonstrated that the acute immune response of *B. glabrata* is capable of mounting specific immune responses depending on the type of challenge. When infections using *E. paraensei* or *S. mansoni* were analyzed at 1, 2, 4, 8, 16 or 32 days post infection, a long-term profile of transcriptional changes emerged. An early increase in immune transcript expression was followed by a sharp decline in the expression of many of the same transcripts. These transcription profiles match closely with earlier experimental studies that both parasites evade immune detection by suppressing the host response. Furthermore, we characterize the transcriptional profile associated with acquired resistance to *E. paraensei* infection. This acquired resistance has been shown to last at least 8 days after sensitization and is associated with the up-regulation of a number of unique immune transcripts that are being further investigated for their possible role in parasite resistance.

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DEVELOPMENT AND CHARACTERIZATION OF VARIABLE EST-SSR MARKERS DELINEATING RESISTANCE/SUSCEPTIBILITY TO *SCHISTOSOMA MANSONI* IN THE INTERMEDIATE SNAIL HOST, *BIOMPHALARIA GLABRATA*

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Biomphalaria glabrata is an important intermediate host of *Schistosoma mansoni*. This parasite is a causative agent of schistosomiasis, a disease that is endemic in over 74 countries. In high transmission areas, patients often have concurrent infections. This reality of co-infections with other pathogens has helped fuel new energy towards blocking transmission at the snail stage of parasite development for long-term control. To understand the molecular genetic basis of the snail/schistosome interaction, crosses were generated between NMRI (susceptible) and BS-90 (resistant) snails to produce F1 and F2 gametes. F2 progenies displayed 58.4% resistance upon *S. mansoni* infection. DNA from segregating resistant/susceptible populations was analyzed for heritability of variable microstellites (MS) using PCR with 37 primers. Results showed a polymorphic region (Bge5_289 and Bge5_292) with significant linkage (7cM). SSRIT and RepeatMasker were used to identify, classify and mask repeats. Thus far, we have developed and characterized several markers that may be helpful for futher linkage analysis. From 1,601 SSH-ESTs, a variable Simple Sequence Repeat (SSR) locus with the (CA)5 motif was identified that also displayed polymorphisms between BS-90 (330 bp) and NMRI (320 bp) snails. More EST-SSR markers were characterized from 52,433 *B. glabrata* ESTs available in GenBank. Using SSRIT to screen for di-, tri-, tetra-, penta-, hexa- nucleotide motifs, we identified 3,583 (6.8%) SSR-containing sequences. However, when low complexity sequences and interspersed repeats were excluded using RepeatMasker only 538 (1.02%) ESTs contained true SSR repeats. The majority of motifs of EST-SSRs were, (TG)n [10.6%], (TC)n [6.3%], (CAT)n [5.5%]. These new SSRs are being evaluated with segregating F2 progenies to determine linkage/mapping of parasite resistant/susceptibility loci on *B. glabrata* chromosomes.

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VARIATIONS WITHIN LOCI CORRESPONDING TO HYDROLYTIC ENZYMES BETWEEN RESISTANT AND SUSCEPTIBLE *BIOMPHALARIA GLABRATA* SNAILS: EFFECTS OF *SCHISTOSOMA MANSONI* EXPOSURE AND AGE OF SNAIL

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Biomphalaria glabrata is an important snail host of the parasitic blood fluke *Schistosoma mansoni* in the Western hemisphere. To elucidate the molecular basis of the host-parasite relationship, studies have used snail stocks that vary in parasite susceptibility. The snail host exhibits a range of susceptibility phenotypes depending on the genetics of the snail and the parasite. Previous studies have suggested hydrolytic enzymes may play a role in the mollusk's defense against invading schistosomes and other pathogens. Genes encoding hydrolytic enzymes, such as cathepsin B, cathepsin L, and legumain have recently been identified from the snail hepatopancreas (Myers et al, 2008). In the present study, using gene specific primers for the above enzymes, PCR was performed to determine, by Amplified Fragment Length Polymorphism (AFLP) analysis, polymorphisms within the corresponding loci of the aforementioned enzymes between resistant (BS-90 and LAC) and susceptible (NMRI) snails, pre- and post- *S mansoni* exposure. Restriction Fragment Length Polymorphism (RFLP) analysis was also used to determine degree of strain variation within these loci between resistant and susceptible snails. Results showed the presence of major variations within the cathepsin B locus that was revealed by the presence of a resistant- specific 0.2kb band with restriction enzyme *Bstx*1. Results also showed strain specific variation within the cathepsin L locus, in this case major intra-strain variation was detected within the susceptible snail stock with snails displaying either a 2.5kb or 1.8kb band after PCR. In addition, variation was detected following parasite exposure (24hr) between juvenile and adult susceptible snails. No intra-strain variation was found in the resistant stock in this locus either after exposure or with age. RT-PCR was used to determine the differential regulation of the transcripts encoding the above enzymes pre- and post- parasite exposure. The effects of age and infection on the modulation of these enzymes between the resistant and susceptible snails will be discussed.

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HUMAN FASCIOLIASIS IN LATIN AMERICA: THE ENDEMIC FOCI IN ANDEAN COUNTRIES AND CARIBBEAN-CENTRAL AMERICA

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Fascioliasis causes serious problems in Latin America. In South America, main human endemic areas are in Bolivian and Peruvian Altiplanos and valleys of Peruvian Andes, where prevalences and intensities in children reach very high levels. Adult subjects show lower rates, and females are

more infected (higher prevalences, higher intensities, or both) than males. Important areas are also known in Chile and more than 400 cases have been reported in Argentina. Lower rates have been recorded from Ecuador and Venezuela. All these areas appear linked to (i) high altitudes where transmission is significantly increased, and (ii) lymnaeid vectors belonging to the *Galba/Fossaria* group. In Central America, health problems have been recently detected in Mexico, children being the most affected. In the Caribbean, Cuba suffers repeated outbreaks. Of less importance is Hispaniola, where cases appear more sporadically. Both Mexico and the Caribbean islands share singular epidemiological characteristics related to *Lymnaea cubensis* and *L. humilis* as main vectors. A WHO initiative has recently been launched against human fascioliasis worldwide, in which the endemic areas in Latin America constitute priority targets. An IAEA initiative is including molecular biology research studies on the domestic animal reservoir species with the aim to decrease human reinfection risks after control campaigns.

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ETIOLOGY AND EPIDEMIOLOGY OF CERCARIAL DERMATITIS IN NORTH AMERICA

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Cercarial dermatitis or swimmer's itch results when cercariae of schistosomes penetrate human skin and initiate inflammatory responses. The trematodes typically die in the skin but in some cases may persist and infect other organs, including regions of the central nervous system. Cercarial dermatitis is caused by a complex and poorly known assemblage of schistosome species, and can occur anywhere where people come in contact with water bodies harboring schistosome-infected snails. Outbreaks are known to occur in both freshwater and marine habitats worldwide. As part of our ongoing studies of schistosome diversity, using morphological traits and sequence data to differentiate species, we have thus far identified 20 genetic lineages of schistosomes from snails across North America, from both freshwater and marine habitats. Five of these were described following dermatitis outbreaks in Stubblefield Reservoir in Northern New Mexico, Prospect Lake in the heart of Colorado Springs, Colorado, and Robert Crown Beach in San Francisco Bay, California. The New Mexico outbreak likely involved two different avian schistosome species, both transmitted by physid snails. The Colorado outbreak was due to *Trichobilharzia brantae*, a species transmitted by Canada geese and the snail *Gyraulus parvus*. Finally the San Francisco outbreak was caused by a previously undescribed marine schistosome developing in a recently introduced exotic snail, *Haminoea japonica*. Our investigations of schistosomes in North America have revealed that the species responsible for outbreaks a) vary across time, space, and habitat, b) use a diverse range of snail intermediate hosts, and c) can occur in exotic snail hosts. Our studies will help to validate unique morphological or molecular features that can be used for routine identification. Our extensive reference collection of schistosomes and the continued application of molecular methods to identify such worms create exciting new opportunities to advance our understanding of this under-appreciated yet widespread public health problem.

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RESULTS FROM SENTINEL SITE SURVEILLANCE FOR TRACHOMA AND SCHISTOSOMIASIS BEFORE PREVENTIVE CHEMOTHERAPY IN BURKINA FASO

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Over 1 billion of the world's poorest citizens continue to be afflicted by neglected tropical diseases (NTDs). A number of international single-disease control initiatives have expanded coverage, and since 2006, there have been moves to integrate these initiatives, mainly using preventive chemotherapy. Monitoring and evaluation of these integrated programs presents a unique set of challenges. Burkina Faso was selected to receive support from the United States Agency for International Development (USAID) funded NTD program for integrated NTD Control, and this country has also combined all the local M&E teams into one integrated group to conduct surveillance in sentinel sites. This study was unertaken to assess potential gender, age and location differences in 4 groups of children (1) with single *Schistosoma haematobium* infection, (2) with single trachoma infection (3) co-infected with *S. haematobium* and trachoma and (4) children with neither infection. The results should contribute valuable information for coordinating the strategy for preventive chemotherapy. Baseline infection data was collected from 3,324 children aged 7 to 11 years in 23 sentinel sites across 11 regions of Burkina Faso and analyzed through a generalized logit model. Probabilities of the risk of belonging to each of the above categories with reference to age, sex and location were estimated. A trachoma case was defined by the presence of Trachomatous inflammation - Follicular; Trachomatous inflammation - Intense in both eyes. *S. haematobium* infection was diagnosed by finding eggs through microscopic examination of urine after filtration. The prevalence rates for single *S. haematobium* infections were 10.95% (95% CI: 9.89 - 12.01); and for single trachoma infections 12.45% (95% CI: 11.33 - 13.58). 0.84% (95% CI: 0.53 - 1.15) were coinfected. Gender and location yielded significant differences in the risk of having *S. haematobium* and trachoma single or/and dual infections. In conclusion, trachoma and *S. haematobium* infections constitute serious public health problems in Burkina Faso and control is strongly recommended. Risk factors for both infections are discussed while a development of common framework for the monitoring of these two infections is also considered.

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PARASITOLOGICAL IMPACT OF ONE YEAR PREVENTIVE MASS CHEMOTHERAPY ON SOIL-TRANSMITTED HELMINTHIASIS AND SCHISTOSOMIASIS IN NORTHERN RWANDA

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A recent baseline data collection in 2 districts located in the Northern Rwanda showed that the overall prevalence of soil transmitted helminthes(STH) was very high (>95%) among schoolchildren. The same study revealed that the prevalence of *S. mansoni* infection was 10.7% and 23.5% in Burera and Musanze districts respectively. Rwandan NTD program conducted a nationwide mass drug administration with albendazole for STHs to all school aged children twice in 2008 while

Praziquantel was additionally given once to school aged children and communities in schistosomiasis endemic areas. The aim of this study was to evaluate the impact of mass anthelminthic chemotherapy. The impact of the treatment program was monitored through a cohort of school children and adults in 2 districts in Northern Rwanda. Their infection status with STHs and *S. mansoni* was determined by parasitological examination at baseline and at year one follow up. The prevalence and intensity of STHs and *S. mansoni* before and after the treatment were analyzed using STATA. A cohort of 1013 schoolchildren (7-16 years old) was successfully monitored. Two rounds of treatment with albendazole have shown an overall reduction of 4.4% in STH prevalence with a significant reduction of hookworm prevalence (82.5%). Infection mean intensity was reduced from 104.3 to 76 eggs par gram(epg), 1315 epg to 427 epg and 12387 epg to 5843 epg for Hookworm, *T. trichiura* and *A. lumbricoides* respectively. The prevalence of *S. mansoni* infection was reduced from 18.4% to 2.5% and 7.7% to 1.2% in Musanze and Burera districts respectively. The proportion of children with heavy *S. mansoni* infection was significantly reduced from 8.3% to 0. In adult cohort, significant reduction in prevalence and intensity of both STHs and *S. mansoni* infection was also observed. In conclusion, the bi-annual mass treatment with albendazole and annual treatment with praziquantel of school aged children and adults at high risk can significantly reduce the prevalence and intensity of infection for STHs and schistosomiasis.

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RANDOMISED CONTROLLED TRIALS (RCTS) OF PRAZIQUANTEL 40 VS. 60 MG/KG FOR TREATING INTESTINAL SCHISTOSOMIASIS

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The ideal dose of praziquantel for treating intestinal schistosomiasis is not fully established and 40 or 60mg/kg doses are variably used depending among other things on the parasite species involved. Single-dose praziquantel at 40 vs. 60mg/kg were compared in RCTs with identical protocol for treating children and adolescents (10-19 years of age) with intestinal schistosomiasis caused by *Schistosoma japonicum* (the Philippines - Mindanao Island) or *S. mansoni* (Mauritania - Trarza region; Tanzania - Mwanza region; and Brazil - Pernambuco state). The primary efficacy parameters were cure rates and egg reduction rates on Day 21. Secondary efficacy parameters were reinfection rates at 6 months and 12 months after treatment, and associated morbidities (by analysing haemoglobin, body weight). Safety was assessed by comparing prevalence and intensity of adverse events. For each study, the sample size was 109/arm (expecting cure rates of 60 and 80%, respectively with 80% power and 95% confidence level, and 20% lost to follow-up). An individual patient meta-analysis of the four study sites is presented based on the intent-to-treat (ITT). Modified ITT and per-protocol datasets for efficacy. The results of this study suggest both national and general policy recommendations for praziquantel dosing.

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INTEGRATED CONTROL STRATEGIES OF SCHISTOSOMIASIS TRANSMISSION INFORMED BY A BAYESIAN MULTILEVEL MODEL AT A LOCAL SCALE

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Schistosomiasis japonica is a zoonotic disease caused by infections with *Schistosoma japonicum* and still remains a public health concern in China. It has long been recognized that schistosome transmission is highly site-specific and its patterns of infections in humans are influenced by local socio-economic and environmental conditions. Evidence-based control strategies are needed especially in mountainous endemic regions of China where resources are relatively limited. Here we report the use of a Bayesian multilevel model to identify risk factors associated with schistosome transmission in a highly heterogenous environment, thereby informing strategies for sustainable control. In this study, 5 061 residents from the 26 villages in Eryuan County were screened for *S. japonicum* infection using indirect hemagglutination assay (IHA) and miracidium hatch test. Demographic information at three hierarchical levels (e.g., individual, family and village) was obtained through questionnaire, while environmental variables (e.g., landscape metrics) were derived from high resolution remote sensing imageries. A Bayesian multilevel model accounting for spatial correlation was built for serological status and the true infection status of *S. japonicum*, respectively. The model including the three level factors and a spatial random effect was the best fitted model. At individual level, all resident were susceptible to infections of *S.japomicum,* and health education should be strengthened. At the family level, reduction in paddy field, installation of biogas digestion systems and sanitary breeding stall for livestock are associated with decreasing prevalence of infections. At the village scale, decreasing heterogeneity of landscape and snail density can link to reduction in sero-prevalence and the true infection rate, respectively. This study illustrates the feasibility of utilizing a Bayesian approach to identify high risk factors associated with schistosome transmission in heterogeneous environments and the value of using such approach to explore control designs.

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EVALUATION OF INTEGRATED MASS DRUG ADMINISTRATIONS FOR NEGLECTED TROPICAL DISEASES IN HAITI

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In Haiti, an estimated eight million people are at risk of lymphatic filariasis (LF) with prevalence of *Wuchereria bancrofti* circulating antigenemia reaching 45% in some communes. Annual mass drug administrations (MDA) with diethylcarbamazine and albendazole can eliminate LF as well as control soil transmitted helminthiasis (STH). In 2008, a MDA for the elimination of LF and control of STH was conducted in 44 highly endemic communes in Haiti. Evaluation of the 2008 MDA was conducted in 11 communes to assess the drug coverage and the efficacy of the health education messages. Communes were chosen based on reportage of high coverage rates, geographic location, and implementing organization. Thirty villages were randomly selected from each commune. Within each village, 10 households were randomly selected for interviews. Age, sex, MDA compliance, and bed net use the previous night was recorded for each household member. One adult individual in each household was randomly selected for more detailed questions regarding their knowledge, attitudes, and practices (KAP) concerning LF and STH. Preliminary results from four communes are summarized here and final results from the 11 communes will be presented. MDA coverage was 67%, with the highest coverage (79.8%) in school aged children. Of those participating in the KAP survey, 62% knew about LF, 74% knew about STH, 42% knew about the 2008 MDA, and 35% knew someone in their community with either elephantiasis or hydrocoele. Medication was identified by 26% of individuals when asked how LF can be prevented. Side effects occurred in 83 (26%) of the 317 individuals reporting they had taken the medication. Univariate predictors of MDA compliance were knowledge of LF (OR=7.2; 95% CI 5.0-10.4), knowledge of STH (OR=7.6; 95% CI 4.9-11.8), knowledge of MDA (OR=23.3; 95% CI 15.6-34.7), and knowledge that medicine prevents LF (OR=3.9; 95% CI 2.7-5.7). Nine percent of the children less than five years of age slept under a bed net the previous night. Of those participating in the KAP survey, 11% reported their household owns at least one bed net. Although variability between areas is expected, the preliminary data from 4 communes indicate that drug coverage and bed net use is sub optimal. This study emphasizes the importance of community awareness during MDAs.

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DIFFERENTIAL DECLINE OF HELMINTH INFECTIONS ON ALOR ISLAND, INDONESIA FOLLOWING SIX ROUNDS OF MASS DRUG ADMINISTRATION USING DIETHYLCARBAMAZINE IN COMBINATION WITH ALBENDAZOLE

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We evaluated a mass drug administration (MDA) program to eliminate lymphatic filariasis on Alor Island, Indonesia. The effect of MDA on filariasis and intestinal helminths was assessed annually over a period of 7 years in one Brugia timori endemic sentinel village. Microfilaremia (MF) was assessed by night blood filtration and filaria specific IgG4 antibodies by the Brugia Rapid™ test. Pre-MDA the microfilaria (MF) prevalence in the population was 26%, and 80% of the residents had antibodies. In 2008, 10 months after the 6th round of MDA the MF rate had dropped to 0.7% and the antibody rate had decreased to 15%. This was the 2nd year in a row with a MF rate of less than 1% and an antibody rate of <15%. The District Health Administration has stopped MDA on the island. The pre-MDA prevalence of Ascaris, hookworm and Trichuris in the sentinel village was 34, 28, and 11%, respectively; these rates after the 6th round were 27, 5 and 3%. Post-MDA infection intensities were very low, and no individual had more than 250 epg. MF and hookworm prevalence rates decreased significantly after the first round of MDA; it took longer for filarial antibody rates and Trichuris to fall (3 to 4 years). Ascaris rates fluctuated during the entire study period. However, the lowest prevalence of intestinal helminths was observed after the 4th round of MDA with 18, 0.7 and 0.7%, respectively. In contrast to B. timori microfilaraemia, the prevalence rates of intestinal helminths appear to increase slowly during the last couple of years. This study has shown that MDA with DEC/albendazole had a major impact on B. timori prevalence rates; this provides a proof of principle that elimination should be feasible with existing tools and policies.

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PERCEPTIONS OF INTEGRATED NEGLECTED TROPICAL DISEASE PROGRAMS AMONG MINISTRY OF HEALTH STAFF IN MALI

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In 2007, the Ministry of Health of Mali (MoH), with assistance from external partners, implemented an integrated mass drug administration (MDA) campaign for five endemic neglected tropical diseases in Bamako and Sikasso Regions. After the MDA campaign, we conducted 16 qualitative interviews with MoH officials at the national, regional, and district levels to evaluate their perceptions about the implementation of this integrated approach. Questions were divided into five subject categories: history, concept, advantages/challenges, effect on the health system and planning, and future of integrated programs. Interviews were recorded digitally, transcribed and analyzed for content manually. Most of the staff interviewed were aware of a shift in global donor interest away from vertical toward integrated disease programs and were overall supportive. Respondents identified several levels of integration. Program integration was seen as effective in temporary, finite situations such as integrated vaccination campaigns. Integration at the country level was seen as an inevitable, but largely positive development that would ensure continued funding for programs and lead to the achievement of disease elimination goals. Integration was also believed to be feasible at the operational level (district) where activities of programs overlap. Co-implementation at that level was thought to maximize efficiencies of resources. A few respondents believed that current integrated programs were co-implementation of activities at the operational level only, without corresponding integration of planning, management and monitoring systems. Nearly all respondents believed that the district should be involved in the planning and monitoring of integrated programs, but that this was not currently happening. Many thought that existing vertical program structures would have to be adapted. The sharing of resources was also seen as potentially destructive for certain programs. National-level respondents questioned the impact of current integrated programs on existing health structures and system. Integrated programs were viewed as having a number of advantages over vertical programs. Establishing a decentralized system with administrative, planning, management and financial structures in place at every level may increase the operational level's engagement and the potential for the long-term success of integrated programs.

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IMPORTANCE OF VIRAL PATHOGENS IN EARLY CHILDHOOD DIARRHEA IN RURAL AND URBAN NEPAL

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Diarrheal disease is a major cause of childhood morbidity and mortality in Nepal. Understanding etiologic agents of disease is fundamental for developing better intervention strategies. We conducted hospital-based surveillance in children 3 months to 5 years of age, 1200 children with diarrhea and 1200 controls, in two hospitals in urban and rural Nepal during 2007-2009. A stool specimen and demographic and clinical background information were collected with informed consent under approved human use protocols. A field laboratory was established where local technicians conducted stool culture and completed initial microbiologic identification of enteric bacterial pathogens. Confirmation of isolates and molecular assays for diarrheagenic *Escherichia coli*, norovirus, and rotavirus were performed at AFRIMS. The most commons organism detected significantly ($p<0.05$) more frequently in cases than controls in both sites were rotavirus (23% vs 3%), *Aeromonas* (9% vs 2%) and *Shigella* (6% vs 1%). In urban Kathmandu, ETEC (11% vs 5%), *Vibrio* (13% vs 1%) and norovirus (12% vs 5%) were more commonly and significantly found in cases than controls. *Giardia lamblia* was significantly more frequently detected among controls than cases from both sites (6% in cases vs 14% in controls). Enteroaggregative *E.coli* was detected in approximately the same proportion of cases (18%) and controls (19%). Similarly, in the urban Kathmandu area, 15% of cases and 13% of controls were infected with Campylobacter. In conclusion, rotavirus and norovirus, causes of viral gastroenteritis are common in Nepal. Asymptomatic infection with organisms linked to intestinal damage, malnutrition and impairment of growth and development are also common among children under 5 years of age in Nepal.

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BURDEN OF ENTEROTOXIGENIC *ESCHERICHIA COLI* (ETEC) DIARRHEA AMONG CHILDREN LESS THAN TWO YEARS IN A RURAL EGYPTIAN COMMUNITY

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Enterotoxigenic *Escherishia coli* (ETEC) is the predominant bacterial cause of diarrhea in Egypt. ETEC adheres to the intestinal epithelium by colonization factor antigens (CFAs) and causes diarrhea by a heat-labile toxin (LT) and/or heat-stable toxin (ST). During January 2004 to April 2007, 348 neonates were followed for two years. Children were visited twice a week. When diarrhea was reported, a rectal swab and a stool specimen were obtained. In addition, a routine stool sample was obtained every two weeks. *E.coli* isolates were screened for LT and ST by GM1 ELISA. ETEC were further typed for CFA by dot-blot assay using a panel of 12 monoclonal antibodies against CFAs (CFA/I, CS1 to CS8, CS12, CS14 and CS17).Incidence was estimated and pathogenicity evaluated by evaluating differential rates of toxin-CF profiles among children with diarrhea [S] and without diarrhea [AS] (adjusted for age, gender, season, mother's education, and crowding). ETEC phenotype with symptomatic diarrhea was performed using logistic regression. Diarrhea episodes were 8.2 per child-year. ETEC was the most common pathogen isolated (incidence rate = 1.26 episode per child-year). Comparison between initial ETEC infection in S and AS children has shown that ETEC strains carrying any of the CFAs were pathogenic. The distribution of toxins produced by ETEC strains among S (43.6% LT, 38.7% ST, and 17.8% LT/ST) varied from the distribution of toxins produced by ETEC strains among AS (55.7% LT, 34.2% ST and9.6% LT/ST. This difference was significant only for LT/ST($p<0.0001$).The distribution of CFAs expressed on ETEC isolates from S and AS children also varied; CFA/I, CS3, CS1+CS3, CS2+CS3, CS5+CS6, CS7 and CS14 were detected in higher frequency in S children, while CS12 had higher frequency among AS children. In conclusion, data confirm that ETEC burden remains high in this Nile delta region in Egypt. Also, LT/ST phenotype and CFA/I, CS3 and CS7 were more commonly found in children with diarrhea compared to AS children; a finding that may be useful for vaccine development.

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IDENTIFICATION OF COLONIZATION FACTOR ANTIGEN IN NON-ENTEROTOXIGENIC *E. COLI* STRAINS

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The colonization factor (CFs) enables bacteria to adhere to and colonize the host intestinal mucosa. There are more than 20 CFs characterized in enterotoxigenic *Escherichia coli* (ETEC); however they have not been described in others types of diarrheagenic *E. coli*. The aim of this study was to determine the presence of CFs in non-ETEC *E. coli* strains isolated from Peruvian children with diarrhea. We have analyzed a randomly selected group of 30 diffusely adherent (DAEC), 30 enteropathogenic (EPEC), 30 enteroaggregative (EAEC) and 5 shiga toxin-producing *E. coli* (STEC) strains previously isolated from a passive surveillance diarrhea cohort study in children < 12 months of age in Lima, Peru. Diarrheagenic *E. coli* were identified by a multiplex real-time PCR: DAEC (*daa*D), EPEC (*eaeA*), EAEC (*Agg*R), STEC (*stx1, stx2*), ETEC (*lt, st*) and enteroinvasive *E. coli* (*lpa*H). Two colonies from each strain were tested for CFs by a dot-blot assay using 21 monoclonal antibodies: CFA/I, CS1-CS7, CS12 (PCF0159), CS14 (PCF0166), CS17, CS18, CS20, CFA/III, CS19, PCF039, PCF071, Ag150, 4264, 4089 and 7162. Three CFs were identified in 95 non-ETEC strains (3%). All CFs were CS20 (coli surface antigen 20), identified in 3 DAEC strains (3/30), from 3 different patients with diarrhea. All other *E. coli* were negative for CFs. The 3 CS20-positive DAEC strains were confirmed to be ETEC-negative by a repeated PCR (*lt* and/or *st* genes) and toxin-negative (LT, ST or LT/ST) by a GM1-ELISA. All DAEC strains showed a diffuse adherence pattern in a HEp-2 cell assay. The CS20-positive DAEC strains correspond to acute diarrheal episodes (9.7days ± 2.1days), as a single pathogen infection. In conclusion, to our knowledge this is the first description of CFs in non-ETEC *E. coli* strains. It is possible that plasmids encoding the structural subunit of CS20 are being transferred to other diarrheagenic *E. coli.* Further studies are needed to evaluate the presence of currently recognized and new CFs in diarrheagenic *E. coli,* in order to identify new and possible vaccine targets.

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ANTI-TOXIN IMMUNITY IN ENTERIC DISEASE: MAINTAINING THE INTEGRITY OF THE INNATE MUCOSAL BARRIER

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The pathogenesis of ETEC begins with ingestion of innocula, elaboration of toxin, bacterial colonization, induction of profuse, watery diarrhea, and dissemination into the environment. The intact, innate gut defenses lay on a scaffold of a mucus layer, are generally sufficient to ward off microbial invasion. Consequently, ETEC organisms have developed a series of strategies to disrupt and modify this barrier, rendering the gut wall susceptible to subsequent colonization or entry. LT toxins enhance ETEC colonization, either after pre-exposure or concomitant secretion by LT containing ETEC. The colonization effects by LT have been demonstrated for LT+, LT+/ST+ and ST+ clinical isolates. In prospective enteric pathogen epidemiology studies, the majority of ETEC isolates are recovered from subjects without diarrhea. This widespread and frequent asymptomatic LT toxin exposure may lead to frequent disruption of the innate mucosal barrier. The hallmark of clinical toxin-mediated secretory diarrheas such as ETEC is a non-inflammatory diarrhea and the absence of microscopically apparent damage. Toxin-induced fluid secretion can be reproduced and quantitated via ligated intestinal loops in several animal models. Using light microscopy, ligated loops from animals exposed to LT demonstrated focal loss of the mucus layer covering the small intestinal wall. Co-localization of LT on the enterocyte in the vicinity of the areas with focal loss of mucus was demonstrated using immunohistochemistry. Scanning EM confirmed the presence of mucus layer disruption. Similarly, visualization of ETEC organisms attached to the microvilli in the focally disrupted regions was seen. These findings can be prevented by pre-immunization with LT via transcutaneous immunization, suggesting that the anti-LT immunity protects against both the direct secretory effects of LT and the more subtle mucosal disruption caused by exposure of the enterocyte to LT. These findings support the hypothesis that toxin mediated fluid secretion disrupts the innate mucosal barrier, leaving the gut surface vulnerable to subsequent colonization. The data are in line with clinical findings that asymptomatic colonization with ETEC is associated with a significant risk of contracting diarrhea, and that the expected protective efficacy against LT+ ETEC using LT as a vaccine antigen is accompanied by protective efficacy against both LT-ETEC and non-ETEC infections.

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CONCOMITANT INFECTION WITH *VIBRIO CHOLERAE* O1 AND ENTEROTOXIGENIC *ESCHERICHIA COLI* INDUCES A MORE ROBUST IMMUNE RESPONSE TO CHOLERA ANTIGENS

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Vibrio cholerae O1 and enterotoxigenic *Escherichia coli* (ETEC) are major bacterial pathogens causing dehydrating diarrhea. Cholera toxin produced by *V. cholerae* O1 (CT), heat labile toxin (LT) and/or heat stable toxin (ST) of ETEC are responsible for the secretory diarrheas characteristic of these illnesses. We have observed that about 5% of hospitalized patients are concomitantly infected with *V. cholerae* O1 and ETEC (CHET group). In order to understand the outcome of such infections on clinical and immunological responses in cholera patients, we studied adults patients infected with *V. cholerae* O1 (n=25), those infected with both *V. cholerae* O1 and ETEC (CHET) (n=25), and those infected with ETEC only (n=25). The CHET group showed more severe dehydration, had higher intake of intravenous fluid, and more severe dehydration than the ETEC group (p=0.01-0.003). The CHET patients showed higher vibriocidal responses, and increased antibody titers to CT and LPS in plasma than did the *V.cholerae* O1 patients (p=0.02-<0.001). The magnitude of antibody titers were higher in CHET than in other groups. All responses in *V.cholerae* O1 and CHET groups were more robust than those seen in the ETEC only group (p=0.01-<0.0010). FINDING: Concomitant presence of ETEC and *V. cholerae* is associated with more severe disease and more prominent immune responses than those associated with the *V.cholerae* O1 or ETEC alone. LT or other factors in ETEC may act as a mucosal adjuvant(s) and enhance immune response to *V. cholerae* O1 during concomitant infection.

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A VARIANT IN LONG PALATE, LUNG AND NASAL EPITHELIUM CLONE 1 IS ASSOCIATED WITH CHOLERA IN A BANGLADESHI POPULATION

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Vibrio cholerae causes a dehydrating diarrheal illness that can be rapidly fatal in the absence of specific treatment. The organism is an historic scourge and, like similar infectious diseases, may have influenced the evolution of the human genome. We report here the results of the first candidate gene association study of cholera. In a family-based study of 76 pedigrees from Dhaka, Bangladesh, we evaluated the association between cholera and five candidate genes -- the cystic fibrosis transmembrane receptor; lactoferrin; long palate, lung and nasal epithelium clone 1 (LPLUNC1); estrogen related receptor α; and calcium activated chloride channel 1. We found a significant association with a marker in the promoter region of LPLUNC1 (rs11906665), a member of a family of evolutionarily conserved innate immunity proteins. A previous microarray-based study of duodenal biopsies revealed significantly increased expression of LPLUNC1 in cholera patients compared to healthy control subjects. Our results suggest that variation in host innate immune responses may influence the outcome of exposure to V. cholerae in an endemic setting.

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FORECASTING CHOLERA EPIDEMICS IN AFRICA

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Previous studies identified factors associated with cholera outbreaks, but most focused on a few predictors and locales. As part of development of an operational prediction and decision support system, we assessed demographic, economic, environmental, and climatic variables associated with Vibrio cholerae infections across Africa (688 provinces) during 1995-2005. Data included reports of cholera outbreaks at province level (ProMED-mail); national economic, health, and demographic data (WHO); high-resolution data on populations (GPW/GRUMP), rainfall (NASA/NOAA), and flood hazard (Center for Hazards and Risk Research); and El Nino indicators (NOAA). We fitted mixed effects logistic regression models to predict the probability of an outbreak for each province for each month. There were 466 outbreaks in 176 of 688 provinces, with 257, 136, 60, 11, and 2 outbreaks in Eastern, Western, Central, Southern, and Northern Africa, respectively. Outbreak seasonality varied markedly by region, with greatest risk during Nov-Jan and Jun-Oct in Eastern and Western Africa, respectively, and little intraannual variation elsewhere. The final multivariate logistic regression model (excluding 1995-96 because of low reporting and Northern Africa because of few outbreaks) included population size (per million; β=0.27, standard error [se]=0.04),% urban constitution (per 10%; β=0.19, se=0.05), mean flood hazard (per decile; β=0.07, se=0.03), gross national income/capita (per international purchasing power parity*1000; β=-0.12, se=0.10), region-specific random effects for rainfall 1 month prior, and country-specific random intercepts. The area under the ROC curve was 0.77, 0.82, 0.87, and 0.93 for Eastern, Western, Central, and Southern Africa, respectively. In conclusion, these preliminary results suggest that accurate forecasting of cholera outbreaks in Africa may be achievable with 1 month lead-time using demographic, economic, environmental, and climatic predictors. Accuracy assessment using independent data and development of more flexible prediction algorithms are underway. The datasets and forecasting approaches used here also may facilitate prediction of other health emergencies in Africa.

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PLEISTOCENE GEOGRAPHIC SEPARATION IN EASTERN PANAMÁ AND NORTHERN COLOMBIA LEADS TO POPULATION STRUCTURE IN *ANOPHELES (NYSSORHYNCHUS) ALBIMANUS* (DIPTERA: CULICIDAE)

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Anopheles albimanus is an important malaria vector throughout the northern Neotropics, and physical barriers to gene flow are expected to restrict its dispersal. To analyze the population history of *An. albimanus*, we used partial sequences of the mitochondrial DNA CO1 gene and mosquitoes from 28 localities in five countries. We hypothesize that *An.*

albimanus is not at demographic equilibrium likely due to population growth and historical geographic fragmentation at a regional scale. Pairwise coalescence analysis of population divergence (MDIV) indicates that migration increases as geographic distance decreases; however, a median-joining network depicted three divergent haplotype groups: (A) Nicaragua, Costa Rica and western Panamá; (B) central-eastern Panamá plus the Caribbean coast of Colombia, and (C) the Colombian Pacific coast plus Ecuador. Groups (A) and (C) are at demographic equilibrium whereas (B) has undergone population expansion. The time since expansion from the mismatch analysis is around 49,000 years ago (95% CI 17,237 - 87,276). Our findings do not support physical barriers to gene flow, but instead, Pleistocene geographic separation in eastern Panama and northern Colombia is the likely cause of population structure in *An. albimanus*.

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GENETIC STRUCTURE OF *AEDES ALBOPICTUS* IN CAMEROON (CENTRAL AFRICA)

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The Asian tiger mosquito, *Aedes albopictus* (Skuse, 1894), is an invasive species that expanded in all continents, including Africa. The species was first detected in Nigeria in 1991, and invaded Central Africa in 2000s, where it was recently implicated in emergences of arborviruses such as chikungunya and dengue. According to their geographical origin, the populations of *Ae. albopictus* are known to vary for biological characters (e.g. anthropophily) which are able to modulated the vectorial capacity of the species. To date, the origin of Central African populations is not determined, since the invasion of this region may have occurred from Nigeria, from a secondary introduction, or both. In order to assess the diversity of *Ae. albopictus* in Central Africa, we undertook a study on the genetic diversity and structure of populations originated from 12 Cameroonian locations. Samples were collected in 2007 according to North-South and West-East geographical transects representing all main bioclimatic regions. In each location, specimens were collected as larvae/pupae and reared to adult stage. We used six microsatellite and two mitochondrial markers (COI and ND5).

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EPIDEMIOLOGICAL IMPORTANCE AND POPULATION GENETICS OF THE HUMAN MALARIA MOSQUITO *ANOPHELES NILI SL* IN AFRICA

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Mosquitoes from the *Anopheles nili* group are major vectors of human malaria in forests and humid savannas of tropical Africa. Despite their epidemiological importance, basic data on their bionomics and genetics are crucially lacking, although such data are a requisite to properly devise and implement efficient and sustainable vector control strategies. Here, we present original data that contribute to fill this gap in our knowledge. *An. nili* specimens were collected in Senegal, Burkina Faso, Ivory Coast, Nigeria, Cameroon and the Democratic Republic of Congo by human landing catches and pyrethrum spray collections. Field-collected specimens were identified by PCR and their feeding preferences and infectious status were determined by ELISA. Eleven recently developed microsatellite loci were used to compare the level of genetic diversity and differentiation between 16 populations. *An. nili s.s.* was highly anthropophilic and partially endophagic and was the only member of the group collected out of Cameroon. Specimens of *An. ovengensis* and *An. carnevalei* collected in South Cameroon were highly exophagic and exophilic. All three species were found infected by *P. falciparum* with mean infection rates ranging 4.3-13.6% in *An. nili s.s.* (N=3646), 0.9% in *An. carnevalei* (N=516) and 1.0% in *An. ovengensis* (N=978). Genetic diversity indices were high in *An. nili s.s.*, and lower in *An. carnevalei* and *An. ovengensis*. High and significant levels of genetic differentiation were estimated across species (Fst>0.19, P<0.001). Within *An. nili s.s.*, the population structure is consistent with isolation by distance, although demographic and/or selective events probably resulted in a higher level of genetic isolation in marginal populations. This study confirmed that *An. nili s.s.* is the major malaria vector of the group and emphasized the exophagic behavior of *An. ovenegensis* and *An. carnevalei*. Genetic structure analyses fully supported former morphological and genetic studies, providing further support for the recent taxonomic classification within this group.

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SPECIES DELIMITATION IN SOUTH EAST ASIAN VECTORS OF MALARIA

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Many vectors of malaria belong to species complexes, which may differ in vector capacity and ecology. So efficient vector control relies on our ability to correctly identify both genetic and geographical species barriers. We have for this purpose developed a method for assessing reproductive boundaries in species complexes, using mitochondrial (cytochrome c oxidase 1) and nuclear genetic data (internal transcribed spacer 2), and we apply the method on three closely related species complexes; Anopheles subpictus (n=300), Anopheles sundaicus (n=375) and Anopheles vagus (n=200), all of which are closely related to each other. The previous morphological and genetic evidence for species delineation in the species complexes was also re-assessed. In all three cases it is found that the number of described species is different from our inferred number of species. Anopheles subpictus is found to contain at least 7 species, two of which are morphologically distinct, and have previously been described as separate species. There are also two cases of shared mitochondrial haplotypes between sympatric species, so identification based on mitochondrial genetic data can in these cases be misleading. ITS2 is found to be the most reliable species marker, but it may overestimate the number of species. Both Anopheles vagus and Anopheles sundaicus species complexes are found to only comprise of one species each, but in both species there are geographically isolated populations, which may have different ecological niches.

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NEOTROPICAL *ANOPHELES TRIANNULATUS* COMPLEX: PHYLOGEOGRAPHY AND DEMOGRAPHIC HISTORY BASED ON MITOCHONDRIAL AND NUCLEAR MARKERS

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Anopheles triannulatus is a complex of at least three sibling species: *An. triannulatus* s.s., *An. halophylus* and *An. triannulatus* "C". *Anopheles triannulatus* s.l. has been incriminated as a malaria vector in some South American countries, especially when it occurs in high densities. Morphological and behavioral differences, such as larval habitat exploitation, have been described. In addition, molecular analysis using isoenzymes detected a barrier to gene flow among the three species in sympatry. To decipher the evolutionary forces that may have led to the current distribution and genetic history of members of *An. triannulatus* s.l., we analyzed the mitochondrial *COI* and the nuclear *white* gene in mosquitoes from 7 countries and 15 different locations, including samples of sympatric *An. halophylus* and *An. triannulatus* "C" from Salobra, (SW Brazil). The median joining network based on the *COI* gene depicted the haplotypes grouping in 7 different lineages with a high number of mutational steps between them, a) Panamanian, N and NW Colombian and Venezuelan, b) Venezuelan c) N and NE Brazilian, d) Ecuadorian, e) NW Colombian, f) SE Brazilian, g) Bolivian, Argentinian and Central Brazilian (including *An.halophylus* and *An triannulatus* "C"). In contrast, statistical parsimony analysis of *white* gene showed 4 different clusters, 1) Panamanian, Venezuelan, Colombian and NE Brazilian (except Ceará), 2) Venezuelan, Ecuadorian and SW Brazilian 3) *An. halophylus* and *An. triannulatus* "C" plus SW Brazilian, 4) NE Brazilian (Ceará). Signatures of population expansion were detected with the *COI* gene in NE Brazil, Venezuela (Casigua), Panamá (Gamboa) and NW Colombia. Estimated time of expansion was during the Pleistocene. Results of Bayesian Inference support network lineages, with stronger posterior probabilities in the *white* gene data. Neither marker supports monophyly of any of the three taxa. Both markers detected Casigua (Venezuela), as an area where lineages converge, supporting the idea of a hotspot for diversity perhaps as a consequence of repeated Andean uplifts.

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LOW LINKAGE DISEQUILIBRIUM IN *ANOPHELES GAMBIAE* S.L. POPULATIONS

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In the malaria vector *Anopheles gambiae*, understanding diversity in population biology and genetic components of important phenotypes like resistance to malaria infection is crucial to develop new malaria transmission blocking strategies and requires the study of polymorphism. Linkage disequilibrium determines the density of Single Nucleotide Polymorphisms (SNPs) to be genotyped to represent the majority of haplotypes present. Here, we aim to determine linkage disequilibrium in *A. gambiae* populations in genes potentially involved in mosquito immune responses against pathogens. We analyzed fragments containing exons and introns of four immune related genes (Gambicin, NOS, REL2 and FBN9) distributed on *A. gambiae* genome in natural populations of seven species of the complex. We used already published and new sequences. Genes were cloned and sequenced for 8 to 16 individuals per population. Detected polymorphisms allowed the measure of linkage disequilibrium decay along the genes. In all tested genes and species, linkage disequilibrium between SNPs was very low: at a distance of less than 200 bp, SNPs were rarely linked to each other. The linkage observed in the *A. gambiae* could be the result of large population sizes and high recombination rates. These results are of great interest in the development of large scale polymorphism studies for population genetics and association studies. It indicates that very fine scale SNP detection will be required to detect association to phenotypes of interest in malaria transmission and to give a general view of genome polymorphism to decipher for example vector immunity, *Anopheles-Plasmodium* interactions or vector behavior.

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POPULATION GENETICS OF *LUTZOMYIA LONGIFLOCOSA* (DIPTERA: PSYCHODIDAE) POPULATIONS FROM COLOMBIA USING THE CYTOCHROME OXIDASE 1 GENE

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During the 2003-2004 epidemic of leishmaniasis in central Colombia, an indoor feeding phlebotomine sand fly, *Lutzomyia longiflocosa*, was found to link the disease to a domestic transmission cycle. Because this sand fly has a distribution beyond the extents of the transmission zone, a molecular genetic assessment was conducted to assess its population structure. Three field populations of *Lu. longiflocosa* from the endemic regions of Tello (n=29), and Chaparral (n=28) (Tolima Province) and San Antonio (n=10) (Cundinamarca Province) were compared with 620 base pairs of the cytochrome oxidase 1 mitochondrial gene. A high-fidelity Taq polymerase along with both forward and reverse sequencing was used to ensure robustness of the sequencing results. A total of 38 variable sites in the sequence were identified in the 67 specimens. Considerable genetic differentiation was revealed between the San Antonio and Chaparral/ Tello samples. Prior morphological analysis identified all samples as *Lu. longiflocosa*. However, the molecular genetic results suggested the occurrence of a genetically distinct taxon in San Antonio. Of the 620 bp sites, San Antonio differed from Chaparral and Tello at 15 segregating sites for all of its 10 samples, along with three additional variable sites. Chaparral and Tello each had seven segregating sites. A total of 20 haplotypes were identified with a haplotype diversity of 0.911. Analysis in pairwise comparisons resulted in an overall nucleotide diversity (Nei's) of 1.3%. The within population nucleotide diversity was highest for San Antonio (0.8%), but similar for Chaparral (0.4%) and Tello (0.3%). The genetic distance was highest between Tello or Chaparral and San Antonio (0.038 and 0.039 respectively) and lowest between Tello and Chaparral (0.005).

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HEPATITIS E OUTBREAK IN A LOW INCOME URBAN COMMUNITY IN BANGLADESH

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On 1 January 2009 a cluster of 10 deaths in women of reproductive age with jaundice was identified in an urban slum, adjacent to Dhaka city. The team investigated to determine the etiology and generate hypotheses about risk factors. Through a household survey, we recorded age and sex of those with jaundice (yellow coloration of eyes or skin) since August 2008 and their illness outcome. We collected epidemiologic and clinical information from all identified deaths and from jaundice cases in the same compound, along with their serum specimens to test for IgM antibodies to hepatitis E (HEV) and hepatitis A viruses (HAV). We tested municipal pump water and tap water in houses of the deceased for fecal coliforms. We explored the perceptions and causal explanations of jaundice. The outbreak occurred in a community with over 50,000 residents at a density of approximately 100,000 people per square kilometer. Many residents work in the garment sector and 20% migrate in or out of the slum each year. The water supply pipelines pass through contaminated surface water and open sewers. There were reports of leakage in the main water lines due to poor maintenance of old pipes and self connections by residents. The slum's water supply is intermittent, resulting in negative pressure on the line that may draw sewage in through breaches in the pipes. The samples from the two municipal pumps were clean; however, water coming from the households' taps was highly contaminated (median of fecal coliform: 38; range: 12-12,000). Since 1 August 2008, 2,756 (5.4%) residents reported new onset of jaundice. We identified 18 jaundice associated deaths; four adult males, 10 adult females (four that were known to be pregnant), two neonates, and two stillborn babies of mothers with jaundice. Eighty four percent (37/44) of the population had IgM antibodies to HEV and 14% (6/44) to HAV. Many residents do not think jaundice is a serious disease or associate drinking contaminated water with it. This large outbreak of HEV caused 18 deaths in the community and is apparently due to contamination of water through poorly maintained water supply systems. As more rural poor in South Asia move into peri-urban settlements, characterized by marginal water and sanitary infrastructure, hepatitis E and other water borne pathogens will continue to kill residents. Practical, affordable and effective approaches to improve drinking water quality in these rapidly growing communities are needed.

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FILOVIRUS SEROURVEY FOLLOWING AN OUTBREAK OF MARBURG HEMORRHAGIC FEVER --- IBANDA AND KAMWENGE DISTRICTS, UGANDA, 2007

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During June-July 2007, three cases of Marburg hemorrhagic fever (MHF) occurred among residents of Kamwenge District, Uganda who worked in a bat-infested mine in neighboring Ibanda District. The filoviruses Marburg virus (MARV) and Ebola virus (EBOV) are both endemic in central Africa; fruit bats are the presumed natural reservoir. We conducted a cross-sectional, household-based serosurvey in the villages surrounding the mine, to estimate the prevalence of antibodies against MARV and EBOV, and assess the associations among reported exposures, illness, and laboratory evidence of prior filovirus infection. During August 28-September 1, 2007, we enrolled all members of randomly selected households, and collected information about prior exposure to the mine, animals, and vectors, and clinical signs and symptoms since the mine reopened in January 2009. Blood samples collected on filter strips by fingerstick from each participant were tested for immunoglobulin G (IgG) antibodies against MARV and EBOV by enzyme-linked immunosorbent assay. We enrolled 606 members in 123 of 130 (95%) selected households. Among household members enrolled, illness was reported in 381 (63%); fever in 301 (50%); any hemorrhagic fever (HF)-like illness (fever and bleeding with or without GI symptoms) in 36 (8%); and severe HF (fever, bleeding, and GI symptoms) in 22 (4%). Four (0.7%) died, one with severe HF, a mortality rate of 5% among those reporting severe HF. We tested blood samples from 564 (93%); 7 (1.2%) had evidence of prior MARV infection, and 7 (1.2%) others had evidence of prior EBOV infection. Prior MARV infection was significantly associated with severe hemorrhagic fever (OR 11.5; 95% CI 2.1-63.3); prior EBOV infection was not associated with any type of illness. Previous mining was significantly associated with prior MARV infection (OR 25.1; 95% CI 5.4-118), but not with prior EBOV infection. The seroprevalence of IgG antibodies against MARV and EBOV were found to be similarly low, despite recent evidence of MARV circulation in this area. The association of mining with prior MARV but not EBOV infection is consistent with the hypothesis that the reservoir hosts for MARV are cave-dwelling fruit bats and those for EBOV are forest dwelling bats. The high percentage of persons reporting HF-like illness highlights the need to distinguish among etiologies of HF-like illness in filovirus-endemic areas.

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EPIDEMIOLOGY OF LASSA FEVER IN THE MANO RIVER UNION COUNTRIES OF WEST AFRICA, 2004-2008

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Lassa fever (LF) is a potentially severe illness caused by Lassa virus, which is maintained in nature and spread to humans by the rodent *Mastomys natalensis*. Person-to-person transmission also occurs. Hundreds of thousands of Lassa virus infections and thousands of deaths are thought to occur yearly across West Africa, with most intense transmission in the Mano River Union (MRU) countries of Sierra Leone, Liberia, and Guinea, and in Nigeria. Civil unrest, human migration and intense perturbation of the landscape, underdeveloped surveillance systems, the non-specific clinical presentation, and the absence of laboratory diagnostics in West Africa have been major impediments to assessing the incidence of infection and disease. However, renewed peace in Sierra Leone and Liberia in recent years and the establishment in 2004 of a new capacity-building project--the Mano River Union Lassa Fever Network--create an opportunity to reestablish surveillance and provide baseline incidence and other epidemiologic data necessary to more accurately assess the burden LF poses to the region. We used government and hospital surveillance data to identify all cases of LF in the MRU region from 2004-2008. Data presented in this abstract are from Sierra Leone, but data from all three MRU countries will be presented at the ASTMH meeting. In Sierra Leone, 729 cases of LF were reported during the 5 year period, with a peak of

296 in 2004 and nadir of 51 in 2006. The nadir most likely represents diminished case finding as previously active relief organizations left the country at the end of the civil war. Most cases were suspected only, since laboratory confirmation was not routinely available until 2008, when 42 confirmed cases were noted. The age group most affected was 25-35 years, with an almost equal number of females and males. The overall 5-year case-fatality ratio was 23%, but rose considerably in 2006 (33%) and 2007 (47%), again likely due to diminished case finding and late presentation for medical care during this period. The incidence of LF was highest in the Chiefdoms of Lower Bambara, Dodo, and Nongowa in the eastern diamond-mining region of the country. Housewives and miners were the most common occupations affected. Although cases were seen throughout the year, peak incidence occurred consistently during the dry season. These baseline epidemiologic data provide a foundation for future systematic monitoring and control of LF in West Africa.

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SEROPREVALENCE OF IGG ANTIBODIES AGAINST PHLEBOVIRUSES IN HUMANS IN NORTHERN AFGHANISTAN

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Phleboviruses form an own genus in the familiy Bunyaviridae and are transmitted by sandflies to humans. They cause febrile illness and meningitis in humans. Although rarely fatal, they are of enormous military importance due to their explosive outbreak patterns. Although actual data on leishmaniasis, also transmitted by sandflies, in Afghanistan are available, information on the prevalence of sandfly fever virus infections is not available. We tested 141 sera from adult inhabitants of Koundouz region in Northern Afghanistan for seroprevalence of IgG antibodies against Sandfly Naples virus, Sandfly Sicilian virus, Toscana virus, and Sandfly Cyprus virus. Sera were taken exclusively from male adults. The test system used was the biochip containing the four virus antigens fixed in infected Vero E6 cells (Euroimmun AG, Lübeck, Germany). Antibodies were detected by indirect immunofluorescence technique. Sera were screened in a dilution of 1:10. Positive sera were end-titrated in two-fold dilution steps. 81/141 sera (57.4%) of the sera tested showed a positive reaction against any of the four phleboviruses. 46/141 (32.6%) sera showed a monospecific or significant (fourfold) IgG titer against Sandfly Sicilian virus. 12/141 (8.5%) sera reacted against Sandfly Cyprus virus. IgG antibodies against Sandfly Naples virus and Toscana virus were prevalent in 5/141 sera (3.5%) each. 13 sera showed non-significant titers against more than one sandfly virus and therefore were classified as indeterminate. Titers ranged from 1:10 to 1:320. Our results present for the first time since several decades the presence and circulation of at least 4 different phleboviruses in Northern Afghanistan. Most prevalent is Sandfly Sicilian virus. One third of the population tested reacted positive against this virus. Lower prevalence rates were found for antibodies against the other three phleboviruses. Our data show an intensive circulation of the four sandfly fever viruses in the human population. These viruses should be included in the differential diagnosis of febrile illness and meningitis.

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A PROFILE OF INFLUENZA VIRUS INFECTION IN ACUTE RESPIRATORY ILLNESS IN GHANA

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Introduction: Influenza surveillance was initiated by routine monitoring of acute respiratory illness in selected health institutions in Ghana as part of national preparedness efforts to the threat of pandemic influenza. Methodology: Under the Ghana Health Service, 8 health facilities in three regions have, since September 2007, served as sentinel sites. Adult patients with abrupt onset of fever >38ºC and at least cough, sore throat, coryza, myalgia and headache were recruited as study subjects and throat swabs/nasopharyngeal aspirates were collected. Children, 5 years and below, presenting with body temperature >38OC, cough and coryza, at 2 hospitals in the Greater Accra Region were also studied. Virus isolation and identification by cell culture and haemagglutination assays were done. Additionally, polymerase chain reaction (PCR) assays, anti-viral susceptibility testing and genetic characterization by sequencing was done. Results: As at 6th May 2009, 410 adults and 358 children under 5 yrs of age (768 patients in total) have been studied with 57 influenza virus isolates. Thirty-nine patients were also positive by PCR for influenza A infection with 15 H1N1 and 5 H3N2 subtypes identified. Amongst the virus isolates, 30 H1N1 and 1 H3N2 influenza A subtypes with 26 influenza type B (Yamagata lineage) strains have been characterized. Two clades of the H1 viruses are apparent and Oseltamivir resistance has averaged 59% amongst influenza A H1N1 isolates. No evidence of H5N1 infection was found with 3 influenza A infections requiring further characterization. Conclusion: Overall, influenza virus infection averaged 12.5% and the profile of influenza viruses contributing to acute respiratory illness in Ghana has been discerned providing a clear snapshot of recent locally circulating influenza viruses. In light of "avian and swine" flu outbreaks worldwide, this is an important contribution to the World Health Organization's global influenza surveillance network. With the Noguchi Memorial Institute for Medical Research established as the 'National Influenza Centre', Ghana's Health Service has enhanced capacity to detect influenza outbreaks, assess the impact of this virus on health and formulate appropriate public health policies.

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INFLUENZA SENTINEL SURVEILLANCE-RWANDA, JULY 2008-MARCH 2009

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Influenza viruses cause significant morbidity and mortality worldwide, however true disease burden is difficult to assess without a surveillance system. In July 2008, Rwanda established an influenza sentinel surveillance system to identify patients with respiratory syndromes, who are then tested for influenza viruses. We used surveillance data to describe Rwanda's influenza epidemiology. Surveillance was conducted at four sentinel sites in Rwanda. Three case definitions were used: influenza-like illness (ILI), severe acute respiratory illness (SARI) adult, and SARI child. ILI was defined as temperature ≥38°C and cough or sore throat. SARI adult was defined as temperature ≥38°C, cough, shortness of breath, hospitalization, and age ≥5 years. SARI child was defined as cough or difficulty breathing, a respiratory danger sign, hospitalization, and age <5 years. For each case identified, a questionnaire was completed with demographic, clinical, and exposure information and two specimens (nasopharyngeal and oropharyngeal) were collected. Specimens were tested by real-time reverse transcriptase polymerase chain reaction (real-time RT-PCR) for influenza A and B virus. Surveillance data for the period from July 2008-March 2009 were analyzed using Epi-Info 3.5.1. In total,127 cases were identified. For all cases, the median age was 7 years (range:<1-64 years) and 76 (59.8%) cases were female. In terms of case classification, 35.4% (n=45) of cases were ILI, 34.6% (n=44) SARI child, and 29.9% (n=38) SARI adult. In total, 91 cases were tested for influenza and 16.5% (n=15) tested positive. Of ILI cases tested (n=28), 14.3% (n=4) were positive for influenza A and 7.1% (n=2) were positive for influenza B. Of SARI adult cases tested (n=29), 24.1% (n=7) were positive for influenza A and none were positive for influenza B. Of 34 SARI child cases tested, 2 (5.9%) were positive for influenza A and none were positive for

influenza B. Of 15 influenza A cases, 13 cases were subtype H3 and 2 cases were unsubtypeable; the median age was 21 years (range: <1-55 years) and 4 (30.8%) were male. In conclusiion, in Rwanda, over an eight-month period, more than 10% of specimens tested positive for influenza, with the majority being influenza A, subtype H3. These results are similar to surveillance data from surrounding countries. Further assessment of seasonal and regional patterns is needed to inform national prevention strategies.

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SPATIAL ANALYSIS OF HEMORRHAGIC FEVER WITH RENAL SYNDROME IN SHANDONG PROVINCE, EASTERN CHINA, 1968-2005

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First human cases of hemorrhagic fever with renal syndrome (HFRS) in Shandong Province, China were reported in Yuquan County in 1968. Since then, the disease has spread across the province where it has become one of the most serious HFRS epidemic areas in mainland China. Causes underlying such rapid spread and wide distribution, however, remain less understood. Here we report a spatio-temporal analysis of human HFRS cases in Shandong using data spanning 1968 to 2005. Data were collected from records of Shandong Center for Disease Prevention and Control. Seasonal incidence maps were generated, velocity vector maps were produced to analyze the spread of HFRS over time, and space-time cluster detection analyses were conducted. Results show a rapid propagation of HFRS from its epicenter in Rizhao, Linyi, Weifang regions in southern Shandong toward north, east, and west parts of the province. Four space-time clusters were detected and three periods were identified to show seasonal shifts of HFRS epidemics. The first cluster was identified in 1968-1982 when the foci of HFRS was located in the south Shandong and the seasonal peak of HFRS cases was between the fall-winter period, representing a typical seasonal characteristic of Hantaan virus (HTNV) infections. The second was in 1983-1985 during which the foci of HFRS spread toward almost all counties on the north and west, and the number of HFRS cases increased rapidly. During this period, although the number of HFRS cases increased in the fall-winter window and remained predominant, the number increased quickly in the spring, especially in new foci, reflecting a characteristic pattern of Seoul virus (SEOV) infections. The last cluster occurred in 1986-2005 during which the foci of HFRS spread north-east to cover all counties, the number of HFRS cases decreased and remained predominant in spring. The findings offer insights into understanding the causes of spread and distribution of HFRS.

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MANAGEMENT OF PNEUMONIA AND MALARIA AT THE COMMUNITY LEVEL IN ZAMBIA

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Pneumonia and malaria are the two leading causes of morbidity and mortality among children under five in Zambia. In rural areas, most sick children including those with malaria and pneumonia are seen by community health workers (CHW) because public health facilities are not readily accessible. Zambia is now deploying artemether-lumefantrine (AL) at the community level for the treatment of malaria, but there are concerns about overuse of this expensive drug. For pneumonia, under current policy, CHWs refer children to the nearest health facility, which may be located some distance away, leading to many children having delayed treatment or no treatment at all. This cluster-randomized controlled study assessed the effectiveness and feasibility of CHWs managing pneumonia and malaria with the aid of rapid diagnostic tests (RDTs) in children 6 months to 5 years. Intervention CHWs performed RDTs, treated positive cases with AL and treated pneumonia with amoxicillin. Control CHWs did not perform RDTs, treated fever with AL and referred pneumonia to the health facility. 3125 children (1017 intervention and 2108 control) with fever and/or difficult/fast breathing were enrolled over a 12 month period. The main presenting complaints were (intervention vs. control): fever 94.7% vs. 98.9%; cough 67.8% vs.63.3%; difficult breathing 16.8% vs. 6.9%; and fast breathing 35.8% vs. 10.2%. 27.5% (265/963) of children with fever in the intervention group were received AL. In contrast, 99.1% (2066/2084) of control children with fever received AL (RR 0.28, 95%CI 0.17 - 0.41). For pneumonia, 68.2% (247/362) in the intervention group received early and appropriate treatment compared to 13.3% 27/203) in the control group (RR 5.13; 95% CI 3.04 - 8.66). The RDT positivity ranged from 58.6% in the rainy season to 9.6% in the dry season. The capacity of CHWs to use RDTs, AL and amoxicillin to manage both malaria and pneumonia at the community level is promising and has the potential to reduce over usage of AL and provide early and appropriate treatment to children with pneumonia.

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ANTIGENIC AND PHYLOGENETIC ANALYSIS OF INFLUENZA VIRUSES IN KENYA FROM 2006-08 WITHIN THE CONTEXT OF REGIONAL AND GLOBAL INFLUENZA DRIFT

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The general consensus has been that distinct influenza virus strains tend to evolve first in SE Asia before global spread, but the emergence of an antigenically distinct strain of Influenza A (H1N1) in North America in 2009 is evidence that important influenza strain drift or shift can occur in other locations in the world. Though historically absent in Africa, sustained sentinel influenza surveillance is critical for the detection of virus evolution and subsequent vaccine selection. The US Army Medical Research Unit-Kenya and the Kenya Medical Research Institute developed an influenza surveillance network at 8 hospitals dispersed throughout Kenya in 2006-08. Nasopharyngeal specimens were screened by real-time RT-PCR and

then identified by HAI after culture. Isolates were characterized by HA1 sequencing. 1213 of 7836 specimens (15%) tested positive for influenza A and 346 (4%) for influenza B. 230 isolates were characterized and submitted to GenBank and WHO. During the first 2 years of surveillance, 3 clades of Kenya viruses were identified which phylogenetically differed significantly from vaccine strains with bootstrap values all over 96%. In two cases--a clade of H3N2 isolates collected from Nairobi slums in 2006 and secondly, among Kenyan 2008 influenza B (Yamagata-like) circulating viruses--the phylogenetic difference did not correspond to an antigenic difference. HAI data indicated 100% and 97% respective correlation with the dominant circulating vaccine strains. The third--all Kenyan H1N1 viruses detected from early 2007--not only formed distinct branches from A/New Caledonia/20/99(H1N1) and A/Solomon Islands/3/2006 (H1N1), the 2007 and 2008 S. Hemisphere vaccine strains, but 44% demonstrated greater than a 4-fold difference in HAI titers. The fact that the 2007 Kenya H1N1 viruses which were phylogenetically similar to influenza A/Brisbane/59/2007 (H1N1), the recommended 2008 global vaccine strain, were circulating much earlier than elsewhere including SE Asia demonstrates that E. Africa may be an epidemiologically interesting site for the emergence of new strains of influenza.

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VALIDATION OF THE MICROSCOPIC-OBSERVATION DRUG-SUSCEPTIBILITY (MODS) TECHNIQUE FOR DRUG-SUSCEPTIBILITY TESTING DURING TUBERCULOSIS THERAPY

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The Microscopic-Observation Drug-Susceptibility (MODS) technique has proven reliability for diagnosis and concurrent direct isoniazid and rifampicin drug-susceptibility testing for patients with suspected tuberculosis (TB). However, MODS has not been validated during TB treatment, when misleading mycobacterial sub-populations may be selected and when antimicrobials in sputum may confound *in vitro* direct drug-susceptibility testing. Consequently, patients whose sputum was collected after treatment commenced (or whose pre-treatment MODS assay failed) miss the opportunity for a rapid MODS drug-susceptibility test. We therefore assessed MODS reliability during TB treatment. Sputum samples were collected from the same patients both prior to and during TB treatment. Sputa were tested with the MODS technique that provided direct concurrent isoniazid and rifampicin drug-susceptibility testing. Subsequently, the sub-cultured TB strain was tested with conventional indirect drug-susceptibility testing with the alamar blue and tetrazolium microplate assays. Results of these direct and indirect tests were compared with reference to the patients' phase of conventional first-line treatment. Paired direct MODS and indirect drug-susceptibility results were available for 1552 samples, 928 in the month prior to treatment, and 624 during therapy. TB therapy had no effect on the accuracy of direct drug-susceptibility testing relative to indirect testing. Specifically, the agreement between direct and indirect testing, respectively, was 92% vs. 91% for isoniazid susceptibility; 97% vs. 97% for rifampicin susceptibility; and 97% vs. 96% for MDRTB testing (all P>0.1). Treatment duration also had no effect on the level of agreement (P>0.1). In conclusion, these results validate MODS drug-susceptibility testing during treatment. Thus, patients who have commenced TB therapy may be offered rapid drug-susceptibility testing with the MODS technique.

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GAPS IN THE GLOBAL USE OF *HAEMOPHILUS INFLUENZAE* TYPE B CONJUGATE VACCINE

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Haemophilus influenzae type b (Hib) causes an estimated 3 million cases of meningitis and pneumonia and almost 400,000 deaths worldwide per year in children under 5 years of age. Although safe and effective vaccines against Hib are available, Hib conjugate vaccines have been underutilized in developing countries where most disease occurs. The GAVI Alliance provides funding to support Hib vaccine use in the poorest of these countries. We assessed progress in global use of Hib vaccine using information from the World Health Organization (WHO), the GAVI Alliance, Ministries of Health, publications, and country press releases. We compared WHO member states' use of, access to, and plans for Hib vaccine over time and stratified by income using World Bank indicators. The number of WHO member states using Hib vaccine increased from 92 (48%) of 192 in 2004 to 143 (74%) of 193 by May 2009; this is projected to increase to 162 (84%) of 193 by the end of 2009. The increase was greatest among the poorest countries [13/75 (17%) in 2004, 49/72 (68%) by May 2009, and 64/72 (89%) projected for 2009]. Higher income countries started introduction of Hib vaccine earlier than countries in lower income strata; for the first time, in 2008 the proportion of low income countries using Hib vaccine was greater than the proportion of lower middle income countries that use the vaccine. Thirty-nine percent (53 million) of the world's 136 million infants had access to Hib vaccine in 2008 compared with 22% in 2006; this is projected to rise to 66% by the end of 2009. The three countries globally with the largest birth cohorts (India, China, and Nigeria) do not currently use Hib vaccine; however, these countries have made steps toward introducing Hib vaccine. The recent increase in the use of Hib vaccine worldwide, particularly in the world's poorest countries, shows that focused strategies to accelerate introduction of new vaccines in developing countries are feasible and effective. The limited increase in Hib vaccine use among lower middle income countries not eligible for GAVI funding and some populous countries highlights the gaps in Hib vaccine use worldwide and where targeted plans are needed.

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TREATING ISONIAZID MONORESISTANT TB WITH STANDARD FIRST-LINE REGIMENS RESULTS IN HIGH RATES OF TREATMENT FAILURE, TB RECURRENCE AND TB-RELATED DEATH

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Optimal management of tuberculosis resistant to either isoniazid or rifampicin that is not multi-drug resistant (MDR) is poorly defined. In resource-poor settings, patients with isoniazid or rifampicin mono-resistant TB are usually treated with standard first line TB-treatment regimens. Little is known about the long term efficacy of such management. 356 patients from a community hospital in a peri-urban shanty town in Lima, Peru were enrolled after diagnosis of pulmonary TB disease; 309 were new TB cases and 47 were cases of retreatment. Patients were tested for resistance to rifampicin and isoniazid, and those resistant to both drugs (MDR-TB, 21 patients) were excluded. All other patients were followed prospectively throughout treatment and interviewed a median of 5 years after treatment completion for determination of the relationship between isoniazid and rifampicin resistance and failure, recurrence after cure, and long term TB-related death. Overall, 39 of 335 (12%) patients had laboratory-confirmed monoresistant TB at diagnosis; 29 patients (8.7%) had isoniazid monoresistant TB and 10 patients (3.0%) had rifampicin monoresistant TB. Among patients with first episodes of TB disease, 28 (10%) and 5 (1.7%) had isoniazid and rifampicin monoresistance, respectively. All new TB patients received six months of standard short-course chemotherapy and all patients with recurrent TB received nine months of first line drugs under the standard national retreatment scheme. At follow-up, only 55% of isoniazid monoresistant and 50% of rifampicin monoresistant TB patients achieved long-term cure with the standard regimens. In addition to poorer treatment outcome, patients with isoniazid monoresistant TB and rifampicin monoresistant TB at diagnosis suffered significantly higher rates of recurrence after cure and long-term TB-related death compared to patients with sensitive strains ($p<0.01$ in all cases). In conclusion, monoresistance to rifampicin and particularly isoniazid are relatively common amongst non-MDR TB patients. Long-term morbidity and mortality is high when patients with isoniazid and rifampicin monoresistant TB are treated with standard first line anti-TB regimens. TB therapy should be augmented for patients with isoniazid and rifampicin monoresistant TB.

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INVESTIGATIONS OF CLOSE CONTACTS OF PATIENTS WITH LABORATORY-CONFIRMED H5N1 INFECTION IN INDONESIA, IN 2007

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Since 2005, 134 patients with H5N1 influenza virus infection have been identified in Indonesia including 108 (81%) who have died. Investigation of patient contacts and prevention of H5N1 virus transmission within the health care setting is a high priority for the Ministry of Health. The objectives of this study were to measure the frequency of H5N1 infection among close contact of patients with laboratory-confirmed H5N1 infection and monitor compliance with MOH guideline of using PPE and recommendations for antiviral prophylaxis for contacts of H5N1 patients. Trained teams investigated family members, neighbors, and health care workers who had close contact to H5N1 patients during the course of clinical illness. Interviews were conducted to characterize exposure to the index patient as well as community exposures to sick poultry. Throat swabs from contacts with influenza-like illness were tested by real time RT-PCR for evidence of H5N1 infection. Acute and convalescent serum samples were tested for antibody to H5N1 virus by hemagglutination inhibition assays. Overall, 694 contacts were investigated including 300 (43%) Health Care Workers (HCW), 243 (35%) family members, and 151 (22%) neighborhood contacts. 54 contacts (21%) reported ILI in the 7 days before the interview. HCW were not more likely (OR=1.42, 0.74 <0r<2.71) to report ILI than other contacts. Two (0.3%) contacts (a family member and a neighbor) tested positive for H5N1 infection and reported exposure to sick poultry. Of 54 contacts with ILI, 7 (13%) took tamiflu within 14 days prior to interview. HCWs were more likely to take tamiflu prophylaxis than other contacts. Only 10 (4%) of HCW using complete PPE when taking care of H5N1 patients. No contacts had antibody to H5N1 virus. In conclusion, we found no evidence of H5N1 virus infection among close contacts of patients laboratory-confirmed H5N1 infection. HCWs are not reported higher ILI than other contacts. More efforts are needed to ensure compliance with MOH recommendations on antiviral prophylaxis and use of PPE.

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BLOOD CULTURES FOR THE DIAGNOSIS OF DRUG-RESISTANT TUBERCULOSIS IN RURAL SOUTH AFRICA

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Multidrug-resistant and extensively drug-resistant tuberculosis (MDR and XDR TB) have emerged as critical pathogens in KwaZulu-Natal (KZN), South Africa, associated with HIV infection and rapid mortality. Alternatives are needed to detect drug-resistant TB in patients (pts) unable to expectorate or with extrapulmonary TB (EPTB). The yield of blood cultures for MDR and XDR TB is unknown and blood may provide additive yield compared to sputum. A retrospective analysis was performed on all pts who had a single mycobacterial blood culture from 09/01/06-12/31/08 at a rural district hospital in KZN. Blood cultures were sent in pts suspected of drug-resistant TB or EPTB. Specimens were inoculated into Bactec MycoF-lytic bottles. Drug-susceptibility testing was performed on all positive specimens. MDR TB was defined as resistance to isoniazid and rifampicin, and XDR TB as additional resistance to a fluoroquinolone and an injectable second-line drug. Sputum samples were analyzed if obtained within 2 weeks before or after the blood culture. Analysis included 130 pts. Median age was 31.5 years (range: 7 months-62 years), with 73 men (56%) and 57 women (44%). 115 pts were HIV-infected (88%), with a median CD4 count of 100 cells/mm^3 (IQR 28-190). 89 pts (68%) were on TB treatment, 42 (32%) had a prior history of TB, and 45 (35%) had signs of EPTB. Blood cultures were positive for *Mycobacterium tuberculosis (M. tb*) in 41 (32%) of 130 patients, of which 8 (20%) were MDR TB and 20 (49%) were XDR TB. Sputum culture was negative or unattainable in 21 (16%) pts, of whom 57% had MDR or XDR TB isolated from the blood. Pts on antiretrovirals were less likely to have *M. tb* in the blood, OR 0.26 [0.11-0.62] p=0.002. Having signs of EPTB was not predictive of mycobacteremia. In conclusion, blood cultures provide a significant additive yield for drug-resistant TB in pts with HIV from rural South Africa. Mycobacteremia was not restricted to pts with signs of EPTB and should be considered in pts suspected of drug-resistant TB, especially those unable to produce sputum.

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IL-15 MAINTAINS A RESERVOIR OF CENTRAL MEMORY CD8 T CELLS THAT ARE REQUIRED FOR PROTRACTED PROTECTION AGAINST *PLASMODIUM BERGHEI* INFECTION

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The maintenance of optimally effective memory T cells is required for long-lasting protective immunity against many pathogens. Protracted protection against human and murine malaria is achieved by multiple immunizations with radiation-attenuated (γ) *Plasmodia* sporozoites (γ-spz). In the *P. berghei* (Pb) γ-spz system, protection is associated with the presence of liver CD8 T cells that comprise two phenotypically and functionally discrete sets: CD8 T effector memory (T_{EM}) cells ($CD44^{hi}CD45RB^{lo}CD62L^{-}$) and CD8 T central memory (T_{CM}) cells ($CD44^{hi}CD45RB^{hi}CD62L^{+}$). CD8 T_{EM} cells secrete IFN-γ upon spz challenge. It is generally accepted that IL-15 is required to maintain homeostatic proliferation of CD8 T_{CM} cells. Although indolent producers of IFN-γ, the CD8 T_{CM} cells from Pbγ-spz- immunized mice express higher levels of CD122, the common β chain shared by the IL-2R and IL-15R, than CD8 T_{EM} cells. In this study we asked if immunization with Pbγ-spz could protect IL-15 KO mice against sporozoite challenge and by extension if CD8 T_{CM} cells are needed for protection. The results demonstrate that following immunization with Pbγ-spz both WT and IL-15 KO mice are protected against an initial challenge. However, unlike WT mice, upon a re-challenge 2 months later, the IL-15 KO mice lose protection, and this is accompanied by a reduced proliferation of CD8 T cells as measured by BrdU incorporation. Interestingly, the capacity of CD8 T cells to produce IFN-γ was lower in the IL-15 KO mice than in the WT mice at both the initial challenge and at re-challenge. On the basis of these observations, we hypothesize that the maintenance of protection induced by Pbγ-spz depends on a conscription process whereby the CD8 T_{CM} cells constitute a reservoir of partly activated T cells that slowly proliferate to IL-15 and that upon re-infection differentiate into CD8 T_{E} cells. Supported by NIH Grant AI 46438 and the U.S. Army Medical Research and Materiel Command.

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LIVER CCD8α⁺DC FROM MICE IMMUNIZED WITH RADIATION-ATTENUATED *PLASMODIUM BERGHEI* SPOROZOITES MEDIATE THE INDUCTION OF LIVER EFFECTOR CD8⁺ T CELLS AGAINST PRE-ERYTHROCYTIC STAGE INFECTION

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Immunization with radiation (γ)-attenuated *Plasmodia* sporozoites (γ-spz) confers sterile and long-lasting immunity against malaria liver-stage infection. In the *Plasmodium berghei (Pb)* γ-spz model, protection is linked to liver $CD8^{+}$ T cells that express an effector/memory (T_{EM}) phenotype, ($CD44^{hi}CD45RB^{lo}CD62L^{lo}$), and produce IFN-γ. However, neither the antigen presenting cells (APC) that activate these $CD8^{+}$ T_{EM} cells nor the site of their induction have been fully investigated. In the present study, we demonstrate that multiple exposures of mice to *Pb* γ -spz lead to a progressive and nearly concurrent accumulation in the liver but not the spleen of both $CD11c^{+}NK1.1^{-}$ DC and $CD8^{+}$ T_{EM} cells. Upon adoptive transfer, liver $CD11c^{+}NK1.1^{-}$ DC from *Pb* γ -spz-immunized mice induced protective immunity against sporozoite challenge. Because conventional (c)CD8α⁺ DC (a subset of $CD11c^{+}$ DC) are considered the major inducers of $CD8^{+}$ T cells, we asked whether the cCD8α⁺DC might be involved in the activation of $CD8^{+}$ T_{EM} cells. In an *in vitro* system, liver cCD8α⁺ DC from Pbγ-spz immune mice induced naïve $CD8^{+}$ T cells to express the $CD8^{+}$ T_{EM} phenotype and to secrete IFN-γ and the $CD8^{+}$ T cell activation was inhibited by anti-MHC class I and anti-IL-12 mAbs. These data suggest that liver cCD8α⁺ DC present liver-stage antigens to activate $CD8^{+}$ T_{EM} cells, the pre-eminent effectors against pre-erythrocytic malaria. These results provide important implications towards a design of anti-malaria vaccines. Supported by NIH Grant AI 46438 and the U.S. Army Medical Research and Materiel Command.

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EXPRESSION OF FOXP3, IL-10 AND TGF-β1 IN IP-10 DEFICIENT C57BL/6 MICE WITH EXPERIMENTAL CEREBRAL MALARIA

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Cerebral malaria (CM) is a major cause of malaria mortality in children in endemic countries. Although the precise mechanism is not known, sequestration of malaria infected red blood cells and leukocytes in brain vessels coupled with the production of pro-inflammatory factors by T cells have been shown to mediate CM. Recently, IP-10, a chemokine involved in recruiting T cells, was reported to be significantly up regulated in cerebrospinal fluids and sera of Ghanaian CM patients and C57BL/6 mice deficient for this chemokine were less susceptible to experimental CM**.** Depletion of IP-10 enhanced the production of regulatory T cells (T regs) and cytokines IL-10 and TGF-β1. Conversely, high expression of IP-10 resulted in loss of regulatory T cells. Regulatory T cells (CD4+CD25+Foxp3+) regulate excessive production of pro-inflammatory factors by secreting suppressive markers IL-10 and TGF-β1. We hypothesized that enhanced T regs production in IP-10 knockout mice will modify the outcome of ECM. To this end, IP-10-/- and wild type (WT; C57BL6) mice were infected with *P. berghei* ANKA parasites after which plasma, peripheral blood mononuclear cells (PBMC) and brain samples were collected at days 2, 4 and 8 post infection. Foxp3, IL-10 and TGF-β1 were evaluated and compared in IP-10-/- and WT mice using ELISA, quantitative real time PCR and *in vitro* restimulation assays. Results confirmed that IP-10-/- mice survived longer than WT during the infection and that IL-10 in infected IP-10-/- plasma was significantly up regulated ($p< 0.05$) compared with infected WT at day 2 and 4 pi. TGF-β1 in plasma was significantly ($p< 0.05$) down regulated in all infected mice compared with the controls. Foxp3 mRNA was induced at day 2 in PBMCs and brains of infected IP-10-/- compared with WT. Thus, *P. berghei* infection up regulates IL-10 in plasma and Foxp3 in PBMCs and brain in IP-10-/- mice than in WT and down regulates plasma levels of TGF-β1. However, there was high production of TGF-β1 by *P. berghei* antigen restimulated IP-10-/- CD4+CD25+ T cells than WT CD4+CD25+ T cells. Interestingly, there was no difference in IL-10 production by *P. berghei* antigen restimulated CD4+CD25+ T cells in both IP-10-/- and WT. We conclude that during *P. berghei* ANKA infection, deleting IP-10 results in increased early expression of Foxp3 in PBMCs and brain with high production of IL-10 in plasma which tends to be protective against fatal ECM.

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INTERFERON-A PROMOTER HAPLOTYPES AND SUSCEPTIBILITY TO SEVERE MALARIAL ANEMIA

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Cytokines are important for regulating the immune response to *Plasmodium falciparum*. Dysregulation in the cytokine balance during falciparum malaria can enhance the development of severe malarial anemia (SMA), particularly in children less than 4 yrs. of age. We have previously shown that interferon (IFN)-α is suppressed in Gabonese children with severe malaria, characterized by reduced hemoglobin (Hb) levels and/or hyperparasitemia. IFN-α is a pleiotropic cytokine of the type I interferon family that bridges innate and adaptive immunity. The role of (IFN)-α in regulating clinical outcomes in children with SMA has

not been reported. The aim of this study was, therefore, to investigate the relationship between IFN-α promoter polymorphisms (-173A/T and -884T/A), functional changes in IFN-α production, and SMA in parasitemic children (n=476; 3-36 mos.) from a holoendemic *P. falciparum* transmission region of western Kenya. Genotypes were determined by 5'-allelic discrimination assay, circulating IFN-α was measured by multiplex analyses, and complete co-infection and hematological parameters were determined by standard methods. Stratification of children according to SMA status revealed that the SMA group (Hb<6.0 g/dL) had lower circulating IFN-α than the non-SMA group (P=0.02). Multivariate logistic regression analyses of individual genotypic variants, controlling for age, gender, HIV-1 status, bacteremia, and sickle-cell trait, showed that heterozygocity at -884 (TA) was associated with an increased risk of SMA [OR, 2.09 (95% CI, 1.19-3.68); P=0.01] and reduced circulating IFN-α relative to wild-type individuals (TT; P=0.03). Haplotypic analyses, controlling for appropriate co-factors, revealed that -173T/-884A was associated with an increased risk of SMA [OR, 2.14 (95% CI, 1.06-4.32); P=0.03] and lower circulating IFN-α (P=0.12). Thus, variation in the IFN-α promoter at -173 and -884 conditions increased susceptibility to SMA and reduced circulating IFN-α, suggesting that appropriate production of IFN-α is required for protection against the development of SMA.

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IDENTIFICATION OF HLA RESTRICTED CD8+ T-CELL EPITOPES ON THE *PLASMODIUM FALCIPARUM* AMA1 PROTEIN

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Plasmodium falciparum apical membrane antigen-1 (AMA1) is a leading malaria vaccine candidate antigen. We used computerized algorithms (NetMHC software) to predict and rank potential AMA1 8-10-mer peptide sequences according to Class I binding affinities for 6 HLA alleles expressed by human volunteers immunized with an experimental AMA1 vaccine. Synthetic peptides representing selected 9-mer epitopes, as well as 15-mer peptide pools spanning AMA1, were screened by ELIspot for capacity to be recognized by recall responses from immunized volunteers. These volunteers were immunized with a single dose of two recombinant adenovectors (serotype 5) encoding PfAMA1 and Pf circumsporozoite protein respectively (1x10e10 particle units each construct). ELISpot responses peaked at 28 days, with summed responses recalled by 12 peptide pools spanning AMA1 averaging 1037 spot forming cells/million PBMC's (range 353 to 2193). Peptides were designated as the predicted minimal epitope (8-11 mer) for the corresponding 15-mer if the predicted binding affinity as measured by the 50% inhibitory concentration (IC50) was less than 500 nM. Fourteen minimal epitopes restricted by a panel of HLA alleles that included HLA-B*1801 (4), HLA-A*0201 (2), HLA-B*4402 (1), HLA-A*0101 (2), HLAB*0801 (2), HLA-A*6802 (2) and HLA-A*3002 (1), as well as the original pools used for initial screening, were assayed by ELISpot and ICS assays. Four of these 14 minimal epitopes restricted by HLA B*1801 (2 epitopes, 2 volunteers), HLA A*0101 (1 epitope, 2 volunteers), or HLA B*0801 (1 epitope, 2 volunteers) were recognized as positive by one or more volunteers. Studies are ongoing to identify additional PfAMA1-specific class I HLA-restricted epitopes as well as class II restricted CD4+ T cell epitopes. Such epitopes have the potential to facilitate the determination of immunogenicity of candidate malaria vaccines as well as to form a component of broad population coverage multi-epitope vaccine.

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EFFECTS OF CONCOMITANT *SCHISTOSOMA HAEMATOBIUM* INFECTION ON THE T REGULATORY CELL RESPONSE ELICITED BY ACUTE *PLASMODIUM FALCIPARUM* MALARIA INFECTION IN MALIAN CHILDREN

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Polyparasitism is common in the developing world. Patent helminth and Plasmodium infections elicit natural T regulatory cells (Tregs). A functional deficit of Tregs cells may be associated with reduced *P. falciparum* acquisition. In a prospective study assessing the effect of underlying *S. haematobium* on the acquisition and frequency of falciparum malaria, we previously demonstrated that schistosomiasis-positive (SP) Malian children, aged 4-8 years, have less malaria, prolonged onset until the first clinical episode and reduced parasitemias during that episode than age, gender and residency-matched schistosomiasis-negative (SN) children. To understand the immunological basis of these observations, immunologic responses at the cellular level were measured using an optimized 10-color flow cytometry protocol. Peripheral blood mononuclear cells (PBMC) derived from 11 SP and 9 SN children presenting with symptomatic malaria during the wet season and again during the dry season were evaluated. Controlling for age, the time to first malaria infection was 105 days after enrollment for SP children compared to 22.6 days in SN children (Mean: 6.3 years). Levels of Tregs ($Vivid^{-}CD3^{+}CD4^{+}CD25^{hi}CD69^{-}FoxP3^{+}$) were noted to be significantly lower in children with dual infection compared to SN children with malaria (0.51 versus 1.52%, P=0.049). Dry season T regs levels were similar in SP and SN children, as well as in SP children who remained malaria free through the malaria transmission season (0.82 vs. 1.25 vs. 1% respectively). Detectable IL-10 and TGF-β production was measured in response to both malaria and schistosomal antigens but did not appear to originate predominantly from T regs. Limited numbers of α-CD25 depletion studies suggest that enhanced IL-2 and IFN-γ is detected after stimulation of depleted PBMC with malaria antigens in both SP and SN children and to schistosomal antigens in SP children. These results support a potential functional deficit of T regs in helminth-infected children which may mediate protection against falciparum malaria.

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ANTENATAL INFECTION WITH SCHISTOSOMIASIS INCREASES SUSCEPTIBILITY TO MALARIA IN KENYAN CHILDREN

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We have previously observed that a subset of offspring of malaria infected women acquire a putatively tolerant immune phenotype in utero that is associated with increased susceptibility to malaria during childhood. We hypothesize that antenatal exposure to parasitic helminths in women co-infected with malaria contributes to this immune tolerant phenotype by downmodulating the fetal immune responses to blood-stage malaria antigens. To examine the impact of helminths (schistosomiasis, lymphatic filariasis and/or hookworm) on malaria susceptibility we undertook a prospective cohort study of 700 newborns in a malaria endemic region of Kenya in which children were examined every 6 months from birth to 36 months of age for *Plasmodium falciparum* infection and the presence of malaria antigen-specific T cell responses. Overall 26% of the pregnant women were co-infected with helminths and malaria, 16% with malaria

and 34% with helminths alone as determined parasitologically. SWAP-specific (adult worm antigen for Schistosoma haematobium) IgG4 was detected in 38% of mothers. There was 2 fold increase in risk of malaria infection in offspring of women co-infected with schistosomiasis based on presence of ova or SWAP IgG4; OR 2.1 (95% CI 1.22-3.6) P=0.007 and OR 2.0 (95% CI 3.46-1.19) P=0.009 respectively, compared to offspring of women without schistosomiasis as measured by frequency of blood smear positivity. Antenatal infection with lymphatic filariasis or intestinal helminths was not associated with increased malaria risk in infants. The increased susceptibility to malaria infection in offspring of women with schistosomiasis and malaria was associated reduced malaria-antigen-driven IFN-γ production by peripheral blood mononuclear cells compared to offspring of women infected with malaria alone OR 0.53 (95% CI 0.86-0.38) P=0.008. Thus, schistosomiasis and malaria co-infections during pregnancy enhance the risk for malaria infection during infancy. Treatment of women for schistosomiasis during pregnancy may have a beneficial effect on malaria susceptibility in childhood.

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COMBINATION OF BENZNIDAZOLE AND NIFURTIMOX PLUS POSACONAZOLE ENHANCES ACTIVITY AGAINST *TRYPANOSOMA CRUZI* IN EXPERIMENTAL CHAGAS DISEASE

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Chemotherapy for Chagas disease (CD) remains unsatisfactory, with limited efficacy and high toxicity reported for the two available drugs, nifurtimox (Nfx) and benznidazole (Bz). Posaconazole (Ps), a novel antifungal triazole derivative, shows potent *in vitro* and *in vivo* anti-*Trypanosoma cruzi* activity and suitable pharmacokinetic properties. Combination therapy of anti-infective drugs is a known strategy to potentially improve treatment efficacy, reduce treatment dose, duration and toxicity, and could also prevent resistance development. A two-staged approach was used to investigate the efficacy of Ps combined with either Bz or Nfx in mice infected with *T. cruzi* Y strain: i) determine sub-optimal doses in monotherapy in murine model of acute Chagas; ii) evaluate additive and synergistic effects of combination of sub-optimal doses. Female Swiss mice were inoculated with $5.0x10^3$ trypomastigotes, tail blood was examined 4 days post-inoculation, and treatment begun after *T. cruzi* detection. Each animal received daily drug suspension by gavage for 7 consecutive days. Treatment doses varied. Time to parasitaemia suppression and reactivation, peak parasitaemia and survival were assessed. The treatment scheme, parasitemia suppression, parasitemia reactivation (day), and parasitemia peak, and survival rate (%) as follows: Bz/Ps (50/10mg) 1.4+0.55 10 21.600 (22nd) 100%; Bz/Ps (50/5mg) 1.33+0.52 10 60.000 (22nd) 100%; Bz/Ps (25/10mg); 1.67+0.52 10 40.166 (26th) 100%; Bz/Ps (25/5mg) 1.16+0.41 7 11.133 (27th) 100%; Nfx/Ps (25/10mg) 1.5+0.55 11 63.333 (27th) 100%; Nfx/Ps (25/5mg) 1.33+0.52 11 27.333 (27th) 100%; Nfx/Ps (12.5/10mg) 1.0+0.0 10 68.666 (27th) 84%; Nfx/Ps (12.5/5mg) 1.16+0.41 9 68.666 (27th) 100%; Bz 100mg 1.33+0.52 5 10.000 (17th) 100%; Nfx 50mg 1.0+0.0 5 8.333 (16th) 100%; and Ps 20mg 1.33+0.51 12 11.333 (24th) 100%. In conclusion, our results demonstrated a clear synergistic effect for both combinations, with reduction of mortality and parasitemia suppression observed in animals treated with ½ and ¼ of the curative dose as compared with mice receiving same dose of individual drug. These data will be confirmed in a 20-day acute murine model.

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CALCULATING DRUG NEEDS AND COSTS FOR TREATING VISCERAL LEISHMANIASIS IN THE INDIAN SUBCONTINENT AND AFRICA USING LOCAL PATIENT ANTHROPOMETRIC DATA

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Antimony is the traditional first-line treatment for visceral leishmaniasis (VL), but is now ineffective in India and Nepal. Presently, other drugs and drug combinations are or are becoming available and a campaign to eliminate VL in the Indian subcontinent has begun. With increasing access, forecasting drug supplies and costs is integral to preparing and sustaining new efforts and delivery. All treatments are dosed in mg/kg body weight. We collected anthropometric data on 5103 patients with VL, of whom age and weight data was available for 4975 patients presenting to clinics in Kenya (n=1765, 33.7%), India (1496, 28.5%), Nepal (1043, 24.9%) and Uganda (671, 12.8%). Median (25-75 percentiles, IQR) age was 12 (7-20, 13) years in Kenya, 14 (8-30, 22) in India, 23 (13-35, 22) in Nepal and 12 (7-20, 13) in Uganda. Mean weight [kg] (std dev) was 30.9 (15.0) in Kenya, 30.4 (14.1) in India, 35.7 (13.2) in Nepal and 31.9 (14.8) in Uganda. Median weight (25-75 percentiles) was 28.5 (17.5-45) in Kenya, 30 (17-42) in India, 39 (26-45) in Nepal and 29.8 (18.6-45) in Uganda. Overall, 63% were male and 37% female. Most women were of child bearing age, especially in India and Nepal. Patients from Nepal were older and heavier. Options for treating VL in these countries are: (i) in Kenya and Uganda: (a) sodium stibogluconate (SbV); (b) liposomal Amphotericin B (AmB) and (ii) in India and Nepal: (a) AmB; (b) miltefosine (MF); (c) paromomycin (PM); (d) combinations of AmB plus MF or PM. We calculated treatment costs related to cost of drug in these various countries based on prospective ITT population of 1000 patients. For AmB the WHO recommends 20mg/kg as split dose (10mg/kg allowed for the Indian subcontinent) the projected drug costs were $163,700 (10mg/kg)-286,000 (20 mg/kg) for India, $194,210-331,820 for Nepal, $297,220 for Kenya, and 298,940 for Uganda. Implications of findings suggects that the drug cost component varies considerably between different treatments. Unit prices are negotiated between international organizations like the WHO and the manufacturer and may depend on volumes. These findings permit (i) calculating population-tailored drug costs and volumes; and (ii) projecting demand (and hence negotiated prices) at both national and international levels.

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SCYX-6759, AN ORALLY BIOAVAILABLE OXABOROLE 6-CARBOXAMIDE, ACHIEVES THERAPEUTICALLY RELEVANT EXPOSURE IN BRAIN AND CSF LEADING TO 100% CURES IN A MOUSE MODEL OF CNS-STAGE HUMAN AFRICAN TRYPANOSOMIASIS

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Human African Trypanosomiasis (HAT) is caused by infection with the protozoan parasite Trypanosoma brucei (T.b.), which is transmitted through the bite of a tsetse fly. The initially blood-borne parasite crosses the blood-brain barrier to establish a potentially fatal infection in the brain. Currently, treatment of the central nervous system (CNS) infection is unreliable because standard of care medications struggle to achieve therapeutically relevant exposure in brain and cerebrospinal fluid (CSF). Here we report rodent plasma:brain:CSF levels for several oxaborole 6-carboxamides that in each compartment sustain levels above an *in vitro* minimum inhibitory concentration (MIC) against T.b. spp. One of the best compounds, SCYX-6759, affords prolonged plasma half-life (>10hr) and low oral (PO) and intra-venous (IV) clearance (10% liver blood flow) in rodents, and brain concentrations above the *in vitro* T.b.brucei and T.b. rhodesiense MIC for 8 and 12 hr in mice and rats, respectively following a single oral 50mg/kg dose. This dose administered intra-peritoneally (IP), twice daily over 14 days achieved a 100% cure rate in a mouse CNS HAT model. In the rat, oral SCYX-6759 achieved dose proportional exposure with 100% bioavailability. Intra-venous volume of distribution was 4.6 L/kg indicating good tissue distribution. Disposition in plasma and CNS compartments will be compared between rodents (following IP and PO delivery), and non-human primates. A preliminary PK-pharmacodynamic (PD) relationship linking exposure in plasma and CNS to efficacy in mouse acute and CNS infections will be discussed. PK-PD modeling will be supported by *in vitro* time-to-kill data.

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IDENTIFICATION OF NEW AMINOQUINOLINE COMPOUNDS ACTIVE AGAINST VISCERAL LEISHMANIASIS USING AN *EX VIVO* MODEL SYSTEM

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The identification of new drugs for visceral leishmaniasis is needed because of the well known toxicity of current drugs and the increasing drug resistance of Leishmania donovani. We have previously evaluated > 4,000 small molecules in a novel medium throughput screening system that utilizes an ex vivo spleen explant of hamsters infected with luciferase-transfected L. donovani. This ex vivo system, unlike others, allows the identification of active compounds in amastigote-laden macrophages in the presence of other immune (Th1-Th2) cells. With this approach we have identified 84 chemically diverse lead compounds with activity against L. donovani as determined by an *in vitro* therapeutic index (IVTI: cytotoxic concentration 50% / effective concentration 50%) >5. Evaluation of 75 lead compounds revealed high discrepancy between the activities determined in the ex vivo model vs. cultured promastigotes (79% of the molecules had higher EC50 in promastigotes). Among the lead compounds quinoline-based molecules were highly represented (~20%). Since quinoline derivatives have strong chemical tractability and good drug-likeness, we further analyzed 65 commercially available aminoquinoline analogs and found some compounds with anti-leishmanial activity in the range of 1 microM concentration. 13% of the analogs (8/59 4-AQ and 1/6 8-AQ) had an IVTI >5 (range 1-63). Preliminary data obtained from a small number of 4-AQ (n=5) to which liver microsomal/cytosolic fraction S9 was added to the ex vivo system suggested that some (2/5) analogs may suffer modifications during first pass metabolism, decreasing its anti-Leishmania activity while others were not affected and probably represent better leads for oral administration. Therefore, the *in vitro* efficacy-activity determinations are currently being complemented by ADME studies before taking leads into the animal preclinical trials. Aminoquinolines constitute a fast track for the identification of novel anti-Leishmania therapeutic agents to treat visceral leishmaniasis.

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ANTILEISHMANIAL ACTIVITY OF SELECTED FDA-APPROVED DRUGS IN A MURINE CUTANEOUS LEISHMANIASIS MODEL

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Current therapeutic options licensed in the U.S. for cutaneous leishmaniasis (CL) are extremely limited. Current intravenous therapies, such as sodium stibogluconate have considerable associated toxicities, and are suboptimal means of treating a self-limited skin disease, albeit a potentially debilitating one. Oral azoles have shown modest efficacy in limited settings. The limited local therapies available are generally suitable only for uncomplicated lesions. There is a need for a safe oral drug for CL. We recently presented a large scale effort to screen already FDA-approved drugs for *in vitro* activity against *Leishmania major*, using infected macrophages. To date 1100 drugs have been screened *in vitro*. We established an *L. major*-infected BALB/c screening model to test drugs with potent *in vivo* activity (IC_{50} less than 10 mcg/mL). The model was optimized to both a foot pad and base of tail inoculation site, and validated against known active positive controls. Candidate drugs were first subjected to a rigorous decision matrix to determine suitability for 2-4 weeks of continuous oral therapy, favorable pharmacokinetics, and prior testing *in vivo* or in humans. Drug screening is currently ongoing. We will report on the results of the top 5-10 candidate drugs in the *in vivo* mouse model. Most of the active substances belong to categories of fungicides, antibiotics, anti-asthmatics, antiprotozoals and antidepressants. The intent of our strategy is to accelerate the process of antileishmanial drug development with reduced cost and shortened timelines.

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NOVEL COMPOUNDS FOR THE TREATMENT OF CHAGAS DISEASE

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In embarking on a project to find a novel antiparasitic drug effective against *Trypanosoma cruzi* (the causative agent of Chagas disease), we have examined a wide variety of structurally diverse "hit" compounds. Many of these compounds have been previously reported in the literature to have activity against *T. cruzi* while others were identified by us using a more speculative process of screening a variety of compounds based on their known biological activity and/or mode of actions against

other organisms. These hits included a number of natural products, pharmaceuticals and agrochemicals. Following a short hit-to-lead programme for each hit involving synthesis and biological testing of the original hit plus a small number of their analogues, we have been able to eliminate most of these hits due to lack of potency, toxicity, or other intractable properties. Only a few of the hits met our selection criteria and were progressed further as "lead" compounds. One lead in particular has already shown considerable promise. Within just a few iterations of analogue synthesis and biological assessment, the IC50 of compounds in this class has fallen to the very low nM range. Some chemistry, biology, structure-activity relationships, and pharmacokinetics of this and other classes of lead compounds will be discussed.

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TRANSPORT OF PENTAMIDINE AND FURAMIDINE IN RAT AND HUMAN HEPATOCYTES

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Aromatic dicationic molecules, e.g. pentamidine and furamidine, exhibited potent antiparasitic activity. However, pentamidine and furamidine accumulated in major drug-elimination organs, including the liver, at substantially higher concentrations than those measured in plasma, as reported previously. The significant and prolonged organ accumulation of these compounds likely contributed, at least in part, to the tissue-specific toxicities. Recently, pentamidine and furamidine were reported previously to be substrates of human organic cation transporter 1 (OCT1) using stably transfected Chinese hamster ovary cells. The objective of this study was to delineate the role of Oct1/OCT1 in the hepatic sinusoidal transport of pentamidine and furamidine using sandwich-cultured rat and human hepatocytes. In both rat and human hepatocytes, the uptake of pentamidine (1 μM) was linear up to 10 min. Uptake of pentamidine was significantly inhibited by 1 mM ranitidine (66%; an OCT1 substrate) and by 30 μM NBMPR (35%; an equilibrative nucleoside transporter (ENT) inhibitor). In rat hepatocytes, the uptake of furamidine (1 μM) was linear up to 10 min. However, no statistically significant inhibition of furamidine uptake was observed with either 1 mM ranitidine or 30 μM NBMPR. These results indicate that Oct1/OCT1 may play a major role in the hepatic sinusoidal uptake of pentamidine, but other transporters like ENT may also contribute. In addition, neither Oct1/OCT1 nor ENT appears to be responsible for the hepatic uptake of furamidine, which is yet to be characterized.

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RESPONSE OF HUMAN SKIN EQUIVALENTS TO *SARCOPTES SCABIEI* MITES AND EXTRACT

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The innate/inflammatory response of the host skin provides the first line of defense to the invasion of the ectoparasitic mite *Sarcoptes scabiei* that encounters and damages keratinocytes and fibroblasts as it burrows into the stratum corneum. In previous studies, keratinocyte and fibroblast monocultures responded to scabies mite extracts by modulating the secretion of an array of cytokines but little is known about the interaction between these cell types in response to scabies mites or extract. This study examined the response of cultured human skin equivalents (HSEs) composed of fibroblasts and keratinocytes in a collagen matrix, to burrowing scabies mites and to mite extracts. HSEs were inoculated on the surface with live mites or mite extract, or with extract added to the medium below the dermal surface. Samples were collected from the media reservoirs to monitor the secretion of selected cytokines during the 48-hr incubation. All HSEs produced increasing levels of IL-6, IL-8, G-CSF, GM-SCF, GROα, and VEGF over time with live mites inducing significantly increased levels (from 2- to 8-fold above controls) of these cytokines by 48 hrs. CTACK and MCSF showed elevated secretion by bottom-inoculated HSEs at 6 and 12 hrs but thereafter all HSEs secreted similarly increasing amounts. Only bottom-inoculated HSEs secreted TGFα and TSLP at early times and these levels waned with time. Although MCP-1 secretion by all HSEs increased over time, the levels produced by surface-inoculated HSEs were always below control levels. No IL-1β was ever detected and only live mites elicited appreciable levels of IL-1α. While mite-induced IL-1RA secretion was significantly increased at 48 hrs, the level of this cytokine waned over time for the other HSEs with bottom-inoculated HSEs exhibiting notably suppressed amounts. This study demonstrates that HSEs, like component monocultures, modulate the secretion of an array of cytokines in response to challenge with scabies mites or extract with the burrowing of live mites resulting in the most robust responses by these tissues.

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SURVEILLANCE OF RICKETTSIAL PATHOGENS ISOLATED FROM TICKS IN THE REPUBLIC OF GEORGIA

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Active surveillance of tick-borne pathogens, including Rickettsial pathogens, have been conducted in the Republic of Georgia using tick surveys. This study analyzed ticks that were collected in the Republic of Georgia, speciated utilizing standard classification techniques, and pooled according to species (n=84). DNA from the tick pools were isolated and real-time PCR analysis was performed to screen the pools for the presence of rickettsial species. Rickettsial species were identified through sequence analysis of multi-locus sequencing of the genes ompA, ompB and geneD. The Rickettsial pathogens, *Rickettsia slovaca* (21.43% of the pools), *R. raoultii (*4.76% of pools), R. *aeschlimanni* (17.86% of pools), and *R. mongolotimonae* (1.19% of pools) were identified and confirmed with the respective percentages as an indicator of prevalence within the collected population. The above pathogens are all members of the spotted fever group of *Rickettsia*. This study points to the fact that spot fever group pathogens in the Republic of Georgia are fairly prevalent and may be responsible for illnesses associated with spot fever like symptoms.

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ULTRASTRUCTURAL STUDY OF *AEDES ALBOPICTUS* SKUSE, 1895 (DIPTERA: CULICIDAE) HEMOCYTES

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Mosquitoes have an efficient defense system against infection. Insect blood cells (hemocytes) play an essential role in cellular defense responses to parasites and other pathogenic organisms. The aim of the present study was to characterize distinct morphological types of hemocytes of *Aedes albopictus* transmission electron microscopy. Hemolymph was obtained from 300 for day old females by perfusing the thorax with fixative solution. Colleted hemocytes were processed using standard techniques. Were found six types of hemocytes in the hemolymph of *Ae. albopictus*: prohemocytes, plasmatocytes, adipohemocytes, granulocytes, oenocytoids, and thrombocytoids. The prohemocytes were the smallest cells with the cytoplasm occupying only a narrow area around the nucleus. The plasmatocytes are polymorphic and exhibit plasma membrane with

irregular processes (pseudopodia and philopodia). The adipohemocytes are the most abundant cell type present. These hemocytes exhibited a large lipid like vesicle and mitochondria are visible. In granulocytes a cytoplasm containing dilated rough endoplasmatic reticulum and a round or elongated mitochondria is observed in electron microscopy. Oenocytoids have many mitochondria and ribosomes are scattered throughout a homogeneous cytoplasm, and abundant rough endoplasmatic reticulum and small smooth endoplasmatic reticulum. Trombocytoids are very fragile cells and few in number. They have a homogeneous cytoplasm with poorly developed organelles, few mitochondria and granules. This study describes the ultrastructure of six types of hemocytes present in *Ae. albopictus* adults female, each with individual morphological characteristics.

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MICROGEOGRAPHICAL ANALYSIS OF GENETIC STRUCTURE AND REINFESTATION DYNAMICS OF *TRIATOMA INFESTANS* POPULATIONS IN NORTHERN ARGENTINA

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The control of *Triatoma infestans*, the main vector of Chagas disease in South America, has had limited success in the Gran Chaco region due to high recolonization rates. A better understanding of the genetic structure, dispersal dynamics and phylogeographic relationships among *T. infestans* populations is needed in order to determine the source of reinfesting bugs and help design improved vector control strategies. We conducted a micro-geographical population structure study, analyzing the multilocus genotype of 780 *T. infestans* collected in houses of rural villages in Santiago del Estero province, northern Argentina during pre and post spraying surveys using 10 microsatellite loci. Genic and genotype diversity were assessed for populations at each capture site, pair-wise comparisons between sites and bayesian based individual assignment were performed. Significant levels of substructure within villages and capture sites were detected. Migration among capture sites and colonization from multiple sources are likely causes of the genetic structure of *T. infestans* populations detected in these communities. A significant impact of vector control actions was reflected in the genetic structure of villages under different control pressure. Taking into account the total genetic pool available from two baseline pre-spraying studies, results showed that most bugs captured post-spraying could have originated from spraying survivors from within each community, and putative source were identified. Thus, these results provide support to the hypothesis of reinfestation by local survivors that actively disperse to neighboring sites. In addition, evidence of gene-flow among villages reinforces the need of establishing vector control efforts beyond the village level.

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A RAPID ASSAY FOR THE DETECTION OF ALL FOUR DENV SEROTYPES IN MOSQUITOES

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Dengue virus infections have undergone dramatic expansion in range, affecting several tropical and subtropical regions of the world. In addition, it is a threat to military forces deployed to endemic areas, causing life-threatening complications characterized by dengue hemorrhagic fever and dengue shock syndrome. Rapid detection assays for DENV-infected mosquitoes are key to the early identification of areas at risk of disease outbreak and will provide for a more timely assessment of the disease threat and the targeting of dengue and vector control efforts in endemic areas; this is especially important when targeting infected adult mosquitoes that pose an immediate threat to non-immune people in the area. Other measures of virus activity that require at least 24 hours and usually several days have limited relevance for adult mosquito control efforts. Because no rapid test systems are available for the detection of dengue virus in the vector, we developed a hand-held immunochromatographic rapid assay similar to the West Nile virus and St. Louis encephalitis virus VecTEST™ assays. We evaluated different combinations of capture and detector monoclonal antibodies, and resultant dipsticks were readily able to detect mosquitoes infected with each of the four DENV serotypes. Data on sensitivity and specificity as well as the relationship between numbers of gene copies and plaque forming units (PFU) will be presented.

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CHARACTERIZATION OF HEMOCYTES FROM *AEDES AEGYPTI* AND *AEDES ALBOPICTUS*

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Insects are important vectors of human disease. Hemocytes mediate cellular responses that defend insects against pathogens such as parasites and other foreign bodies. This work aims to characterize the hemocytes of *Aedes aegypti* and *Ae. albopictus*, two important dengue vectors. Collection and identification of hemocytes is difficult due to the limited amount of hemolymph and cells present in circulation in small insects. Hemocytes were harvested from hemolymph obtained by mosquito thorax perfusion. Cells were spread in glass slides, processed analyzed by light microscopy. Hemocytes were fixed and also labelad with FITC-fluorescent lectins (ConA, WGA, LPL, PNA, HPA and BS1), and analyzed laser scanning microscopy. Phagocytosis assays were conducted by injecting FITC-latex beads into the mosquito thoraxes followed by hemolymph collection. Our results show that *Ae. aegypti* and *Ae. albopictus* hemocyte populations are composed of six different cell types that vary in size and morphology (prohemocytes, adipohemocytes, granulocytes, plasmatocytes, oenocytoids, and thrombocytoids). Hemocytes isolated from *Ae. albopictus* express ligands to ConA, WGA, BS1 and HPA lectins, but PNA and LPL lectins did not bind in any hemocytes. All fluorescent lectins (ConA, WGA, LPL, PNA, HPA and BS1) labeled *Ae. aegypti* hemocytes. The phagocytosis assay showed that only granulocytes and plasmatocytes ingest latex beads in *Ae. albopictus.* However, in *Ae. aegypti*, only granulocytes internalized the latex beads. The present study describes two new types of hemocytes in *Ae. aegypti* (prohemocytes and plasmatocytes) and describes six types of hemocytes of *Ae. albopictus*. These results begin to form a knowledge base for our ongoing studies on hemocyte function, and their involvement in controlling infections.

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EVALUATION RESTING BEHAVIOR OF *AEDES AEGYPTI* USING MARK-RELEASE-RECAPTURE DESIGN AND EXPERIMENTAL HUTS, PERU

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Dengue is one of the most important disease currently. *Aedes aegypti* is the primary vector of dengue. Preventive measures in the adult stage are the most effective method for reducing disease transmission however chemical control is becoming difficult due to: environmental concerns, adverse health effects, and insecticide resistance. For these reasons the efforts underway to identify a novel compounds for use against the adult stage. Identify preferred mosquito resting in the indoor sites will help to development new strategies for adult *Ae. aegypti* prevention inside homes. This study is part of larger research program focused on dengue vector control whose long-term objective is develop a novel Insecticide treated material system to reduce the mosquito inside homes. That was developed in experimental huts in Iquitos, Peru. The objective of this study was determined the preferred resting location of marked female adult *Ae. aegypti* inside experimental huts to report variation in resting behaviour in response to material texture: (dark vs. Light material) in different varying coverage ratios of both materials (75%, 50%, and 25%). The results from this study will be used to guide experiments: hut entry and exit behavioural studies with chemically treated materials.

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ORAL-MEDIATED GENE SILENCING OF A FAT BODY GENE OF *RHODNIUS PROLIXUS*

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Rhodnius prolixus, a vector of Chagas disease, is an obligate hematophagous insect used as a model to study insect physiology. Intrathoracic delivery of dsRNA in fourth instars is effective at silencing salivary gland heme proteins. However, oral delivery of dsRNA was less effective. The dose and route of dsRNA delivery are important variables in gene silencing efficacy for genes expressed in different tissues. Oral-mediated silencing of the *Rhodnius* heme binding protein gene, a gene expressed in the fat body, was evaluated.

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EHRLICHIAE AND SPOTTED-FEVER GROUP *RICKETTSIAE* IN TICKS FROM TENNESSEE

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Amblyomma americanum (L.)*, A. maculatum* Koch, *Dermacentor variabilis* (Say)*, Ixodes texanus* (Banks)*, I. cookei* Packard*,* and *I. scapularis* (Say) were collected from 31 counties in Tennessee by the United States Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services (USDA-APHIS-WS) and the Tennessee Department of Health (TDH) by examining wild animals and dragging flannel sheets through vegetation. Ticks were collected from April 2007 to September 2008 and sent to the TDH Vector-Borne Diseases Laboratory for identification and pathogen detection via molecular testing. Ticks were individually homogenized and DNA was extracted. For detection of *Ehrlichia chaffeensis,* a real-time polymerase chain reaction (PCR) targeting the 16S rRNA gene was conducted and for *Ehrlichia ewingii*, a nested, species-specific conventional PCR targeting the 16S rRNA gene was conducted. A PCR targeting the RompA gene of spotted fever group rickettsiae (SFGR) was conducted as previously described using primers Rr190.602n and Rr190.70p. To identify rickettsiae to species, positive samples were subjected to a restriction fragment length polymorphism assay (RFLP) by digestion of the amplicons with *Rsa*1 and *Pst*1. All positive *E. chaffeensis, E. ewingii* and SFGR samples were verified by sequence analysis. Overall, *E. chaffeensis* was detected in 2.6% (2/309) of *A. americanum* and *E. ewingii* in 0.8% (2/238)*. Ehrlichia* spp. DNA was not detected in any tick species other than *A. americanum*. All positive ticks were identified in the Interior Plateau and Southeastern Plains ecoregions where the majority of human ehrlichiosis cases are reported in Tennessee. A single *A. americanum* adult male (0.2% of total) was positive for *R. parkeri.* This is the first report of *R. parkeri* identified in Tennessee. Only two specimens of *A. maculatum*, the primary vector of *R. parkeri,* were collected in our study period, supporting previous reports that this tick species is uncommon in Tennessee. In contrast, *A. americanum* is ubiquitous in high densities throughout the southeastern United States. SFGR DNA was detected in 27% of ticks. Three rickettsial species, *Rickettsia montana*, *R. amblyommii,* and *R. cooleyi,* were identified by RFLP and sequence analysis. *Rickettsia rickettsii* was not detected suggesting that some RMSF cases reported in Tennessee may be due to cross-reactivity with other SFGR antigenically related to *R. rickettsii.*

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DEMONSTRATION OF PARATRANSGENIC *PHLEBOTOMUS ARGENTIPES*

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Leishmaniasis is a global health concern with an estimated 12 million people infected and 367 million at risk. Visceral leishmaniasis (VL) is the most devastating form of the disease and, if left untreated, has a mortality approaching 100% within two years. The disease is caused by a protozoan kinetoplastid flagellate and transmitted through the bite of an infected sand fly. The greatest number of cases of VL are reported in Bihar, India. Two hundred fifty thousand cases are reported in India annually and, of those, 90% are from the state of Bihar. In India, the causative agent is *Leishmania donovani* and the leading vector is *Phlebotomus argentipes*. Our group has been developing a novel strategy for targeting *L. donovani* within the vector, termed paratransgenesis. In paratransgenesis, commensal or symbiotic bacteria found at mucosal sites of transmission are isolated and genetically altered to elaborate molecules that kill the infectious agents. Transgenic bacteria are delivered back to insect vectors to block pathogen transmission. A microbiology analysis of the gut flora of *P. argentipes* flora was performed and revealed several nonpathogenic soil bacteria that could be used in the paratransgenic system including *Bacillus megaterium*, *Bacillus subtilis*, and *Bacillus pumilus*. These bacteria were transformed with a shuttle plasmid that expresses GFP and with three antimicrobial peptides with leishmaniacidal activity: mellitin, magainin, and cecropin. When fed to 4th instar *P.argentipes* larvae, we demonstrated the transstadial passage of transformed *B. subtilis* to the emergent sandfly. These results suggest that the addition of the transgenic bacteria to the soil at *P. argentipes* breeding sites could be a feasible paratransgenic approach to control of vector transmission of *L. donovani*.

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PROMISING STRATEGY FOR VISCERAL LEISHMANIASIS CONTROL UTILIZING NANOPARTICLE DELIVERY OF EFFECTOR MOLECULES

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Engineered nanoparticles provide efficient means to deliver a payload with increased specificity. Dextran sulfate-chitosan nanoparticles possess a number of desirable properties including stability at low pH, mucoadhesion, low toxicity, biodegradability and high zeta potential. In this study we suggest a novel application for dextran sulfate-chitosan nanoparticles: targeting *Leishmania donovani* within the sand fly vector,

Phlebotomus argentipes, and/or targeting the vector itself. *L. donovani* is the leading causative agent of visceral leishmaniasis (VL), a devastating and understudied disease which disproportionally afflicts the poor. Methods for VL disease prevention have been hindered by sand fly resistance to DDT. The female sand fly acquires the parasite during a blood meal and transmission occurs during a second blood meal. Prior to the blood meal, the female sand fly will feed on a sugar meal. Dextran sulfate-chitosan particles are stable in acidic dextrose solution and *P. argentipes* acquire the nanoparticles within their gut when fed the nanoparticles suspended in a dextrose solution. Additionally, the nanoparticles remain stable within the acidic gut environment. The particles release their payload with an increase in gut pH, mimicking the change in gut pH which occurs with a blood meal. Dextran sulfate-chitosan particles delivered via baited traps may provide an effective means to deliver leishmaniacidal or insecticidal molecules to *P. argentipes*.

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CLINICAL FACTORS CORRELATED TO POSITIVE SEROLOGIC TEST IN MENINGITIC ANGIOSTRONGYLIASIS

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Meningitic angiostrongyliasis, caused by Angiostrongylus cantonensis, can be diagnosed by clinical criteria. Confirmatory serologic tests are not widely available and have varied sensitivity. We would like to find clinical predictive factors for the positive serologic result in clinically-diagnosed meningitic angiostrongyliasis patients. A case-control study was conducted at Khon Kaen University, Thailand. Consecutive adults clinically diagnosed with meningitic angiostrongyliasis were enrolled. Subjects with positive and negative serologic test were defined as cases and controls, respectively. Multiple logistic regression analysis was used to assess the clinical variables predictive of the positive serologic result including exposure history, clinical signs and symptoms, and laboratory data. Between 1996 and 2007, 75 patients diagnosed as meningitic angiostrongyliasis had serologic results. Of those, there were 49 cases and 26 unmatched controls. Baseline characteristics and laboratory results were comparable. There were three clinical factors remaining in the final model predictive for having positive serologic result; including male gender, cerebrospinal fluid protein more than 100 mg/dL and cerebrospinal fluid eosinophils more than 40%. Only cerebrospinal fluid eosinophils had significant adjusted odds ratio of 4.970 (95% confidence interval 1.337-18.477). In conclusion, in facilities with paucity of serologic test, having cerebrospinal fluid eosinophils more than 40% was predictive for having positive serologic test for meningitic angiostrongyliasis.

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A RETROSPECTIVE ANALYSIS OF HEALTH AND SCHOOL OUTCOMES OF A SCHOOL BASED MALARIA PROGRAMME IN MANGOCHI DISTRICT, MALAWI

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We investigated retrospectively the effect of the school malaria treatment programme on mortality, sickness, repetition and drop out of school. The intervention involved training teachers in presumptive management of malaria cases among students, community engagement in advocacy and supervision, monitoring and evaluation. The intervention was implemented in a malaria perennial district of Mangochi in Malawi since 2000. The development of the intervention took place from 200 to 2001; actual implementation started in the year 2002 to 2006. The evaluation covered the academic years from 2001/2002 to 2005/2006. Propensity score matching was used to select schools and control for covariates of selection. Difference-in-difference was used to evaluate the effectiveness of the intervention. Survival analysis for drop out and repetition was undertaken using Kaplan Meier. The results showed that the intervention had a significant effect in reducing repetition and drop out of schools. The mortality rates between intervention and non-intervention schools were not significant. Future research activities should consider investigating the feasibility of training teachers to use rapid diagnostics tests to improve case finding in the context of the new expensive first line drug, ACT.

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DETECTION OF *ENTAMOEBA HISTOLYTICA*, *GIARDIA INTESTINALIS* AND *CRYPTOSPORIDIUM* SPP. ANTIGEN BY USE OF LUMINEX XMAP TECHNOLOGY

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Entamoeba histolytica, *Giardia intestinalis*, and *Cryptosporidium* spp. are the three most important and common diarrhea-causing parasitic protozoa. Microscopic diagnosis of these parasites is neither sensitive nor specific. Recently, more specific and sensitive alternative molecular methods (PCR, real-time PCR and antigen detection tests) have been introduced for all three of these parasitic infections. Recently, the Luminex xMAP technology has been employed for genotyping and detection of microrganisms. Luminex xMAP technology (Luminex Corp., Austin, Texas) is a microsphere-based multiplexing system where microsphere are internally dyed with various proportions of red and infrared dyes, producing different spectral that are detected by two lasers of Luminex machine. In this study, we have evaluated a Luminex multiplex assay that simultaneously detects *E. histolytica*, *G. intestinalis* and *Cryptosporidium* spp. in one reaction. Twenty three *E. histolytica*, 38 *G. intestinalis* and 25 *Cryptosporidium* spp. positive stool samples and 40 stool samples negative for these three parasites as detected by the TechLab's ELISA based antigen detection tests (TechLab, Blacksburg, Virginia) were tested by this Luminex multiplex assay in one reaction. All antigen detection tests positive stool samples were also positive by this Luminex assay and none of the 41 stool samples negative by the antigen detection tests was positive by this Luminex assay. More evaluation of this Luminex multiplex assay in comparison to other molecular tests for detection of these intestinal protozoan parasites is currently underway and data will be presented.

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LABORATORY CONFIRMED DIAGNOSES OF ACUTE FEBRILE ILLNESS IN GHANA

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The most common diagnosis of acute febrile illness in sub-Saharan Africa is malaria. Because this infection is so common in this area, and because there are limited laboratory tests available at most locations, the diagnosis is often a clinical one. In Ghana, the National Malaria Control Program has set a goal of increased laboratory confirmation of malaria diagnoses. Since June 2008, we have collected samples from all patients who agreed to participate and who presented to one of two participating hospitals in the Greater Accra region of Ghana with acute febrile illness. Acute febrile illness is defined as history of fever present for between two and fourteen days with documented T > 38 C. One hundred and ninety eight patients have been enrolled so far, and one hundred and seventy two have both clinical diagnosis and final laboratory results for all currently performed tests. These tests include malaria microscopy, blood culture and serology. Serologic tests for Q fever, rickettsia, leptospirosis,

brucellosis, West Nile fever, and the Widal test are currently performed. Laboratory confirmed malaria is defined as clinical diagnosis of malaria with positive microscopy. Positive blood cultures with bacteria other than coagulase negative staphylococcus are considered a laboratory confirmed diagnosis. Positive serologic tests are determined by fourfold increase in titer, where convalescent samples were obtained, or by strongly positive acute samples. The positive acute samples are classified as probable or confirmed cases. Of the one hundred and seventy two cases with full data, 73, or 42%, meet definition for laboratory confirmed diagnosis. The most common diagnosis is malaria, which accounts for 122 (71%) of the clinical diagnoses, with 40 of these being laboratory confirmed. Other laboratory confirmed diagnoses in the 172 cases have been typhoid fever (7.6%), Q-fever (8.1%), rickettsia (4.6%), other bacteria (3.5%), and leptospirosis (1.7%). This shows that while malaria is the most common etiology, only 33% of clinical diagnoses of malaria were confirmed by microscopy. It also suggests that co-infections may be common - occurring in 11% of clinical diagnoses and 16% of laboratory confirmed cases. Lastly, it suggests the need for further investigation, since only 42% of cases had a laboratory confirmed diagnosis, an important fact for physicians who will be seeing more febrile patients with negative malaria tests.

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ETIOLOGY OF FEBRILE DIARRHEA IN PERÚ AND PARAGUAY, 2001-2009

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Fever and diarrhea are common in the developing world. Initial clinical management may be challenging, especially in resource-constrained settings with limited diagnostics. We describe here the results of an ongoing surveillance program in patients in Perú and Paraguay presenting with fever and diarrhea. Patients with fever >38.0 C and diarrhea for less than seven days were offered enrollment. Those participants enrolling from malaria-endemic regions underwent thick and thin blood smear testing for *Plasmodium*. Acute and convalescent serum specimens were obtained along with stool specimens. Species identification of bacterial enteropathogens was confirmed through routine biochemical and serologic procedures, with antimicrobial susceptibility testing performed by Kirby-Bauer disk diffusion testing per CLSI guidelines. Serum specimens were tested by viral culture and PCR for arboviruses and by paired acute and convalescent IgM ELISA for viral and rickettsial antibodies. A total of 621 patients with fever and diarrhea were identified. In Perú, 296 were from Cusco, 204 from Iquitos, 36 from Piura, and 13 from Tumbes; an additional 72 were from Asunción, Paraguay. Bacterial enteropathogens were identified in 235/621 (37.8%). *Shigella* species were a majority of stool isolates (170/235, 72.3%), most often *S. flexneri* (122/170), followed by *Salmonella* (51/235, including 2/235 *S. typhi*), ETEC (31/235), and *Campylobacter* (6/235). *S. flexneri* isolates showed high rates of resistance to ampicillin (69.2%), erythromycin (50.8%), and cotrimoxazole (64.2%). Ciprofloxacin resistance was noted in 4.5% of ETEC but 71.4% of *Campylobacter*. Arboviral sources of infection were detected by PCR, viral culture, or serology 85 cases (13.7% of the total 621 patients), mostly dengue viruses (48/85, 56.5%). One case of *Plasmodium vivax* infection but no cases of *P. falciparum* were identified from this survey. In conclusion, *Shigella*, *Salmonella*, and dengue viruses were the most commonly identified sources of fever and diarrhea in patients presenting in this passive surveillance network. In rural settings with higher incidences of both *Plasmodium vivax* and falciparum, greater emphasis on malaria may be warranted. Resistance to older antibiotics was common in bacterial enteropathogens. The differential diagnosis of fever and diagnosis remains broad and requires a thorough diagnostic evaluation.

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CHILDHOOD KWASHIORKOR IN MADAGASCAR: A CASE REPORT

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Protein-energy malnutrition of early childhood is an umbrella term for a range of syndromes. Specifically, in Madagascar, 52.8% of young children were stunted in growth and 36.8% were under weight due to malnutrition. Kwashiorkor is not only a disorder that afflicts undeveloped nations, but can also occur in affluent countries. Severe nutritional disorders, in general, are relatively uncommon in the United States in comparison to undeveloped nations. However, of those cases that are observed, Kwashiorkor is the most common disorder seen in hospitalized children in the United States. It is often seen with extreme dietary restrictions (vegetarianism), introduction of new health food milk alternatives, neglect, or malabsorption syndromes. Our patient is a 4 year old Malagasy male who was seen initially at a local hospital in Antananarivo, Madagascar at one year of age. At that time he presented with dermatoses, edema of the face and feet, enlarged edematous abdomen, enlarged liver, temple wasting, red straight hair, diarrhea, upper respiratory tract infection and dehydration. Based on the WHO classification of malnutrition in children, the patient was determined to have severe malnutrition. A diagnosis of kwashiorkor was made based on clinical features and history. The patient was rehydrated with the World Health Organization (WHO) rehydration oral solution, was given WHO vitamin mix and a vitamin A injection. He received antibiotic prophylaxis for infection. The patient vomited due to an atrophied stomach and had numerous episodes of diarrhea. The diet was advanced as tolerated to wheat, rice, vegetables, milk, eggs, meat. Although he recovered from initial protein deficiency and malnutrition, he continues to struggle with frequent respiratory infections, dermatitis, and acute diarrheal episodes every 1-2 months. United States physicians are generally unfamiliar with typical clinical features of nutritional disorders. As a result there is often a low index of suspicion when patients present with symptoms of kwashiorkor. The intent of this report is to increase the primary care physician's awareness of the clinical features, implications and treatment of the disorder.

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IMMIGRANT SCREENING IN THE INPATIENT SETTING IN A MUNICIPAL HOSPITAL

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Screening asymptomatic immigrants in an inpatient setting can identify patients with long term parasitic infectious diseases. Immigrants admitted to the medical ward were approached and asked brief questions about immigration history, travel and high risk behaviors. Serologies were obtained *Strongyloides*, Chagas and schistosomiasis and hepatitis based on travel/immigration history. Individuals that screened positive were compared with those that screened negative. Over a 5 month period 141 patients were screened. The mean age and time to immigration was 57±15and 26±18 years, respectively. The regions of origin were in order of decreasing frequency: Caribbean, South America, Mexico, Sub-Saharan Africa, Asia, Southern Asia, Eastern Europe, and the Middle East. Thirty seven% of individuals (52/141) screened positive on one or more serologies. There were no differences in age, sex and time from immigration between those who screened positive and those who screened negative. *Strongyloides* serology was positive in 43/128 screened

(34%) with 21% having eosinophilia and 6 patients (14%) on steroids. Thirty seven percent (16/43) had stools performed and all were negative. There was no difference in eosinophilia in strongyloides positive and negative patients. Of the 13 patients screened for Chagas disease, one patient was positive. One of 5 patients from Sub-Saharan Africa screened positive for *Schistosoma haematobium*. Of the 135 patients screened 4 were positive for hepatitis B Ag. 3/93 patients tested positive for hepatitis c virus. Only 46 (33%) of house staff obtained a travel or immigration history. Thirty-six were tested for HTLV-1 and one was positive (co-infected with Strongyloides). In conclusion, screening asymptomatic immigrants from endemic regions for strongyloides and other tropical diseases on an inpatient ward identifies patients with asymptomatic infection. An admission question and screens on immigration/travel should be considered for inpatients on hospitalization.

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VARICELLA STATUS IN TRAVELERS SEEN IN THE BOSTON AREA TRAVEL MEDICINE NETWORK (BATMN)

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Greater than 90% of adults in the United States are immune to varicella zoster virus (VZV) by age 20; this rate is lower for adults raised in tropical areas (27.9-56%). VZV seroprevalence rates increase with age but are lower in all age groups when compared to temperate climates. We reviewed records of 1217 individuals presenting to the BATMN from December 18 2008-February 28 2009 to determine whether their immunity to varicella was identified for need of immunization. The Boston Area Travel Medicine Network (BATMN) is a research collaboration of five travel clinics in the greater Boston area seeing about 7,500 travelers per year in urban, suburban, academic, university-affiliated and independent facilities. Demographic data and trip information were entered into a database for all travelers. Data were analyzed for individuals at risk for varicella infection, including available varicella test results. 1012/1217 (83.2%) subjects in the database contained varicella-related information. 149/1012 (14.7%) were born in non-OECD countries. 42/ 1012 had no known history of VZV infection or vaccination. 8/148 (5.4%) of foreign-born travelers reported never having chickenpox. Demographic characteristics of all VZV risk travelers showed: 17/42 (40.5%) male, mean age 26.0 years IQR (17 to 34), 27/42 (64.3%) were 20-60 years old, and 13/42 (31.0%) were non-white. 33/42 (78.6%) of the travelers were born in the US and 16/39 (41.0%) of their parents were born outside US. Primary language was English for 34/42 (81.0%). Reason for travel: VFR 6/42 (14.3%), tourism 16/42 (38.1%), business 2/42 (4.8%). None had previously had varicella testing done. In conclusion, 5.4% of foreign-born travelers reported no history of VZV infection and therefore would benefit from varicella vaccine. 64/149 (43.0%) were women of child bearing age. 4/64 (6.25%) were VZV risk travelers and if became pregnant, were at higher risk from complications from chickenpox. Travel clinics provide an untapped opportunity to address an unmet health need (screening for VZV and immunizing when appropriate) in immigrant travelers. The results for varicella serology obtained at the pre-travel visit will be presented.

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PREVALENCE OF *PLASMODIUM FALCIPARUM* INFECTION AMONG PATIENTS WITH CHRONIC RENAL FAILURE (CRF) IN MALI

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The prevalence of chronic renal failure (CRF) in Mali has increased from 1.9% in 1990 to 12.9% in 2003. Because the immunosuppresion produced by CRF potentially increases the risk of infections such as *Plasmodium falciparum* malaria, we prospectively examined 116 patients with CRF who were receiving their care at the Renal Unit of the Hôpital Point G in Bamako. Testing performed on patients with CRF included ultrasound examination of the kidneys, ureters and bladder, blood smears for malaria parasites and blood chemistry tests related to metabolic and renal parameters such creatinine, uric acid and protenuria. Testing for significant differences was performed with the Pearson X^2 using an α of 0.05. The mean age of the study population (subjects with CRF) was 43 ± 16 years (13-92 years), and there were more males than females, 58% vs. 42%. Ultrasound examination revealed symmetrically small kidneys without evidence of obstruction, and did not correlate with parasitemia. Of the 116 CRF patients examined, 81% lived in Bamako and 28% (32 of 116) were receiving dialysis. Based on Giemsa-stained blood smears, the frequency of *P. falciparum* infection was higher in adults with CRF, in whom it was 33% (38 out of 116) than in healthy adult controls (15%, p=0.04). However, the parasite density did not exceed 1,000 parasites per µl blood. Quinine and ACT were the antimalarials used most frequently (37% total) followed by the combination of Quinine plus Sulfadoxine-Pyrimethamine (26%). Among the symptoms and signs examined, dysuria, proteinuria and anemia were associated with parasitemia (X^2=5.294, p=0.03). These results indicate that malarial parasitemia is more prevalent among adults with CRF in Mali than among healthy adult controls. The dysuria and proteinuria in CRF patients with parasitemia are of uncertain cause and should be studied further to determine whether they are caused by *P. falciparum* infection.

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LEPTOSPIROSIS IN THE REPUBLIC OF GEORGIA

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Leptospirosis is a zoonotic infection with a world-wide distribution. This infection is suspected to occur in the Caucasus, but limited information is available. The clinical presentation is non-specific and highly variable, and complications may develop if left untreated. Sentinel surveillance for acute febrile illness was initiated at 5 hospitals in Georgia. Patients >4 years of age with fever ≥38°C for 48 hours or more were considered for inclusion. Blood culture and serologic testing (IgM ELISA; PanBio, Inc.) was conducted for leptospira, and ELISA results of ≥9 PanBio units were confirmed with microscopic agglutination testing (MAT). Confirmed leptospirosis was defined as MAT titer of ≥800 or a ≥ fourfold change between paired samples. A MAT titer of 400 or positive PanBio serologic results (pending confirmation) was considered possible leptospirosis. One hundred and thirty one (131) patients were enrolled since May 2008. Sixty four samples had ELISA results of ≥9 PanBio units, 38 have been tested by MAT to date. Leptospirosis was confirmed in 2 patients and the possible diagnosis for 27 patients. The identified serovars were *L. autumnalis*, *L. mankarso*, and *L. bataviae*. The predominant symptoms were fever (100%), rigors (87%), fatigue (87%), excessive sweating (74%), and headache (57%). Leptospirosis cases made up a disproportionate percentage (>25%) of the following physical exam findings: mental status changes, neurological findings, neck stiffness, icterus, jaundice, respiratory crackles, and abdominal distention. Mental status changes included short term altered consciousness and somnolence. One probable leptospirosis patient was suspected for meningitis. Patients that reported frequent contact with cattle (OR=3.5, p=0.03), chickens (OR=3.4, p=0.01), or exposure to forested areas (OR=9.2, p=0.002) were at increased odds for leptospirosis. In conclusion, leptospirosis is an important cause of acute febrile illness in Georgia. Clinical awareness and laboratory capacity are

essential to diagnose this infection. Further information is needed to develop prevention and control strategies in the Caucasus.

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CANDIDAEMIA IN A TERTIARY CARE HOSPITAL IN KOLKATA, INDIA

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Candida infection is increasing throughout the globe and there is gradual increase of non-albicans *Candida* spp. In South-East Asian countries there are reports of significant increase of *C. tropicalis* infection. Candidaemia showed very high morbidity and mortality rates. Thus, in this study we have analyzed retrospective Candidaemia cases in a tertiary care hospital in Kolkata, India. All *Candida* isolates from Candidaemia cases in the year 2008 were analyzed to find out common isolated species and their antifungal sensitivity pattern was also studied as per CLSI guidelines for Amphotericin B and fluconazole. Eighteen Candidaemia cases were found in 2008 in our hospital. Eight isolates were *C. glabrata*, 7 isolates were *C. tropicalis* and three isolates were *C. albicans*. Most of the isolates were resistant to fluconazole while all of them were sensitive to Amphotericin B. In conclusion, candidaemia in Kolkata is mostly due to *C. glabrata* and *C. tropicalis* and most of them were fluconazole resistant.

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DENGUE AMONG PATIENTS WITH UNDIFFERENTIATED FEVER IN SOUTHERN SRI LANKA

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Dengue virus (DV) is an emerging pathogen of global importance which presents as undifferentiated fever (DF). Despite transmission of all 4 serotypes (DEN1-4) for ≥ 40 years in Sri Lanka, epidemic dengue, with an increased incidence of dengue hemorrhagic fever, began in 1989 and cases have increased since. Epidemiologic study to date has focused on the capital, Colombo. However, over the last decade, cases have been increasingly reported elsewhere. To evaluate the burden of DF among patients ≥ 2 years old presenting to hospital with fever in Galle (Southern Sri Lanka) in 2007, we obtained sera at presentation and 2-4 week follow-up. We tested paired sera for IgG and IgM antibody to DV by ELISA, and defined acute infection as seroconversion or rising levels of DV-reactive IgM and/or IgG. We further defined DF as primary or secondary DF by the absence or presence, respectively, of DV-reactive IgG in the acute serum sample. Acute DF was confirmed by isolating virus in insect cells and by reverse transcriptase PCR, which also defined DV serotype. Overall, 456/846 (54%) of patients had serologic evidence of current or prior DF, and this increased with age (10% by age 5, 14% by age 10, 23% by age 20, 50% by age 25, 65% by age 40, and 75% by age 70). We identified 48 cases (5.7%) of acute DF [primary (27) and secondary(21)] among the 846 paired sera tested. The mean age of those with primary and secondary acute DF was similar (30.0 vs 30.8 years, respectively, p=0.84). We isolated DV serotypes 2, 3 and 4 from a subset. Among those with DF, the likelihood of isolating DV was greater in those with fewer days of fever (mean 2.9 vs 4.9 days, p=0.01). The mean white blood cell count was similar among those with and without DF, but the mean platelet count was significantly lower in those with DF (203,955 vs 247,918, p=0.003). We conclude that dengue accounts for substantial (5.7%) illness among those seeking care for undifferentiated fever in Southern Sri Lanka, that most cases occur in young adults, and that at least 3 of the 4 serotypes are circulating in that area.

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COST OF DENGUE VECTOR CONTROL ACTIVITIES IN PUERTO RICO

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Dengue is a major public health problem in Puerto Rico. Approximately 5,000 dengue cases were reported annually from 2002-2006, and 10,508 cases were reported in 2007. Vector control (fumigation and preventive educational campaigns) is currently the only approach to mitigate the disease. As part of a study of the economic burden of dengue in Puerto Rico, the annual cost of public sector vector control activities was estimated. A telephone survey was conducted of all 78 municipalities to identify those with active vector control programs. A structured questionnaire inquiring about personnel, other inputs, and services performed was developed and validated in the municipality of Carolina. For each staff member, the questionnaire elicited the distribution of efforts between dengue control and other activities. The questionnaire was then used to gather cost data for the years 2002-07. On-site interviews with vector control personnel of the Department of Health and all 11 municipalities with independent active programs were initiated. After reviewing the data for internal consistency and agreement with published reports, the authors contacted agencies to review data. Preliminary estimates of island-wide cost per year for vector control activities for the years 2002 through 2007 showed that Puerto Rico spent $2.77 million/yr ($0.72 per capita). Of this, the state spent an average of $1.41 million/yr, while the municipalities averaged $1.27 million/yr. The top 4 municipality programs (Carolina, Guaynabo, Bayamon and Toa Baja) served only 16% of the island's population but represented 35% of the island's costs. Alternative estimates about the dengue share of certain joint vector control activities gave a range of $2.55 million ($0.66 per capita) to $3.08 million ($0.83 per capita) for total costs. While Puerto Rico's per capita spending on vector control is only half of other endemic countries in the region (e.g., Panama $1.56 in 2005), dengue vector control nevertheless represents a substantial expenditure.

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AGGREGATE ECONOMIC COST OF DENGUE IN PUERTO RICO

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Calculation of the economic cost of an illness can guide prevention and treatment policies. For Puerto Rico, the only such study was a limited analysis from a single year, 1977. To inform stakeholders involved with vaccines and vector control technologies, we estimated the average annual cost of dengue in Puerto Rico, using data from 2002 through 2009. To determine the prevention cost (surveillance and vector control), a structured questionnaire was developed, validated and used to estimate expenditures by the state government and municipalities with active control programs from 2002 through 2007. To estimate the illness cost of dengue, a structured interview questionnaire was developed, validated and used with a prospective sample of dengue hospitalized and ambulatory patients since July 2008. The data provided the direct and indirect cost of a dengue episode. Additionally, published literature and hospitals records were used to validate and complement patient recall. Sensitivity analyses employed alternative assumptions about rates of treatment among non-reported cases. Preliminary results gave lower and upper bounds (± standard deviation across years) of $15(±9) million and $32(±20) million, respectively. Corresponding per capita amounts were $4(±2) and $8(±5), respectively, with a highest yearly value of $18 (in 2007). Illness costs represented 82% to 91% of this total, with hospitalized and ambulatory cases responsible for 61% and 39% of

illness costs, respectively. The average length of hospitalization on the first 35 cases was 6.2(±6.3) days. Vector control activities were divided approximately equally between municipalities and the state department of health. The economic burden of dengue and the variability, as shown by standard deviations, are both substantial. Costs are several times the comparable amount for Panama during an epidemic year ($5). Thus, considerable investments for the development and implementation of effective technologies would be economically justified.

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EFFECT OF METYLPREDNISOLONE IN PREVENTING DENGUE COMPLICATIONS: A SINGLE-CENTER RANDOMIZED PLACEBO-CONTROLLED TRIAL

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Dengue, the most frequent arbovirus infection globally, has no specific treatment currently. Although corticosteroids may be beneficial, its efficacy in preventing complications when administered during the early phase of the disease remains uncertain. The objective of this study was to evaluate the impact of Methylprednisolone (MP) on dengue severity (spontaneous haemorrhages and vascular permeability). We conducted a factorial 2x2, randomized, placebo-controlled trial, blinded for participants, care givers and outcome adjudicators. Eligible patients were ≥5 years old with dengue-like acute febrile syndrome for less than 120 hours of evolution, with diagnosis of dengue based on a validated clinical score or immunochromatography test, and free of clinical signs of plasma leakage. We randomly assigned patients by strata of age (<15 or ≥15 years old), platelet count (≤100.000 or >100.000 platelet/mm^3) to MP, N-acetylcistein or their matching placebos. This report refers to the MP arm, where patients received a single oral dose of MP (1.5 mg/kg) or placebo. Participants had a daily follow up for eight days. Data analysis followed the intention-to-treat principle. We enrolled 189 participants between July, 2006 and January, 2009 (35.3% of 536 potentially-eligible cases screened) in Bucaramanga (Colombia). There were less cases developing spontaneous haemorrhages and ascitis among those receiving MP (32/87, 36.8% and 0/87, 0%), compared with those receiving placebo (47/91, 51.7% and 4/91 4.4%, RR: 0.71, CI95% 0.5-1.0, p=0.046, p=0.048, respectively). The MP group had also less frequent and shorter hospitalizations (9/87, 10.3% and mean time in-hospital 37.3 hours) as compared with placebo (15/92, 16.5%, and 55.1 hours, RR: 0.63 CI95% 0.29-1.36, p=0.25). There were no adverse or severe events or mortality. Our results suggest that MP reduces spontaneous haemorrhages, ascitis and hospitalizations, among patients with dengue. A multi-centric, larger study should confirm these encouraging results.

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NATURAL STRAIN VARIATION AND THE NEUTRALIZATION OF DENGUE SEROTYPE 3 VIRUSES

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Dengue viruses (DENVs) are emerging, mosquito-borne flaviviruses and the agents of dengue fever and dengue hemorrhagic fever. The DENV group consists of 4 serotypes designated DENV1-DENV4. Current dogma is that the main neutralizing epitopes recognized by antibodies are preserved within each serotype but not between serotypes. However, there is considerable genetic diversity within each serotype and surprisingly little work has been done to explore how naturally occurring strain variation influences the neutralization of DENVs belonging to the same serotype. We compared the amino acid sequence of 175 envelope (E) proteins representing the different genotypes of DENV3. We found that many surface exposed amino acids located at or near known antibody epitopes were not conserved between DENV3 strains. The variable amino acids include 6 residues at the lateral ridge of domain III of E protein (EDIII). This region of E protein is the target of type-specific antibodies that strongly neutralize dengue and other flaviviruses. To determine if natural amino acid variation in DENV3 EDIII has an effect on neutralizing antibody, we obtained a panel of 5 mouse monoclonal antibodies (Mabs) that bound to DENV EDIII. The binding sites of the antibodies were mapped using mutant recombinant proteins. Two of the Mabs bound to a serotype-specific epitope on the lateral ridge that was centered on the N terminal linker and BC loop of DENV3 EDIII. One Mab was sub-complex specific (DENV3 and DENV1) and bound to an epitope close to the lateral ridge that included the A strand of EDIII. Two Mabs cross reacted with all 4 serotypes (complex-specific) and bound to an epitope outside the lateral ridge. The binding and neutralization capacity of the 2 type specific lateral ridge Mabs were strongly influenced by naturally occurring mutations on the lateral ridge of DENV3 EDIII. Our data demonstrate that the lateral ridge "type specific" epitopes is not conserved between strains of DENV3. This variability should be considered when designing DENV vaccines, especially those targeting EDIII.

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PAEDIATRIC DENGUE SURVEILLANCE IN COLOMBO, SRI LANKA

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Although dengue viruses (DENVs) have been endemic to Sri Lanka for over 40 years, dengue fever/ dengue hemorrhagic fever (DF/DHF) epidemics are a recent phenomenon on the Island. The first epidemic of DF/DHF was in 1989 and regular epidemics have been documented every year after 1989. Despite the gravity of the DENV problem in Sri Lanka, we currently do not have reliable estimates of the incidence of infection and disease in Sri Lanka. Such information is crucial for understanding the emergence of severe dengue disease and for designing trials to evaluate vaccines. The Paediatric Dengue Vaccine Initiative (PDVI) and the Sri Lanka Ministry of Health (SL-MOH) have been conducting a community based enhanced passive surveillance study to estimate burden of DENV infection and disease. The SL-MOH/PDVI study is based in Colombo as this is the district with the highest number (~25%) of reported cases. A census of households in Municipal Council Ward 33, Colombo, was conducted in October 2008. From this population, 800 randomly-selected children aged ≤12 years have been recruited to be followed up for fever events over one year. During the initial recruitment, finger-prick blood samples were collected onto filter paper discs to determine baseline flavivirus sero-status. A repeat finger-prick sample will be collected at 12 months to determine the annual sero-conversion rate. All children with ≤ 7 days of fever are being referred to dedicated healthcare centers for further investigation. Acute and convalescence blood samples are being collected from all children with fever for laboratory testing by serology (IgM and IgG ELISA) and PCR. The baseline blood samples indicate that the flavivirus sero-prevalence in the cohort is 52% and ranges from 9% in children below 1 year to 80% in children aged 12 years. Within the first three months of the study, a total of 154 samples were collected from children with fever. When a subset of 76 acute and convalescent blood sample pairs were tested, 14 cases (18%) of acute dengue infection were detected by serology and/or PCR. The 14 cases consisted of 4 primary and 10

secondary infections. Serotypes 2 and 3 were responsible for all except 1 case. These results demonstrate intense transmission of multiple serotypes among children in Colombo. We will present data from the first year of the study, including the first population based estimates for the incidence of DENV infection and disease in Colombo.

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DETECTION OF ASYMPTOMATIC INFECTION AMONG RELATIVES OF DENGUE PATIENTS

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Dengue viruses are the most important arboviruses of public health significance. These viruses usually cause dengue fever (DF), but some patients experience a more severe form of the illness, dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS). Besides these clinical presentations, a high number of asymptomatic disease is supposed to occur, but the percentage of the dengue virus infections resulting in asymptomatic disease is unclear. In order to approach this problem, we studied patients with clinical manifestations suggestive of dengue and their asymptomatic next of kin, especially those in close contact with the patient. Thus, the aim of this study was to detect asymptomatic patients among relatives of acutely infected patients. Blood was drawn from all participants of the study and their sera submitted to a diagnostic test capable of making the diagnosis of an acute infection, RT-PCR and NS1 detection during the viremic phase and IgM detection after 6 days of disease onset. The relatives of all probable dengue patients were submitted to a questionnaire to enquire about possible dengue clinical manifestations, and were included on the study if they had had no dengue symptoms during a period ranging from 2 weeks before up to the moment of the interview. Among all patients with clinical manifestations suggestive of dengue and whose all asymptomatic relatives agreed to participate on the study, we were able to enroll only 15 patients. Out of these, dengue was confirmed in 11 patients and these patients provided 27 relatives in whose dengue was confirmed in 10 persons (2 by NS1 detection and 8 by a positive IgM). On the other hand, the patients in whom dengue diagnosis was ruled out had 10 asymptomatic relatives, and none of them were infected by the dengue viruses (3 of them were IgG-positive). Thus, in our study 37% of the relatives of a dengue patient showed an evidence for asymptomatic dengue infection, and the importance of dengue case in the silent transmission of dengue viruses is evidenced by the lack of asymptomatic patients among the relatives of those patients in whom dengue was ruled out. This asymptomatic/symptomatic rate of dengue infection is very high and may be explained by our small sample size and by the stringent patient selection of our study. A study with a bigger sample is necessary to confirm our preliminary data, but if they hold true, these findings provide an explanation on why dengue outbreaks are so explosive.

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INVESTIGATING NOVEL DENGUE VIRUS INFECTION BIOMARKERS USING PROTEOMIC METHODS: VITRONECTIN PRECURSOR PROTEIN AS A NEW LEAD

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Plasma from pediatric Thai patients with confirmed primary (1°) or secondary (2°) dengue fever (DF) or dengue hemorrhagic fever (DHF) as well as samples from Thai children admitted to hospital with other febrile illnesses (OFI) were screened by SELDI-TOF mass spectrometry. Using a cut-off significance level of p-value < 0.001, 24 diagnostic and prognostic biomarkers were detected. Several of the most informative biomarkers were tentatively identified using SDS-PAGE and mass spectrometric microsequencing. Our study focuses on Vitronectin (Vn) precursor protein as a potential biomarker to differentiate between 1°DF and DHF. Vn is an acute phase glycoprotein thought to be involved in vascular inflammation and complement activation. Since haemorrhages and plasma leakage are hallmark manifestations of the more severe form of dengue virus (DV) infection, it is interesting to note that Vn can bind to and stabilize type 1 plasminogen activator inhibitor (PAI-1) to inhibit fibrinolysis. In SELDI analysis, we found a peak at 53.8kDa, matching Vn precursor protein. This peak correlates with the WB results and achieves a p-value of 8.47E-9 using the non-parametric Kruskal-Wallis H test. By Western blot, the precursor form was found in healthy subjects as well as patients with OFI or 1°DF but was not detectable in plasma samples from patients with 1°DHF, 2° DF, or 2° DHF. However, total Vn plasma concentrations were found to be consistently lower in all patients infected with DV (68 ± 24 µg/ml) compared to healthy or OFI controls (228 ± 63 µg/ml) by EIA. To further investigate the role of Vn in the DV pathology, an *in vitro* model using HepG2 cells (human hepatocytes) was developed. Studies of the impact of DV infection on Vn protein and its binding partners, including PAI-1 are currently underway.

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BABOON MODEL FOR DENGUE VIRUS INFECTION AND VACCINE EVALUATION

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Animal models of Dengue (DEN) virus infection, immunity and pathogenesis are essential in order to understand the basic biology of the virus and to evaluate candidate vaccines. Non-human primate models, including baboons (genus papio) offer unique insights into viral pathogenesis. Non-human primates are more human-like than rodents. We previously established baboons as an animal model for West-Nile Virus (WNV), as reported previously, and have no expanded our explorations to DEN. 5 animals were infected with a cocktail of 4 DEN3 isolates at a total infectious dose of 4x 10^6 FFU. Animals responded with a mild, but significant drop in platelet counts, as well as leucopenia. No overt signs of distress were noticeable upon primary infection. Heterotypic challenge is in progress. The baboons developed a prototypical immune response to DEN3: we observed high titer IgM antibodies, which peaked and subsequently declined; we observed a lesser but sustained IgG response; and we observed strictly type-specific neutralizing antibodies. Unlike WNV infection, which resulted in transient, but robust systemic viremia, DEN3 infection resulted in only barely detectable viral load in plasma. We also infected 3 animals, which were chemically immune compromised. These showed exacerbated hematological responses. Further details of their immune response and viral load data will be presented. This data establishes baboons as a suitable animal model to study the immune response to DEN infection.

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SEROPREVALENCE OF DENGUE IN UNITED STATES ARMY SPECIAL OPERATIONS COMMAND PERSONNEL

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Worldwide endemicity and epidemics of dengue are on the rise, especially in Central and South America. As a result, dengue is a threat of particular concern to our United States Army Special Operations (USASOC) soldiers. These personnel routinely deploy to areas with an endemicity of multiple dengue serotypes. This exposure pattern increases the likelihood of multi-

serotype exposures, potentiallly increasing the risk of more severe disease manifestations in our soldiers and loss or degradation of operational capability. Since October 2008, five USASOC soldiers have been diagnosed with dengue fever; four of them with multiple serotypes. We hypothesize that many more USASOC personnel have been exposed to dengue. In order to characterize the risk, a study using approximately 1000 sera samples from the DoD Serum Repository is being conducted to determine the seroprevalence of dengue in USASOC personnel who have served in the SOUTHCOM area of responsibility for at least 30 days from 2006 through 2008. The results from this study will provide an epidemiological baseline for dengue in deployed troops, guide commanders' medical threat planning in endemic areas, and help guide development of preventive countermeasures. It will also serve as a foundation for prospective studies to further explore exposure rates and evaluate current force health protection effectiveness.

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MOLECULAR DETERMINANTS OF DENGUE VIRUS ENVELOPE PROTEIN IN VIRUS INFECTION

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We engineered site-specific mutations in four functional domains of the DENV2 E-protein: 1) fusion peptide (FP), 2) hinge region, 3) VEPG loop, and 4) surface heparan sulfate binding (HSB) domains. We recovered 7 stable FP mutant viruses and 4 non-infectious FP mutant particles and demonstrated the direct involvement of the FP in virus mediated cell membrane fusion. Most of the FP mutants exhibited different growth kinetics and/or genetic stabilities in mammalian cells, mosquito cells, and mosquitoes, suggesting that the requirements for efficient membrane fusion may vary with cell type. Ten viable hinge mutants were recovered, but different AA substitutions had different effects on virus infection in cells and mosquitoes. The VEPG loop of the DENV2 E-glycoprotein was previously implicated as an attachment ligand for mosquito C6/36 cells, implying that they may play a critical role for infection of mosquitoes. Surprisingly, all 4 of the VEPG loop mutants, including the VEPG deletion mutant, were viable in both C6/36 and Vero cells. All 4 VEPG loop mutants were also competent to orally infect *Aedes aegypti*, but their infection efficiency was less than that of wild-type DENV2. The positively-charged HSB clusters are thought to be involved in the initial, nonspecific binding of DENV to cultured mammalian cells. Although HS on cells appears to be involved in DENV2 viral attachment, molecular knockout of subsets of the putative HSB on the E protein do not significantly decrease virus attachment. In addition to HS binding, some of the positively charged AAs may also be involved in other functional domains that regulate specific virus-receptor binding, internalization, and/or down stream viral replication.

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PHENOTYPIC ANALYSIS OF DENGUE VIRUS ISOLATES ASSOCIATED WITH DENGUE FEVER AND DENGUE HEMORRHAGIC FEVER FOR CELLULAR ATTACHMENT, REPLICATION AND INTERFERON SIGNALING ABILITY

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Eighteen dengue virus (DENVs) representing all four serotypes, isolated from pediatric patients at children's hospital, Queen Sirikit National Institute of Child Health, Bangkok, Thailand exhibiting a diverse spectrum of disease ranging from uncomplicated dengue fever (DF) to severe dengue hemorrhagic fever (DHF), were tested for their ability to attach to host cells, replicate and interfere with the IFNα signaling pathway by interfering with signal transducer and activator of transcription 1 (STAT-1). Although most isolates suppressed IFNα-induced STAT-1 phosphorylation, our results showed no difference between DENV strains associated with DF and those associated with DHF. However, the DHF isolates tended replicate to higher titers in dendritic cells (DCs) than the DF isolates, but this ability was independent of their cell binding capability. Our results suggest that the emergence early in infection of viruses with a high degree of replication fitness may play an important role in DENV pathogenesis.

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NEEDLE-FREE DELIVERY OF A TETRAVALENT DENGUE VACCINE (DENVAX): SAFETY AND EFFICACY IN NON-HUMAN PRIMATES

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The immunological advantages of intradermal (ID) vaccine delivery and the potential benefits of needle-free vaccination prompted us to examine the delivery of a tetravalent dengue (DEN) vaccine in non-human primates using the PharmaJet device. PharmaJet's needle-free device permits routine ID delivery and provides significant advantages including: 1) improved safety due to an auto-disable design that eliminates needle reuse issues in resource poor settings; 2) decreased injection site pain; and 3) easier and speedier vaccine delivery. Flavivirus naïve cynomolgus macaques received primary and secondary immunizations (sixty days apart) of DENVax, a tetravalent chimeric DEN-2 PDK-53-based dengue vaccine. Four animals were immunized ID with a DENVax formulation containing 10^5 PFU each of the four DENVax components using the PharmaJet device; control animals received the same formulation subcutaneously by needle and syringe. The animals were challenged subcutaneously 30 days after the boost with 10^5 PFU of wild-type DEN-1 West Pacific (WP) or DEN-2 New Guinea C (NGC). ID administration of the tetravalent DENVax vaccine with the PharmaJet device had no significant effects on body weights, temperature or hematological parameters of the animals. Plaque neutralization reduction titers (PRNT) against all four DEN serotypes were greater when the vaccine was delivered ID. Following challenge with DEN-1 WP or DEN-2 NGC, all animals were free of viremia, highlighting the protective efficacy of the PharmaJet-delivered DENVax. Our findings highlight the potential of the PharmaJet device for easy, needle-free ID delivery of DENVax. ID administration of a live attenuated DEN vaccine, mimicking natural virus infection by mosquito bite, enhances the immunogenicity of the vaccine. A vaccine delivery system that provides ease of administration without compromising vaccine safety and efficacy would facilitate vaccination campaigns against dengue and other infectious diseases.

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INHIBITION OF THE TYPE I IFN PRODUCTION IN DENDRITIC CELLS BY DENGUE VIRUS

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Dengue virus (DenV) is an emerging human pathogen that infects millions of people worldwide. 2.5 billion people are at risk for dengue disease, which causes systemic disease that includes dengue fever and the more severe forms dengue hemorrhagic fever and dengue sock

syndrome. Several cell types have been proposed as a primary target of DenV infection *in vivo*, including monocytes, macrophages and dendritic cells (DCs). Because DenV infects those cells several mechanisms have been reported about how DenV evades immune response in humans, including the inhibition of type I Interferon (IFN) signaling in infected cells. However little is known about how DenV interferes with the activation pathway of the DCs, including production of type I IFN. Previous work in our laboratory has shown that monocyte derived DCs infected with DenV produce high levels of proinflammatory cytokines and chemokines (TNFα, RANTES, MIP1β) but no type I IFN, measured either by qRT-PCR or ELISA. In this study we report that DenV reduces the ability of DCs to produce type I IFN in response to different inductors such as infection with other viruses like Newcastle Disease Virus (NDV) or a NS1 deleted influenza A virus (DeltaNS1), or other TLR ligands, indicating that DenV is also able to block the production of type I IFN in primary DCs after several stimuli. This inhibition of the type I IFN production is dose dependent and requires DenV replication and/or translation. Also the inhibition seems to be specific for the type I IFN pathway since the expression of other genes like TNFα or IL-6, dependent of the NFκB pathway are not affected. These results suggest a new mechanism by which DenV can evade the immune system in humans.

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DENGUE TRENDS BY AGE AND SEX IN PUERTO RICO A HISTORICAL ANALYSIS FROM 1990 TO 2008

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Historically, dengue has been a childhood disease and is the leading cause of pediatric hospitalizations in several endemic countries. Recent evidence suggests an increasing incidence among older age groups and a shift in the modal age. In contrast, there is little agreement in the literature on sex differences in infection rates and severity of disease. A change in case demographics is important to identify in order to better design and target interventions. We used data from the Puerto Rico Department of Health and the Centers for Disease Control and Prevention's passive dengue surveillance system to calculate annual suspected and laboratory-positive dengue incidence rates by age and sex from 1990-2008. Rates per 10,000 population were calculated using US Census data. Ordinary Least Square methods were used to determine if there was a significant change in rates over time by age and sex. Mean and modal ages of infection overall and by sex were determined. The mean and modal age of suspected dengue infections was 22.4 and <1 year in 1990 and 26.7 and 16 years in 2008; for laboratory-positive infections these figures were 24.3 and 12 years, and 25.3 and 14 years, respectively. There was a significant increasing trend in mean age for laboratory-positive females (24.4 to 25.7 years) and males (24.1 to 25.1 years) overtime. Rates of suspected dengue among males versus females was 23.2 per 10,000 and 22.9 in 1990 compared to 9.9 and 7.8, respectively in 2008; the rates of laboratory-positive dengue were 5.8 and 5.9 in 1990 compared to 2.5 and 1.8, in 2008, for male and females respectively. Our data suggest that there was an increase in mean age of cases, and that in some years, there was a difference in rates by sex.

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CHARACTERIZATION OF A PANEL OF DENV-3 INFECTIOUS CLONES

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Dengue viruses (DENV) are enveloped single stranded positive sense RNA viruses transmitted by *Aedes spp* mosquitoes. The viral genome is approximately 11kb and encodes 3 structural and 7 nonstructural genes. There are four genetically distinct serotypes designated DENV-1 through DENV-4. DENV infection causes a spectrum of illness ranging from asymptomatic viremia to hemorrhage, hypovolemic shock and death. DENV occur throughout the tropics, infecting approximately 50 million persons annually and causing 15,000 to 30,000 deaths. All four serotypes cause severe disease, but genetic differences within serotypes have been associated with mild or severe disease. Here we describe the characterization of 4 isogenic DENV-3 infectious clone constructs derived by replacing the parent envelope (E) glycoprotein gene with E genes based on four genetically and geographically distinct DENV-3 genotypes. Virus infectivity and growth kinetics were assessed in several cell lines, including Vero, C6/36 and DC-SIGN expressing U937 cells. Additionally we characterize the infectious clones' neutralization profiles using human polyclonal immune sera and mouse and human monoclonal antibodies. The availability of a DENV-3 molecular clone and panel of isogenic DENV-3 recombinant viruses provides new resources for illuminating the pathogenic mechanisms and biology of this important human pathogen.

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TOWARDS DEVELOPING AN ANIMAL MODEL TO EVALUATE THE PROTECTIVE EFFICACY OF ANTIBODIES RAISED AGAINST CANDIDATE DENGUE VACCINES

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Animal models provide the best means of assessing the safety and efficacy of prospective vaccines intended for human use. Candidate dengue (DEN) vaccines have typically been evaluated in non-human primates (NHP). While NHP infected with DEN develop viremia and virus-specific neutralizing antibodies, they do not develop signs of dengue disease. In contrast, AG129 mice infected with certain strains of DEN viruses develop viremia and other characteristics of dengue disease. In this study we sought to examine the potential of benefiting from the merits of both animal models in assessing the role of protective antibody responses to candidate DEN vaccines. To establish and evaluate this system we tested the immunogenicity of each DEN virus serotype in groups of two flavivirus naïve cynomolgus macaques. All four DEN virus serotypes elicited robust primary neutralizing antibody responses that were boosted following a second injection by day 63. To assess the protective efficacy of these antibody responses, NHP serum samples from each group were diluted to a neutralizing antibody titer of 1:200 against the homologous virus and were passively transferred to groups of AG129 mice. The next day all animals were challenged with 106 PFU of mouse adapted DEN-2 New Guinea C virus. Following challenge all mice receiving anti-DEN-1, anti-

DEN-3 anti-DEN-4 antibodies succumbed to infection by days 5-7 (median survival time 4.5 days). In contrast, mice receiving anti-DEN-2 antibodies remained healthy for 10 days and slowly succumbed to infection by day 18 (median survival time 13.5 days). Studies are currently underway to evaluate the protective efficacy of these antibodies against challenge with DEN-1 virus in AG129 mice and define correlates of protection related to humoral responses. These findings highlight the merits of these two animal models and set a precedent for evaluation of protective humoral immune responses to candidate DEN vaccines.

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IMMUNE-SUPPRESSIVE ABILITY OF DENGUE VIRUS IN AN *AEDES AEGYPTI* CELL LINE

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Little is known about the molecular biology of mosquito immune responses and defense reactions against dengue virus (DENV). Our group has shown that DENV infection in whole mosquitoes triggers an antiviral response involving the Toll and Jak-STAT pathways, as reported previously, and that inactivation of these pathways results in higher levels of virus replication in the mosquito. However, the presence of multiple tissues and cell types in whole mosquitoes complicates the molecular dissection of these immune responses and other pathways. We have thus established an immune-competent *Aedes aegypti* cell line system that can be used to more accurately dissect anti-dengue immune pathways and identify potential downstream anti-viral effectors. A preliminary microarray gene expression analysis carried out with cells infected with live DENV revealed an unexpected down-regulation of many immune genes, including antimicrobial peptides, serine proteases, thio-ester containing proteins (TEPs), and pattern recognition receptors. Interestingly, infection with heat-inactivated (HI) DENV induced twice the number of immune genes as live DENV infection, but down-regulated only half as many immune genes. These data suggest that live DENV may be capable of immune suppression in the cell line. We are currently using DENV-bacteria coinfections to investigate this possibility, and are particularly interested to determine if known mechanisms of DENV immune suppression in mammalian cells are also at work in invertebrate cells. In mammalian cell lines, it is known that some DENV subtypes are capable of immune suppression while others are not; we plan to test these subtypes in our Aedes cell line and in live mosquitoes to determine if the same holds true in the invertebrate system. These experiments will shed light on how conserved the mechanisms of DENV-mediated immune suppression are in the vertebrate and invertebrate systems, and on the ability of arboviruses to optimally adapt themselves to both hosts.

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KINETIC ASSESSMENT OF DENGUE VIRUS CELLULAR TROPISM

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The four serotypes of dengue virus (DENV1-4) infect over 50 million people per year and induce disease ranging from mild to lethal. Despite the high number of cases, the virus-host interactions that ultimately determine disease and the cell types that support replication *in vivo* have not been characterized in detail. In this study, the cellular hosts of DENV replication, as indicated by intense cytoplasmic non-structural protein staining, were assessed by immunohistochemistry and flow cytometry using a mouse model of DENV infection. In lymphoid tissues, replication was observed first in the spleen 6 hours after infection, followed by replication in the lymph nodes, Peyer's patches and bone marrow. Replication in these tissues peaked at approximately 2 days post-infection and was nearly undetectable by 3 days post-infection. In nearly all of the other tissues, replication was detected by 2 days post-infection and continued to increase beyond 3 days post-infection, at which time mice became moribund. These data provide a framework for understanding the virus-host interactions that modulate the DENV infection kinetics and suggest that different factors mediate and control viral replication in the lymphoid versus non-lymphoid tissues.

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FREQUENCY OF DENGUE FEVER AMONG FEBRILE PATIENTS PRESENTING TO AN URBAN HOSPITAL IN MEDELLIN, COLOMBIA: STUDY RESULTS

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In 2007, the incidence of dengue in Medellin, Colombia was 21.2/10,000. Peak dengue season in Medellin is September through January of the following year. Due to difficulties with clinical diagnosis and lack of funding to support laboratory testing, we hypothesized that significant under reporting of dengue was occurring. Objective: Determine the proportion of febrile illness due to dengue among patient presenting to ambulatory clinics. We established fever surveillance in three facilities in 2008 in a barrio of Medellin: A private clinic, a public clinic, and San Javier Hospital. Healthcare providers at these facilities were asked to refer all febrile patients (defined either by history of fever in the previous 7 days or having a core temperature >38.0 C on exam), to a study physician posted in the clinic. A standardized history and physical and an (acute) venous blood sample were obtained from all patients. A convalescent serum sample was collected 14-21 days after fever onset. Because the study physician rotated between clinics, surveillance was not continuous at any one site. A dengue case was defined as a patient presenting to a study clinic with fever and a serum sample positive for dengue by RT-PCR or MAC ELISA. From March 20, 2008 to March 20, 2009, 781 patients were recruited; 66 (8%) were laboratory-positive for a recent dengue infection: 26 patients were confirmed by RT-PCR: 8 were DENV2 and 18 were DENV3; 40 patients by MAC ELISA. Median age of dengue-positive patients was 3years (range 1 month-89 years). Eight patients required hospitalization. None met WHO criteria for DHF; however only 9 had a platelet count and 4 had chest imaging for effusions completed. Only 2 patients were diagnosed by the treating physician with dengue. In conclusion, dengue virus infection was indentified in 8.5% of patients presenting with fever in Medellin; however, dengue infection was rarely suspected. The results suggest that the burden of dengue in Medellin is greater than indicated by surveillance data.

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USE OF AG129 MICE TO ASSESS THE SAFETY OF LIVE, ATTENUATED DENGUE VACCINES

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A significant obstacle in development and evaluation of candidate DEN virus vaccines has been the lack of a suitable animal model that mimics dengue pathogenesis in humans. Recently, interferon (IFN)-α/β + IFN-γ receptor knockout (AG129) mice have been used as a small animal model for DEN virus pathogenesis and vaccine testing. In a pilot toxicology study, we compared the effects of a GMP-quality tetravalent DEN vaccine

(DENVax, a chimeric DEN-2 PDK-53-based vaccine) to those of a wild-type DEN- 2 virus (strain 16681) in AG129 mice. Animals were subcutaneously dosed with a DENVax, DEN-2 16681, or with an excipient control. Injected animals were evaluated on a daily basis for weights and clinical abnormalities. On days 0, 3, 5, 7, 9 and 11, subsets of animals in each treatment group were anesthetized, and blood samples were obtained for hematology, plasma chemistry and virology. The anesthetized mice then were euthanized for gross necropsy, histopathological evaluation of various tissues and measurement of virus levels in the brain. While there was no mortality in any of the study groups, infection with the wild type DEN-2 resulted in abnormal clinical signs and reduced body weight on Day 7. In contrast, DENVax vaccinated animals had no abnormal clinical signs. The main hematological and histological observations in the DEN-2 and DENVax groups were consistent with the altered immune status of these interferon-deficient mice. In both DEN-2 infected and DENVax vaccinated groups, peak viremia was observed on Day 3 post-vaccination. Peak titers and duration of viremia were lower in the DENVax group than in the DEN-2 group. Finally, virus was isolated from clarified brain homogenates only in the DEN-2 group and was not detectable in brain homogenates of the DENVax vaccinated mice. These findings highlight the utility of the AG129 mouse model for evaluating attenuation of dengue and other viral vaccines. The data also support the safety profile of DENVax and its use as a vaccine to protect against dengue fever.

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SUSCEPTIBLE RECONSTRUCTION AND SEROTYPE SPECIFIC ESTIMATES OF THE TRANSMISSIBILITY AND SEASONALITY OF TRANSMISSION OF DENGUE VIRUSES IN THAILAND

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The incidence of dengue and dengue hemorrhagic fever are greatly influenced by the level of immunity present in a population. The level of immunity in a population varies over time and is difficult to measure. Here we present a statistical method to reconstruct susceptible dynamics similar to methods that have been used in measles, influenza and cholera dynamics. The proportion of the population susceptible to primary and secondary infection with dengue is estimated using serotype specific incidence data collected at the Queen Sirikit National Institute of Children's Health over 30 years. The model provides a statistical framework for making inference about specific aspects of dengue transmission dynamics. We have used the model to estimate the seasonality of transmission for each of the dengue serotypes. We find that transmission coefficients for each of the dengue serotypes vary throughout the year (serotype 1: 0.80-1.44, serotype 2: 0.81-1.18, serotype 3: 0.64-1.82 and serotype 4: 0.71-1.62). We also use this model to estimate the impact of short term cross protection between dengue serotypes. The model recreates multi-annual oscillations present in incidence data. Our model can be used to make approximate forecasts of incidence far enough in advance to provide time to make operational changes in response to impending epidemics.

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DENGUE INFECTION IN HOSPITALIZED PATIENTS WITH FEBRILE SYNDROME. MEDELLIN, COLOMBIA

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Dengue Fever (DF) is hyperendemic in Medellín, Colombia. During 1998-2007 circulation of all four DENV serotypes was documented, with an average incidence of 57 cases/100,000; 2% of cases progressed to dengue hemorrhagic fever (DHF). We hypothesized the incidence of DF in Medellin was higher than reported because dengue surveillance is passive, clinical symptoms are not specific, and access to dengue diagnostics is limited. The study was undertaken to determine the proportion of hospital admissions for febrile illness due to DF. From March 20, 2008 to March 20, 2009 all patients admitted to Unidad Hospitalaria de San Javier for a febrile illness in the previous 7 days documented either by history or by physical exam were included in this study regardless of the examining physician's diagnosis. A standardized history and physical, as well as venous whole blood sample were obtained from all admitted patients. Patients were examined daily for clinical changes during their hospitalization and convalescent blood samples were obtained at discharge. Dengue diagnosis was confirmed using RT-PCR or a dengue-specific IgM ELISA. Among the136 patients admitted for febrile illnesses, 9 were confirmed dengue infection (7%); median age, 4 years (range 2 months-48 years); 22% were female. One patient met WHO diagnostic criteria for DHF; there were no deaths. The patient with DHF was the only one of the nine confirmed cases that was diagnosed by the treating physician as dengue. This patient was also presumptively diagnosed and treated for leptospirosis. The diagnoses of the other confirmed cases were bronchitis and pneumonia (3), viral syndrome (1), febrile convulsion (1), mastitis (1), diarrhea (1), and cellulitis (1). Of the other 125 patients, only one was presumptively diagnosed with dengue. In conclusion, a significant proportion of hospital admissions for fever at Unidad Hospitalaria de San Javier in Medellin are due to dengue. However, in most cases dengue was not considered. This has important implications for patient treatment and assessments of dengue disease burden.

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USEFULNESS OF COMMERCIALLY AVAILABLE GPS DATA-LOGGERS FOR TRACKING HUMAN MOVEMENT AND RISK OF DENGUE VIRUS INFECTION

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Our understanding of the effects of human movement on dengue virus spread remains limited in part due to the lack of precise tools to monitor the time-dependent location of individuals. We determined the utility of a new, commercially available, GPS data-logger for long-term tracking of human movements in Iquitos, Peru. We conducted a series of evaluations focused on GPS device attributes key to reliable use and accuracy. Positional point and line accuracy were 4.4 and 10.3 m, respectively. GPS wearing mode increased spatial point error by 6.9 m. Units worn by two volunteers for 14-16 days allowed the identification of locations visited by them and the frequency and duration of each visit. Cost, battery life, size, programmability and ease of wear are unprecedented from previously tested units, proving the usefulness of our units for linking movement of individuals and transmission risk of dengue virus and other infectious agents.

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DENGUE SURVEILLANCE IN A TERTIARY HOSPITAL IN CEBU CITY, PHILIPPINES

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Dengue-like illness (DLI) cases were enrolled and characterized in a cross-sectional, tertiary hospital-based passive surveillance study in Cebu City, Philippines. Male and female hospitalized patients presenting with DLI and who satisfied the study inclusion and exclusion criteria were enrolled in the study. From January to December 2008, 176 patients were enrolled into the study. AFRIMS JE/ dengue EIA and/ or dengue RT-PCR data was available on 173 of the subjects with 154/173 (89%) confirmed as dengue infections. Demographics of the confirmed dengue cases are as follows: male to female ratio was 1.2; age range was 2 to 32 years old with majority (96%) less than 15 years old. 144 (94%) subjects were diagnosed as acute secondary infections and only 6 (4%) cases were diagnosed as acute primary infections. The remaining cases were either suggestive of secondary dengue infection (2%) or unable to be differentiated whether it was a secondary or primary dengue infection (1%). Specific breakdown of the clinical diagnosis of the laboratory confirmed dengue cases as follows: 69 (45%) DHF gr 2, 43 (28%) DHF gr 1, 33 (21%) DSS (DHF gr 3/gr 4), 5 (3%) DF, and 4 (3%) systemic viral infection. Serotype data was available on 85 (55%) of the subjects with all 4 serotypes documented to be circulating with distribution as follows: 65 (76%) DEN-3; 10 (12%) DEN-1; 9 (11%) DEN-2; 1 (1%) DEN-4.

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ESTIMATING THE GLOBAL BURDEN OF DENGUE

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Current WHO estimates show 2 billion people living in dengue endemic countries and up to 100 million dengue fever (DF) cases annually. We identified countries with populations at risk for dengue during the last 25 years using three methods: [1] PubMed, country Ministry of Health and World Health Organization websites for reports of dengue incidence data, outbreaks, or serologic surveys to identify country-specific laboratory confirmed dengue infections; [2] published literature on laboratory-confirmed dengue among travelers, and [3] maps to identify additional counties at risk that have no published data but are contiguous to with at least two countries reporting dengue disease and share $\geq$ 50% of their border. We estimated the total population at risk for dengue using 2007 estimated census data of identified countries. For countries where dengue is limited to certain regions (e.g. China, Argentina) we only include the populations of those regions. To estimate the total annual DF and dengue hemorrhagic fever (DHF) cases in endemic countries, we applied incidence data from dengue cohort studies to the at risk population by region. Africa was not included in DHF calculations. To estimate the total dengue deaths we applied an average case fatality rate of 1% to the total DHF estimate. We identified 113 countries with reported dengue in both types of published reports. The contiguous-country method added an additional 11 countries, all in Africa. We estimated 3.5 billion people live in areas at risk for dengue. The contiguous country method increased this to 3.6 billion people at risk. 55% of the world's population is living in countries at risk for locally acquired dengue infection. Applying expansion factors from cohort studies and the 0.1% CFR, each year an estimated 34 million DF cases and 2 million DHF cases occur globally with 21,000 deaths. In conclusion, these estimates are at 2-4 times larger than previous estimates of populations at risk of dengue. DF may be a greater public health problem than previously considered.

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USE OF HAND HELD COMPUTERS FOR PRIMARY DATA COLLECTION IN A DENGUE FEVER SURVEILLANCE STUDY, MEDELLIN, COLOMBIA

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Hand held computers use the same operating systems and applications as desktop computers. Their small size and weight makes them ideal for use in the field. Data entered in the field is immediately available on downloading. However, replacing paper case report forms with hand held computer applications could raise multiple logistical, security, and regulatory issues during a clinical trail. We are conducting fever surveillance in Medellin, Colombia in preparation for possible clinical trails. In the pilot phase of the fever study, we introduced hand held computers to be used instead of paper forms. The hand held computers were programmed with data entry software. Study physicians were provided with these devices which were programmed with the standardized history physical exams questions (in Spanish).We compare data entry time and completion rate before and after introduction of the devices. We chronicled the logistical and other issues that occurred with use of the devices and the solutions to these issues. During the pilot phase of the study (December 12, 2007-February 20, 2008), 138 patients were recruited. The case report data was collected on paper for 57 of these patients, the remaining 81 were gathered using a hand held. The time taken to complete a history and physical exam (30-40 minutes) did not change going from a paper form to a hand held computer. However the number of incomplete forms was greatly reduced from 40% to 0%. Data entry and accompanying transcription errors were eliminated by the introduction of the hand held computers. Logistical issues encountered included risk of data loss, device malfunction, and issues with data transfer and storage. In conclusion, in the pilot phase of a fever surveillance study in Medellin, Colombia we replaced paper forms with programmed hand held computers. Savings in time and staff occurred through the elimination of data entry from form to computer. Missing data was also greatly reduced. Use of this technology greatly improved data quality in the study.

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EPIDEMIOLOGIC CONDITIONS ASSOCIATED WITH CASES OF DENGUE HEMORRHAGIC FEVER AND CONTROL EFFORTS: APPLYING TAIWAN'S EXPERIENCES TO GLOBAL CONTROL

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The number of dengue epidemics involving the more severe dengue hemorrhagic fever (DHF) has been increasing worldwide. Using epidemiological findings to prevent and control global epidemics of DHF is a primary public health concern. While most research has typically used incidence to describe epidemics, spatial-temporal preconditions for severe DHF epidemics have not been thoroughly understood. In Taiwan, where dengue is not endemic, we have had an unprecedented opportunity to develop spatial-temporal indices in Kaohsiung, southern Taiwan that may help identify risk patterns for predicting large-scale epidemics and the emergence of DHF.We used three new indices, probability of case-occurrence (dengue weeks/52 weeks), duration (mean weeks/dengue

wave), and transmission intensity (incidence/ number of waves) to map the risk patterns of DHF cases during a large-scale 2002 dengue epidemic in Kaohsiung, Taiwan. The percentage of DHF/total dengue cases was used as an index of severity. Greater risk for large-scale dengue epidemic was predicted by viral waves with both long durations (above the range of 0.7 to 1.5 weeks per wave) and high intensities (above the range of 0.2-1.2 dengue cases per 1,000). Our research revealed greater risk conditions associated with DHF emergence in high versus low populated urban areas. In areas with high population density, DHF cases were associated with a longer lasting waves or waves with more intense transmission, despite low annual incidence (< 8.31 dengue cases /1,000) in these areas. In less densely populated areas, DHF cases were associated with both the waves of longer duration and higher intensity. Our efforts to assess the potential severity of dengue epidemics involved identifying fundamental risk patterns that contributed to the emergence of DHF cases. By honing in on identifiable spatial-temporal variables and their effect on the progression of DHF, we apply our research to preventing and minimizing the severity of future global epidemics.

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DENGUE IN CEBU PROVINCE FOR OVER A DECADE AND PUPAL PRODUCTIVITY OF DENGUE MOSQUITO VECTORS (DIPTERA: CULICIDAE) IN A RURAL AREA IN CEBU, PHILIPPINES

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This study aims to examine the patterns of dengue cases and deaths in Cebu province, Philippines recorded at sentinel hospitals of the Department of Health Regional Epidemiological Surveillance Unit in 1997-2008, and to characterize the pupal productivity of *Aedes aegypti* and *Aedes albopictus* in rural Guba, Cebu City. A total of 33,771 dengue cases and 831 deaths occurred in Cebu province in 1997-2008. Year-to-year differences of dengue cases, deaths and case-fatality rates (CFR=1.19-3.73%) were significant. Cebu (33.37%) and Mandaue (8.33%) were the top two cities in dengue cases yearly, whereas Talisay (5.31%), Lapulapu (3.55%), Toledo (3.48%), Minglanilla (3.11%), Danao (3.0%), Consolacion (2.65%), Liloan (2.48%), and Naga (1.94%) comprised the rest of the top ten cities or municipalities. Cebu City differed in dengue cases and deaths among them but not in CFR. Dengue cases and deaths increased in the rainy season yearly. One- to five-year old children (31.7%) were the most affected followed by six- to ten-year old (30.59%). Female and male patients did not differ. Pupal survey was conducted in 2008 rainy season in Guba so that dengue control focuses on eliminating the most productive breeding sites. Approximately 2,500 pupae were collected in 554 breeding sites in 725 premises. Ten types of breeding sites yielded 41.16% *Ae. aegypti*, whereas nine types yielded 14.26% *Ae. albopictus*. Plastic (40.15%) and metal drums (29.62%), plastic containers (10.53%) were the three most productive for *Ae. aegypti*, whereas coconut shells (0.10%), plant parts (0.94%), and bamboos (1.88%) were the least productive. The top most productive for *Ae. albopictus* were bamboos (28.52%), plastic barrels (21.14%), and rubber tires (19.13%); the least were cemented tanks (1.34%), plant parts and discarded containers (2.01%). Dengue in Cebu province, Philippines is attributed to growing urbanization and population, inadequate public health infrastructure, poor solid waste management, insufficient water supply in rural areas, and lack of effective mosquito surveillance.

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ACUTE ENCEPHALITIS SYNDROME SURVEILLANCE FOR JAPANESE ENCEPHALITIS --- INDIA, MAY 2007-APRIL 2008

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Japanese encephalitis (JE) virus is a leading cause of acute encephalitis syndrome (AES) in Asia, but the true burden of disease in India is unknown. Since 2006, the Government of India (GOI) has conducted JE vaccination campaigns among children (<15 years) in select endemic areas. In 2007, GOI established sentinel surveillance to better define the epidemiology and burden of JE in two endemic districts. From May 2007-April 2008, active surveillance was performed for AES cases among children (<15 years) at the district hospitals of Bellary and Bardhaman. Serum and cerebrospinal fluid specimens were evaluated using JE IgM ELISA and plaque reduction neutralization tests. We analyzed JE vaccination status data and calculated minimum AES and JE incidence using resident cases and district-level census data. We identified 709 AES cases, including 439 (62%) in Bellary and 270 (38%) in Bardhaman. Overall, 440 (62%) case-patients were under five years, 419 (59%) were male, 550 (78%) were rural residents, and 111 (16%) reported previous JE vaccination. Among district residents, the estimated AES incidence per 100,000 children was 40 for Bellary and 7 for Bardhaman. Of 677 AES case-patients with available specimens, 63 (9%) had laboratory evidence of recent JE, including 6% (26/409) in Bellary and 14% (37/268) in Bardhaman (p=0.001). Six (10%) JE cases reported a history of JE vaccination. The estimated incidence of JE per 100,000 children was 1.8 for Bellary and 0.7 for Bardhaman. In conclusion, JE is a significant cause of AES among children in Bellary and Bardhaman districts. Future surveillance should focus on defining the impact of JE vaccination and identifying other etiologies of AES.

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IN SITU REVERSE-TRANSCRIPTION LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (*IN SITU* RT-LAMP) FOR DETECTION OF JAPANESE ENCEPHALITIS VIRAL RNA IN HOST CELLS

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Clinical diagnosis of Japanese encephalitis is usually difficult due to non-specific signs at the early and acute stages of the infection. Virus isolation from peripheral blood is also not possible because of the short period and low level of transient viremia even in the acute stage of the disease. It is thus urgent to develop a feasible and convenient method for laboratory diagnosis of the infection. This study was undertaken to establish a newly designed molecular approach that can be used to detect intracellular Japanese encephalitis viral RNA in host cells. The method was established and then was carried out to test its efficacy in cultured BHK-21 cells, subsequently in peripheral blood mononuclear cells (PBMCs) isolated from mice that have been inoculated with JE virus suspension. In this study, *in situ* reverse-transcription loop-mediated isothermal amplification (*in situ* RT-LAMP) was established; which combines merits of recently developed loop-mediated isothermal amplification (LAMP) and *in situ* reverse-transcriptase polymerase chain reaction (*in situ* RT-PCR). In conclusion, the newly-designed method can detect viral RNAs in peripheral blood mononuclear cells (PBMCs) in a short time with high sensitivity and efficiency.

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DEVELOPMENT OF A CONSENSUS MICROARRAY METHOD FOR IDENTIFICATION OF SOME SEVERE INFECTIVE VIRUSES

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Chikungunya virus, Japanese encephalitis virus, Yellow fever virus, Dengue virus, and other virus such as hanta virus, SARS-CoV, H5N1 avian influenza virus, can cause severe infectious diseases. However the consensus detective method is still not well established. Here we report a rapid and sensitive microarray technology to detect these viruses. A panel of specific probes both at genus level and species level were designed. The probes cover 9 genus and 16 virus species with the 70-mer oligonucleotides for genus level and 50-mer oligonucleotides for species level respectively. To decrease the interference of host genome in hybridization, the consensus genus primers were designed and utilized to specially carry out reverse transcription of the virus genome. The synthesis of the second strand was carried out by random primer (5-GTTTCCCAGTAGGTCTCNNNNNNNN-3). The amplified products were labeled and proceeded to microarray analysis. This microarray based method utilized the highly conserved consensus primers to specially synthesize cDNA of virus could effectively identify 16 severe infective viruses, and furthermore, an unknown virus isolated from pig brain in Shanxi province. This approach would have potential application in clinical diagnosis.

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THE CHANGE OF THE HSP70-RELATED GENES AND ITS POTENTIAL ROLE IN C6/36 CELL INFECTED BY JAPANESE ENCEPHALITIS VIRUS

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Japanese encephalitis (JE) virus is a member of Flavivirus; frequently causes encephalitic diseases through mosquito bites. The genome of JE virus contains linear, single-stranded positive-sense RNA (~11 kb in length) that encodes 3 structural proteins in the order of the nucleocapsid (C), membrane (preM/M), and envelope (E), followed by 7 non-structural proteins (NS1~NS5). It is known that virus infection may induce changes in the expression of host genes. We recently found that the HSC70 gene were up-regulated in JE virus-infected C6/36 cells 6~24h post-infection. The HSP70 is a gene family; which is composed of four highly conserved proteins: HSP70, HSC70, GRP75 and GRP78. These proteins has been reported to play a variety of roles, such as acting as molecular chaperones facilitating the assembly of multi-protein complexes, participating in the translocation of polypeptides across cell membranes and to the nucleus, and aiding in the proper folding of nascent polypeptide chains. Additionally, the HSP70/HSC70 has been reported to involve in virus replication in some plant RNA viruses, resulting in improved production of virus genome. Not only for this, HSP70/HSC70 was also mentioned directly interacting with the JE virus E protein on the cell surface, suggesting that it participates the process of virus entry. Because this gene increases in its expression in response to JE virus infection to C6/36 cells, we are now investigating how it molecularly interacts with C6/36 cells during infection by the JE virus.

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DIFFERENTIAL INTERACTION OF DENDRITIC CELLS AND MACROPHAGES TO RUSSIAN SPRING-SUMMER ENCEPHALITIS AND OMSK HEMORRHAGIC FEVER VIRUSES

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Omsk hemorrhagic fever virus (OHFV) and Russian spring-summer encephalitis virus (RSSEV), genus *Flavivirus*, have 90% homology at their amino acid level although they have different disease outcomes. Infection with OHFV mainly causes hemorrhagic fever, whereas RSSEV infection results in a severe form of CNS disorder. Understanding the interaction between these viruses and the host immune response will help in identifying potential new targets for vaccine production. Dendritic cells (DC) and macrophages play a critical role in both innate and adaptive immunity. Both function as antigen presenting cells to T-cells by upregulating expression of costimulatory and MHC molecules, and cytokine production. We exposed murine bone marrow DC and peritoneal macrophages to OHFV/RSSEV and examined their activation markers, and cytokines and chemokines responses. RSSEV replication was restricted in infected cells as the viral titer decreased over time. OHFV titer increased at 48hr and goes down over time. Neither virus blocked maturation of antigen presenting cells as evidenced by an increase in CD80, CD86 and MHC II. OHFV/RSSEV infection of macrophages and DC resulted in the secretion of a broad array of cytokines and chemokines. OHFV infected bone marrow DC had a robust production of IL-6, IL-10 and MIP-1α response as compared to mock or RSSEV infected bone marrow DC. Our results showed that macrophages and DC are important targets of OHFV/RSSEV infection that modulate the host immune responses.

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DETECTION AND CHARACTERIZATION OF TICK-BORNE ENCEPHALITIS VIRUS AND ITS RESERVOIR IN THE KYRGYZ REPUBLIC

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Tick-Borne Encephalitis Virus (TBEV) is an RNA virus belonging to the family flaviviridae that causes dangerous neuroinfections in an endemic zone extending from Central Europe through Siberia to Japan. In the summer of 2007 we undertook a field trip to the Kyrgyz Republic where we harvested animals and ticks, to initiate establishment of a baseline for viral epizootiology and epidemiology studies. We collected tissues from 185 specimens of rodents and shrews from 4 collecting localities. We hypothesize that due to environmental similarities to Alpine regions in Central Europe, the Kyrgyz Republic represents a locality of TBEV risk. To evaluate the presence of TBEV we analyzed samples using RT-PCR and serological screening techniques. We detected a Siberian subtype TBEV in a Himalayan field mouse *(Apodemus pallipes).* Initial sequencing of the NS5 gene demonstrated similarity to Kokkola strain from Western Finland. This is, to our knowledge the southernmost description of Siberian subtype TBEV and the first description of TBEV in the Himalayan field mouse. We are currently isolating viral genomes for sequencing. We will correlate viral genomic information with serology and the phylogeny of rodent and insectivore hosts. We are also evaluating the risk of human infection using risk map models and serological data from human patients.

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JAPANESE ENCEPHALITIS IN THE PHILIPPINES: CHART REVIEW AND LABORATORY CONFIRMED HOSPITALIZED CASES

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Japanese Encephalitis (JE) is a serious and disabling viral neurological infection. Human infection presents as asymptomatic, mild ILI or a life-

threatening disease. A significant number of hospitalized cases result in death or if they recover, develop residual neurologic disabilities. A safe and effective JE vaccine is available and vaccination is considered the most effective method for long-term protection. In the Philippines, the incidence of JE is unknown with JE reported under the viral encephalitis/ meningitis disease group. Diagnosis is usually based on clinical presentation since laboratory based tests are not widely available. A chart review was done for all admitted cases of meningitis/encephalitis/ meningoencephalitis cases in 4 tertiary hospitals (2 in Region 3, 1 in NCR and 1 in Region 6) covering 2004-2006. Classification was based on the final clinical diagnosis. For the 3 year duration among the 4 sites, 658 cases were admitted (484 meningitis, 124 encephalitis, 50 meningoencephalitis). Mortality ranged from 10-36% and recovery with residual disability was as high as 22%. Majority of the cases were <15 y.o. Cases started to increase in June and peaked in July of each year. Concurrently, a study was done in a tertiary hospital in Manila to document JE in Manila and nearby provinces. Data was collected from Sep 2005-Dec 2006. Fifteen patients were enrolled with 6(40%) cases from Region 3, CALABARZON, and Metro Manila confirmed with JE using the AFRIMS dengue/JE EIA. For the confirmed JE cases M:F ratio was 2:4, age range was 3-14 y.o. and confirmed JE was highest in the month of July. JE is endemic in the Philippines with possible evidence of seasonal peak of transmission but disease burden is unknown and surveillance is not laboratory based. There is a need to establish standardized and affordable diagnostic tests for JE and also data is needed to establish lab confirmed prevalence and incidence to determine whether JE vaccine should be incorporated in the national EPI.

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DEVELOPMENT OF A BROAD SPECTRUM *FLAVIVIRUS* QUANTITATIVE DETECTION ASSAY USING RT-PCR/ELECTROSPRAY IONIZATION MASS SPECTROMETRY ON THE IBIS T5000 PLATFORM

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Flaviviruses are a highly diverse group of viruses classified within the genus *Flavivirus*, family *Flaviviridae*. Flaviviruses are non-segmented, enveloped, positive-sense RNA viruses and most are arthropod-borne, requiring a mosquito or tick vector. The high genetic diversity of flaviviruses makes them extremely challenging to identify and several flaviviruses are highly pathogenic to humans and listed as NIH/CDC biothreat agents. In this study, we developed and evaluated a broad-range *Flavivirus* assay designed to detect both tick- and mosquito-borne flaviviruses. The assay consists of eight broad-range PCR primers and is performed in a 96-well plate format using RT-PCR combined with electrospray ionization mass spectrometry (RT-PCR/ESI-MS) on the Ibis T5000 platform. The assay was evaluated using a panel of thirteen different flaviviruses and all samples were correctly identified to the species level. To determine the limit of detection for the six mosquito-borne primer pairs, total RNA from West Nile virus (WNV) was serially diluted and assayed in replicates of 10. The mosquito-borne virus primer pairs could detect WNV to an equivalent viral titer of 0.2 PFU/mL. Similar results were obtained for the tick-borne primer pairs using the tick-borne flavivirus, Karshi virus. Analysis of flaviviruses in their natural background matrices included testing *Aedes aegypti* mosquitoes that were laboratory-infected with Dengue-1 virus. Not only could the assay accurately identifiy the virus within the infected mosquitoes, but we could determine that the average viral genome per mosquito was 5.4 x 10^6. Finally, using human blood, serum, and urine spiked with WNV and mouse blood and brain tissues from Karshi virus-infected mice, we determined that these clinical matrices did not inhibit the assay and the viruses were clearly detected. In summary, we developed a single high-throughput *Flavivirus* assay that could detect multiple tick- and mosquito-borne flaviviruses and thus provides a new analytical tool for their diagnosis and to study their ecology and epidemiology.

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DETECTION OF WEST NILE VIRUS RNA BY ONE-STEP REAL TIME RT-PCR

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West Nile virus (WNV) is a mosquito-borne flavivirus. West Nile virus possesses a single stranded plus-sense RNA genome. In this study, we developed a new TaqMan based one-step real time reverse transcriptase polymerase chain reaction (RT-PCR) assay for detection and quantification of WNV RNA. Oligos targeting 3' non coding region of WNV were designed by using oligo design and analysis software, OligoYap 4.0 (GATA, Turkey). None of the primer sequences showed genomic cross-reactivity with other viruses or cells in a web based BLAST (NCBI) search analysis. Oligos's specificity was tested with WNV positive samples obtained from Faculty of Veterinary, University of Ankara. To confirm the specificity of the TaqMan PCR assay, CMV, EBV, HCV, CCHFV, RSV, measles, and mumps viruses were tested, and no cross-reactivity was observed between WNV and tested viruses. The reproducibility of assay was performed several times, and the same results were achieved. The new assay was able to quantify the WNV genes in different concentrations ranging from 10^1 to 10^8 copies per reaction by plasmid standards. The first WNV was detected in a blood sample of a patient with acute graft-versus-host disease in our hospital. It can be concluded that our one-step real time RT-PCR method is a rapid and convenient diagnostic tool for clinical virology laboratories.

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A CPE BASED HIGH THROUGHPUT SCREEN FOR ANTI-WEST NILE VIRUS: MLPCN CAMPAIGN

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A mosquito-born pathogen, West Nile virus (WNV) causes significant health problem such as West Nile fever and neuroinvasive disease. Since the outbreak in New York in 1999 has been reported, WNV has spread and expands from the east coast to the Midwest of North America. In 2003 nearly 10,000 cases were reported in the USA. Currently there are no antivirals or vaccines available. Safe and effective anti-virals are required immediately. Recently we have established a cell based, high throughput screening (HTS) system to develop antivirals against WNV. Unlike other HTS systems using a reporter expression or target based, *in vitro* enzyme assay, a cell based system utilizing a live infectious virus can identify compounds active in any target of virus replication cycles. The assay was adapted into a 384-well plate format and optimized with variations in assay conditions. The Z' value, a coefficient to evaluate the quality of the assay, was greater than 0.7 indicating the assay is robust and suitable for HTS. We used the HTS as a primary screening method for screening a proprietary 13K compound library and a 300K compound library from the Molecular Libraries-Small Molecule Repository within the NIH molecular library probe production network (MLPCN). In a pilot scale screen with 13K compounds, 110 compounds were selected and then analyzed further by dose response, time of addition, titer reduction and mechanism of action studies. Seven compounds were validated as initial probes; specific to the viral targets, reduction in progeny virus titers over 10 fold and EC90 less than 20 μM. A variety of chemical classes were identified including nucleoside analogues and N-phenylanthranilic acid chemical cores. Two compounds were found to be active in an *in vitro* protease assay using WNV NS2b-NS3. The primary screening data from the 300K compound library and the dose response data from selected compounds

were deposited at PubChem website. Currently, hit compounds from the 300K compound library are being characterized in a titer reduction assay. A similar approach using the cell based-HTS with an infectious virus may warrant the search for more potent agents that selectively abrogate production of the infectious virus.

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A MULTICENTER EVALUATION OF A NEW ANTIBODY TEST KIT FOR LYMPHATIC FILARIASIS EMPLOYING RECOMBINANT *BRUGIA MALAYI* ANTIGEN BM-14

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Antibody tests are useful for mapping the distribution of lymphatic filariasis (LF) in countries and regions and for monitoring progress in elimination programs based on mass drug administration. Previous antibody tests have suffered from poor sensitivity and/or specificity or from a lack of standardization. We conducted a multicenter evaluation of a new commercial ELISA that detects IgG4 antibodies to the recombinant filarial antigen Bm14. Four laboratories tested a shared panel of coded sera that included 56 samples from people with microfilaremic Wuchereria bancrofti or Brugia infections and 25 control samples. Qualitative results were identical in all four test sites. In addition, each laboratory tested samples from their own serum banks. The assay detected antibodies in 32 of 36 sera (91%) from people with Brugian filariasis and in 96 of 98 sera (98%) from people with Bancroftian filariasis. Specificity testing showed that many sera from patients with other filarial infections such as onchocerciasis had positive antibody tests. Specificity was otherwise excellent, although 3 of 30 sera from patients with ascariasis and 4 of 51 with strongyloidiasis had positive antibody tests; it is possible that some of these people had previously lived in filariasis-endemic areas. Antibody test results obtained with eluates from blood dried on filter paper were similar to those obtained with serum tested at the same dilution. This test may be helpful for diagnosing LF, especially for patients with clinical signs of filariasis. The test also has attractive features for use in public health programs in LF endemic countries, namely robustness, high throughput, and the ability to test filter paper blood samples. These features should help to make antibody testing a practical tool for monitoring the progress of filariasis elimination programs and for post-MDA surveillance.

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SOCIO-ECONOMIC ANALYSIS OF NTD AT INDIVIDUAL AND HOUSEHOLD LEVEL: SELECTED EVIDENCES, KNOWLEDGE GAPS AND ALTERNATIVE PATTERNS OF IMPACT ASSESSMENT

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The research question deals with understanding the extent of socio-economic assessments dealing with Neglected Tropical Diseases (NTDs) in terms of type of evaluation, applied techniques and research trends from a socio-economic perspective. The ultimate goal of the review is two-fold. First, to identify knowledge gaps and research areas which have not been addressed so far through the lens of socio-economic assessment tools. Second, to describe and propose a different framework of analysis addressing the identified gaps of knowledge. For the purpose of this document, this review is a first synthesis of evidences and is designed to highlight some key evidences to open further assessment patterns. The use of the illness-poverty schemes provides a first understanding of the bidirectional relationship between NTDs and poverty. However, by reviewing the available socio-economic studies on NTDs impact, some knowledge gaps can be identified. This calls for new patterns of socio-economic estimations to measure NTDs effects and the impact of health interventions to tackle the burden of these diseases. The proposed new framework moves from the poverty focus to the vulnerability lens, leading the following implications affecting assessment design and orienting operational decisions: (1) The assessment of vulnerability over time focuses on contextual factors changes and how these same changes may differently impact on the risk of infection and re-infection. Vulnerability analysis would address contextual factors such as seasonality within the broad context of socio-economic issues, thus going beyond epidemiological approaches that focus biological responses to infectious disease; (2) Vulnerability assessment helps extend the assessment of disease impact, thus addressing not only the cause of disease, but also the disability condition caused by the chronic nature of diseases such as NTDs; and (3) Knowledge of contextual change is a critical factor to assess how households differently modify their coping mechanisms over time. For instance, households may cope by making temporary adjustments to their expenditures in the short term. However, in the long term the same coping strategies can have negative implications for households, thus increasing levels of vulnerability and poverty for those households unable to cope with the costs of disease.

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NOVEL BORON-CONTAINING SMALL MOLECULES AS POTENTIAL THERAPEUTICS AGAINST HUMAN LYMPHATIC FILARIASIS

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Lymphatic filariasis is a parasitic infection of humans caused by filarial nematodes of the genera *Brugia* and *Wuchereria*. These organisms infect more than 120 million people in 80 countries in tropical and subtropical regions. Nematodes are transmitted by mosquitoes and more than one billion people live in areas where mosquito-borne transmission is active. Currently available medicines neither prevent filarial infection nor permanently disrupt the parasite life cycle, and resistance to the existing therapeutics is emerging. New compounds are urgently needed with the capability to kill the adult worm form. Anacor Pharmaceuticals has developed a novel class of oxaborole compounds, some of which target leucyl aminoacyl tRNA synthetase in bacteria and fungi. In an *in vitro* screen against adult worms of the filarial nematode, *Brugia malayi*, a selection of these oxaboroles have shown excellent activity. The rate of *in vitro* killing by these oxaboroles was compared to that of a known antifilarial compound, albendazole, which at 100 μM requires 15-19 days to kill adult *B. malayi*. Fifteen oxaboroles killed adult worms faster than albendazole, and two structurally-related oxaboroles killed *B. malayi* at nM concentrations in 24 h. In preparation for *in vivo* efficacy studies, pharmacokinetic profiles were measured on the most active compound, AN3031. After oral dosing at 22 mg/kg in mice, AN3031 showed an oral bioavailability of 43%, an AUC of 37.5 h*μg/mL and a terminal $t_{1/2}$ of 3.0 h. This compound is now progressing to *in vivo* efficacy studies. Structure-activity relationships for the tested oxaboroles with activity against the adult worms will be reported.

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OPTIMISING ANTIBIOTIC TREATMENT OF *BRUGIA MALAYI WOLBACHIA* IN INFECTED JIRDS

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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. One major obstacle threatening the success of the filariasis control programmes is the absence of a drug with macrofilaricidal or permanent sterilizing activity. Anti-biotic targeting of filarial *Wolbachia*, an essential bacterial symbiont, has recently provided a novel treatment for filariasis with such activity. Here we report on experiments aimed at optimising different antibiotic treatment regimens (25, 100, 200 or 400 mg/kg/day for 32 or 46 days), in Mongolian jirds (*Meriones unguiculatus*) infected with adult *Brugia malayi* and the effects on worm recovery, embryogenesis, microfilaria (Mf) release, and *Wolbachia* load. The reduction in numbers of *Wolbachia* are determined using quantitative real-time PCR (qPCR) and expressed as *Wolbachia wsp:Brugia gst* ratios. The log drop in the ratio of *wsp:gst* gives a quantitative measure of the effect on *Wolbachia* loads. qPCR analysis showed significant decreases in *Wolbachia* levels compared with controls in worms recovered from both doxycycline-treated and minocycline-treated jirds. In addition, parasitological analysis showed significant decreases in Mf production and clear disruption of embryogenesis leading to shifts in the development of embryonic stages in both treatments. The effects on Mf, embryogenesis and *Wolbachia* following treatment with doxycycline are more dependent on the length of treatment period than on the dose. Treatment with minocycline is more effective than doxycycline, producing significant effects on Mf, embryogenesis and *Wolbachia* at both lower doses and shorter treatment times. The effects of a combination of doxycycline and ivermectin and a comparison with *B. pahangi* will also be presented.

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PROSPECTS FOR THE INTERRUPTION OF ONCHOCERCIASIS TRANSMISSION IN MOUNT ELGON FOCUS, EASTERN UGANDA

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The Mount Elgon onchocerciasis focus is a highly isolated, having an area of 250 km^2, and is located in eastern Uganda close to the border with Kenya. The vector is *Simulium neavei s.s.*, whose larvae develop in a phoretic association with the fresh water crab *Potamonautes granviki*. The focus has been treated with annual ivermectin mass drug administration (MDA) through community-directed treatment since 1994. When the ministry of health of Uganda launched its onchocerciasis elimination program in 2007, MDA was increased from once to twice per year, and vector elimination activities using the larvicide temephos (Abate®) commenced in early 2008. Sentinel village examinations with skin snips to measure microfilardermia were conducted in 1994 and 2005. Baseline crab trapping and examinations, and 11-hour black fly landing catches at the four established catching sites and fresh fly dissections began in 2007 and have been repeated monthly. Ground application of Abate®) at the rate of 0.2-0.4 mg/l were conducted in the focus. MDA coverage has been over 90% of eligibles since the 1994 and was unaffected by the switch to twice per year treatment. Microfilardermia dropped from over 50% to under 2%. The results from the larviciding campaign revealed that crab infestation with larvae and pupae of *S. neavei* drastically reduced from 30.2% pre-control in 2007 to 0% in March 2009. Adult fly biting rate reduced from 5 FMH in 2007 to 0 FMH in March 2009. Fly infection rate also drop from 7% in 2007 to undetermined in July 2008 when there was no more flies to dissect. Onchocerciasis transmission has been interrupted in the Elgon focus. However, entomological surveillance and semi annual treatment with ivermectin will continue for undetermined period of time until it is clear that elimination of onchocerciasis has been achieved.

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COST-EFFECTIVENESS ANALYSIS OF MICROSCOPY, OG4C3 ELISA AND PCR FOR THE MONITORING OF LYMPHATIC FILARIASIS MASS DRUG ADMINISTRATION INTERVENTION

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Lymphatic filariasis (LF) is a disease, resulting from infection with the nematode parasite Wuchereria bancrofti and transmitted by mosquitoes. It is a profoundly disfiguring disease that has major social and economic impact on afflicted communities. WHO necessitated the adoption of the mass drug administration (MDA) intervention as a global programme for the elimination of the disease. A number of diagnostic tools are used to monitor the programme but each has its own limitations. To help define an end point to disease transmission in order to stop MDA, it is important to assess the sensitivity of currently used monitoring tools in areas with low LF infection rates following MDA. This study sought to evaluate the relative performance and cost effectiveness of microscopy, ELISA and PCR following MDA. The study was conducted in Ankwanda and Brenu Akyinimu, two LF endemic villages located in the KEEA district in the Central Region of Ghana that had undergone at least three rounds of MDA. Blood was collected at night from 115 individuals and was used to prepare smears which were observed under the microscope. Some of the blood was blotted on filter papers which were used for ELISA and PCR tests. Capital and recurrent cost for the three diagnostic tools were determined and calculated using standard methods. In all, there were no positives recorded for microscopy. ELISA and PCR tests showed 1.7% and 2.6% respectively for positives. The cost of microscopy was $136.94, $1.2 per test, ELISA was $335.28, $2.9 per test, with PCR costing $466.4 and $4.1 per test. Compared with Og4C3 and microscopy, PCR was found to be the most cost-effective tool to help define an endpoint to disease transmission for MDA to be stopped.

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STRUCTURAL AND IMMUNOLOGIC CROSS REACTIVITY AMONG ALLERGENS AND HOMOLOGOUS HELMINTH ANTIGENS: LESSONS LEARNED FROM TROPOMYOSIN AND THEIR IMPLICATION FOR THE HYGIENE HYPOTHESIS

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With allergy increasing in both resource-rich and -poor countries and with the mechanisms underlying this increase being multi-factorial and poorly understood, it has been suggested that the relationship between allergy and control of helminth infection is the major determinant (so-called "Hygiene hypothesis"). Nevertheless, there are data to show that helminth infection can aggravate or induce allergic reactivity. To address specifically the relationship between the structure of particular helminth antigens and their important homologues among known allergens, we assessed the immunological cross-reactivity of parasite and non-parasite tropomyosin, as tropomyosin is an important allergen that is common to a variety of organisms including crustaceans, mites and cockroach. To this end, tropomyosins from *Onchocerca volvulus* (Ov) and *Dermatophagoids pteronyssius* (Der p) were assessed for reactivity of IgE and IgG antibodies

in 58 individuals with helminth infection and 21 non-infected individuals allergic to house dust mite. Our data showed a strong correlation between serum levels of both for tropomyosin-specific IgE and IgG ($p<0.0001$, $r=0.97$). Pre-incubation of sera from Der p allergic patients with Ov tropomyosin depleted IgE and IgG anti-Der p tropomyosin by 100 percent when compared to pre-incubation with Der p tropomyosin itself. Moreover, Ov-tropomysin inhibited the binding of Der p-specific antibodies to Der p tropomyosin using an interference ELISA, whereas an unrelated antigen failed to inhibit at all. Taken together, our data suggest a strong cross reactivity between Der p and Ov tropomyosin that may provide insight into the relationship between helminths and allergic disease.

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NATURAL PROGRESSION OF LYMPHEDEMA IN TOGO BETWEEN 2004 AND 2007

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Lymphatic filariasis (LF) is a mosquito-borne disease of global concern. Globally, over 14 million people suffer LF-associated lymphedema for which effective management strategies are currently available. In preparation for a national morbidity management program in Togo, a baseline survey was performed to identify lymphedema burden and treatment preferences and cost. Due to a three year delay in release of program funding, the baseline survey had to be redone, providing the opportunity to assess the natural progression of the disease. A cohort consisting of a convenience sample of 188 people with lymphedema was interviewed in 2004 and 2007. Symptoms, treatment preferences, frequency of acute attacks, and physical and psychological burden of leg lymphedema were assessed at both times. Pictures were taken of cohort participants' legs in 2004 and 2007, allowing for the comparison of stage and individual symptoms to survey responses. From 2004 to 2007, 36 patients were lost to follow up. Preliminary analysis showed no discernible pattern of disease evolution over the three years. The slight decrease in prevalence of lymphedema between 2004 and 2007 was not statistically significant: self-reported swelling of the right leg went from 67% to 63% (OR=1.22, p=0.49) and self-reported swelling of the left leg went from 66% to 60% (OR=1.29, p=0.38). When looking at the individual patient, we saw no statistical significance between people who improved and people who experienced worsened conditions. Even in the absence of a morbidity management program, the number of Togolese with swollen legs who self-reported using no treatment declined (- 73%). The use of recommended treatments increased, in particular elevation (+ 266%) and use of medication for acute attacks (+ 360%), suggesting that the interviewers may have recommended management strategies to patients. Even after excluding those patients from the cohort who adopted morbidity management techniques, there was still no statistically significant difference in lymphedema improvement or deterioration within the three years. More detailed analyses will be included in the final presentation. Preliminary analyses indicate variable progression of the disease over time. Further research is needed to better investigate the natural history of lymphedema. This information is necessary to evaluate lymphedema management programs.

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EVALUATION OF THE LARVAL MIGRATION INHIBITION ASSAY FOR DETECTING IVERMECTIN RESISTANCE IN *BRUGIA PAHANGI*

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Programs for the control and elimination of lymphatic filariasis, with high coverage rates and repeated treatments, are expected to place strong selective pressures for the development of anthelmintic resistance. Similar strategies for parasite control in livestock have produced high levels of resistance that threaten animal health and productivity worldwide. Though the potential for resistance in human filarioid parasites remains a concern, there are currently no diagnostic assays that can detect or measure this. The larval migration inhibition assay (LMIA), which separates nematode larvae on the basis of their motility, may potentially serve this purpose through the generation and comparison of dose-response curves. We tested multiple assay parameters to determine optimal migration conditions for third-stage *Brugia pahangi* larvae collected from mosquitoes fed on blood from infected dogs. These conditions include a three hour incubation of larvae in the presence of various ivermectin concentration followed by a three hour migration through 20µm mesh, both at 37°C with a 5% CO_2 atmosphere. We are evaluating the repeatability of the dose response to ivermectin. Preliminary results suggest that the LMIA may have potential for detecting resistance to ivermectin in *B. pahangi*.

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STATUS OF NEGLECTED TROPICAL DISEASES IN MOROGORO REGION, TANZANIA MAINLAND

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The World Health Organization estimates that, at least one billion people are affected by one or more of Neglected Tropical Diseases(NTDs) and a further two billion are at risk in tropical and subtropical countries. These diseases affect impoverished people living in rural and poor urban areas. The control these disease namely, schistosomiasis, soil-transmitted helminthiasis, lymphatic Filariasis and onchocerciasis is largely dependent on preventive chemotherapy. Several parasitic diseases overlap in Tanzania and multiple species of parasites co exist in most individuals. The control of these diseases are coordinated by the Ministry of Health and Social Welfare and implemented at district level. As part of an integrated approach to baseline data collection for the NTD program, information on all of these disease infections and their morbidity indicators in was carried out in two districts endemic for more than 2 NTD's in Morogoro Region. The aim of the study was to obtain information on these diseases and their morbidity indicators in order to assess the impact of mass chemotherapy. 12 villages in the two districts of Kilombero (4 villages) and Kilosa (8 villages) were selected as the study areas. Blood samples were taken for Circulating Filarial Antigen (CFA), Microfilaria, Haemoglobin and Malaria parasites. Urine for Schistosomiasis haematobium while stool for was for schistosomiasis mansoni and STH tests. A total of 756 people were sampled 486 Kilosa and 270 Kilombero. Circulating Filarial Antigen (CFA) prevalence was 18.5% in Kilombero and 30.7% in Kilosa. Prevalance of *Schistosoma mansoni* in Kilosa was 2.7% while in Kilombero 3.7%.A.lumbricoides and Trichuris prevalence were less than 1% in both districts while hookworm infection was 0% in Kilombero and 5% in Kilosa. These data serve as baseline data on NTDs burden and distribution in communities, which in turn will facilitate the assessment of the impact

of mass chemotherapy on these infections prevalences and morbidity. The extent and impact of polyparasitism in these areas will be discussed.

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THE NEMATODE PARASITE *ONCHOCERCA VOLVULUS* AND OTHER FILARIAE GENERATE THE TRANSFORMING GROWTH FACTOR-β (TGF-β)

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Transforming growth factor-β (TGF-β) is a highly conserved cytokine that has a well-known regulatory role in human and murine immunity, but also in organ development and tissue homeostasis of most animal species including helminths. Homologous *tgf-b* genes and mRNA have been detected in the filaria *Brugia malayi*. However, the in situ protein expression is unknown for filariae. Therefore, we examined several filariae for the expression and localization of latent (stable) TGF-β in adult and larval stages. A specific goat anti-human latency associated protein (LAP, TGF-β 1) antibody, purified by affinity chromatography, was used for light and electron microscopic immunohistochemistry. Adult *Onchocerca volvulus*, *O. gibsoni*, *O. ochengi*, *O. armillata*, *O. fasciata*, *O. flexuosa*, *Wuchereria bancrofti*, *Dirofilaria* sp., *B. malayi*, and infective larvae of *W. bancrofti* reacted with the antibody. Labeling of worm tissues varied between negative and all degrees of positive reactions, but the pattern of reactivity was the same among different worms and different filaria species. Latent TGF-β was most strongly expressed adjacent to the cell membranes of the hypodermis, the epithelia and muscles, particularly of the uterus, adjacent to some laminae and to many nuclei in all organs of vital immature and mature female as well as male worms. The basal, but not median or cortical layer of the adult worms' cuticle was labeled. Degenerated uterine microfilariae were TGF-β+, whereas vital mf, oocytes and embryonic stages were negative. TGF-β was well expressed in worms without *Wolbachia* endobacteria eliminated by doxycycline treatment. Some moribund, but no dead adult worms were labeled as well as pleomorphic neoplasms in *O. volvulus*. The other species demonstrated the same staining pattern. We conclude that latent TGF-β protein is expressed by filariae independently of *Wolbachia*, transported and secreted internally possibly regulating organ development and worm tissue homeostasis.

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DEVELOPMENT OF GMP PROTOCOL FOR THE PRODUCTION OF R-BM14 BASED IGG4 ELISA KIT

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Reliable, cost effective diagnostic tools are an essential part of the Global Program to Eliminate Lymphatic Filariasis. Previous research has indicated that the suite of tools available should contain a standardized, commercially-available assay to detect anti-filarial antibodies.
The recombinant based Bm14 antibody detection Enzyme Linked Immunosorbent Assay (ELISA) was developed to meet this need. This poster describes the development and optimisation of the assay, and the methods used to evaluate the reliability and stability of the prototype kits prior to field evaluation trials.

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CONGENITAL TRANSMISSION OF *TRYPANOSOMA CRUZI* IN SECOND GENERATION WISTAR RATS

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Mothers infected with *Trypanosoma cruzi* can congenitally transmit this parasite to their progeny in successive generations. In this work, we research the congenital transmission of second generation *T. cruzi* in the offspring of the Wistar rats (*Rattus norvegicus*), using parasitological, serological, histopathological, immunohistochemical and molecular diagnostic tests. Direct blood exams and cultures carried out on the offspring were negative while the xenodiagnosis revealed 18.2% to be positive. The IFI and ELISA serologies applied to each one of the offspring serum showed a seropositivity of 31.8% and 34.1% respectively. The histopathological study shown the restoration of an acute myocarditis (26.19%) and myositis (38.09%), with inflammatory processes of variable intensity, sin tissue parasitism. The immunohistochemestry study in weaves dealt with PAP reaction, revealed the presence of abundant antigenic deposits on the cardiac muscle (40.47%) and in the skeletal muscle (45.23%). Also, with the IIF fluorescent antigenic deposits were detected in the hearts (30.95%) and skeletal muscle sections (9.52%). The DNA extracted from the serum and the tissue of the offspring was used to evaluate the *T. cruzi* infection using the PCR technique with species specific oligonucleotides. In the serums, 6.8% of the positive samples were observed to be positive while the rest of the DNA of the parasite detected in the heart and skeletal muscle sections was 54.5 and 45.4%, respectively. The diagnostic tests used confirmed the presence of *T. cruzi* in blood circulation, in the serum, and in the tissue of the offspring. The most sensitive technique was PCR and the organ most affected was the heart. In conclusion, second generation congenital transmission of the *T. cruzi* occurred in the experimental model of Wistar rats. These results support the use of the bimolecular technique for the study of the epidemiology in Chagas'disease.

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IMPORTANCE OF URINARY KINETIC TO VALUE THE DISPOSITION OF EXPERIMENTAL PENTAVALENT ANTIMONY ULAMINA®

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Pentavalent antimony has demonstrated therapeutically effectiveness against distinct forms of leishmaniasis. The urinary kinetics of the generic pentavalent antimony Ulamina was studied following intramuscular administration in a single dose of 5 $mg.kg^{-1}$ of weight to five healthy volunteers. The urinary samples were collected and cuantificated at the follow intervals: 0-6, 6-12, 12-18 and 18-24 hours following treatment. Determination of trivalent antimony (SbIII), pentavalent antimony (SbV) and total antimony in urine samples was carried out using atomic absorption spectrometer. The volunteers was monitoring before and after administration of Ulamina® by clinical history, physical examinations, corporal weight, tests for hepatic and kidney functions. Didn't have evident of adverse o toxics reactions. Its make curves of accumulated drugs in urine vs. time (du vs. t). The pharmacokinetic parameters calculated were amount remaining to be excreted (ARE) vs. time (Sigma-minus graphic), rate of elimination (K_{el}) = 0,056 h^{-1}, half-life ($t_{1/2}$)= 13, 68 h, rate of urinary excretion of first order process (K_u)= 0.014 mg/Lxh^{-1} and renal clearance (V)= 0, 11 L/h. All urinary samples had SbIII as a metabolite in a value of 20, 22 percent, in some cases the relationship between SbV/SbIII were half of total antimony biodisponibility. Of the doses of total antimony administered it's was excreted 20, 70% in 24 hours. Finally,

the plasmatic $t_{1/2}$ reported is 13.46h, without significative difference with obtained following urinary data.

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LEAD OPTIMIZATION OF NOVEL BORON-CONTAINING DRUG CANDIDATES FOR THE TREATMENT OF HUMAN AFRICAN TRYPANOSOMIASIS

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Human African Trypanosomiasis (HAT) represents a significant public health problem in sub-Saharan Africa affecting hundreds of thousands of individuals. An urgent need exists for the discovery and development of new, safe, and effective drugs to treat HAT, as existing therapies suffer from poor safety profiles, difficult treatment regimens, limited effectiveness, and a high cost of goods. From a collaborative effort between SCYNEXIS, Anacor Pharmaceuticals, Pace University, and DND*i*, we report ongoing lead optimization efforts on a novel class of small molecule boron-containing compounds, exemplified by SCYX-6759. These compounds inhibit *in vitro* growth of *T. brucei* with IC_{50}'s ~100 nM, are not cytotoxic to mammalian cells, and exhibit good physiochemical and pharmacokinetic properties. In a murine model of CNS-stage disease utilizing the TREU667 strain of *T.b.brucei*, treatment with 50 mg/kg of SCYX-6759 BID for 14 days has demonstrated 100% efficacy, resulting in absence of blood parasites for >180 days. Development of a structure-activity relationship (SAR) profile for this chemical series and efforts to improve biological and pharmacokinetic profiles in the CNS-disease model through chemical modifications are reported herein.

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COST-EFFECTIVENESS ANALYSIS OF COMBINATION THERAPIES FOR VISCERAL LEISHMANIASIS ON THE INDIAN SUBCONTINENT

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A visceral leishmaniasis elimination campaign is underway in the Indian subcontinent. Early diagnosis and treatment are essential components of this initiative, but antimony has been lost to resistance in Bihar and Nepal. Miltefosine (MF) and Paromomycin (PM) are now available but might rapidly select for resistance if widely used on their own. Combining drugs, practice now for other infectious diseases, may provide mutual protection and prevent or delay parasite resistance. Also, shorter treatment duration may decrease costs incurred by patients and improve adherence to treatment. We assessed from a societal perspective the cost-effectiveness of 4 combination therapies (CTx): liposomal Amphotericin B (L-AmB) with MF, L-AmB with PM, MF with PM and antimonials and PM, and 5 monotherapies: MF, PM, conventional Amphotericin B (AmB), L-AmB and antimonials. Effectiveness and probability values were derived from clinical trials (systematic review) and clinical experts (Delphi survey). We considered direct costs incurred by providers to deliver the intervention, and direct and indirect costs by patients and relatives to receive treatment. Sensitivity and threshold analysis was performed on all variables to analyze the effect of varying parameter values on the rank-ordering of strategies. Preliminary findings show that MF+PM is the most cost-effective CTx (US$77 per death averted) followed by PM (US$107 per death averted). All other strategies were dominated, except AmB but has a high incremental cost-effectiveness ratio. Results are sensitive to adherence to MF and the price of L-AmB. L-AmB+PM is the best combination therapy if the price of L-AmB falls to US$6,5/vial and adherence to MF is below 90%. Effectiveness of combination therapies is mainly affected by the adherence to treatment which is especially relevant for MF. In the base case analysis MF+PM is the best strategy from an economic point of view. However, in sensitivity analysis the low cost of MF+PM offsets decreases in efficacy to levels which may render this treatment unsuitable.

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LOOK TO THE LESION: SYSTEMIC ANTIMONIAL THERAPY DOES NOT CURE EARLY PREULCERATIVE AMERICAN CUTANEOUS LEISHMANIASIS IN NORTHEASTERN BRAZIL

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Recent reports demonstrate markedly lower cure rates for early American Cutaneous Leishmaniasis (ACL) compared to classic ulcerative disease. Little is known about risk factors for clinical progression and therapeutic failure in early ACL. We evaluate clinical and immunological risk factors for the development of ulcerative disease and trea tment failure in early ACL caused by *Leishmania braziliensis*.This is a prospective cohort of 44 patients enrolled between 2002 and 2006 with early nonulcerative ACL in an endemic area in Bahia, Brazil. Cases were 13-60 years of age with less than 30 days of ACL confirmed by intradermal skin test and/or culture. Patients with previous or non-cutaneous forms of leishmaniasis or chronic diseases were excluded. Peripheral blood was collected to measure levels of IFN-γ, TNF-α, IL-10 and IL-5. All participants were treated with antimony (20 mg/kg/day x 20 days). Outcomes were lesion activity and cure by 90 days without recurrence. Early nonulcerative forms of ACL were plaques (46.3%), papules (46.3%) and nodules (3.3%). These were primarily single lesions (72.7%) and located on the lower extremity (67.4%). Treatment failure occurred in 59.1% of all patients; the majority of whom (57.7%) developed ulcerated lesions. Treatment failure was associated with younger age ($p=0.05$) and lesion ulceration ($p<0.001$) and was potentially associated with smaller leishmania skin test (LST) area ($p=0.07$), regional lymphadenopathy ($p=0.06$), and higher levels of TNF-α ($p=0.06$). There was no correlation with outcome and other cytokines or the size, location, type or number of primary lesions. Early treatment of ACL is associated with high rates of treatment failure. We demonstrate that lesion ulceration is the strongest predictor of treatment failure. Although important, markers of systemic inflammatory response appear less predictive of outcome in early ACL than in classic ulcerative disease. The study of local inflammatory responses may provide further evidence into mechanisms of lesion formation and therapeutic response to antimony therapy in early ACL.

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EFFICACY OF MILTEFOSINE IN THE TREATMENT OF AMERICAN CUTANEOUS LEISHMANIASIS CAUSED BY *LEISHMANIA BRAZILIENSIS* IN BRAZIL

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Miltefosine has been used in the treatment of visceral leishmaniasis in India. Recently, miltefosine has been tested in New World cutaneous leishmaniasis (CL) and Old World CL. The cure rate from the studies in New World CL in Colombia and Guatemala ranged from 91% (*Leishmania panamensis*) to 33% (*L. braziliensis*). There are no data regarding miltefosine use in CL caused by *L. braziliensis* in Brazil. This is a phase II randomized trial with 90 CL patients from the endemic area of Corte de Pedra in Bahia, Brazil. Patients included presented 1 to 3 months of untreated CL, with 1 to 5 ulcerative lesions. Diagnosis was confirmed by a positive culture or polymerase chain reaction methods and by intradermal leishmania skin test. After randomization 30 participants were treated with parenteral meglumine antimoniate (20mg/kg/day x 20 days) and 60 with miltefosine administered orally (2.5mg/kg/day x 28 days). Outcome measures were cure rate or complete cicatrization of the ulcer 2 and 6 months after the end of the treatment. Cure rate at 2 months for the antimony group was 65% and for the miltefosine group 75%. The final cure rate at 6 months was 58% in the animony group and 62% in the miltefosine arm. Intent to treat analysis showed no difference regarding the primary and final cure rate in both groups. Adverse events occurred in 80% of antimony patients compared to 77% in miltefosine patients. In the antimony group the most common side effects were headache (43%), fever (23%), arthralgia (22%) and mialgia (22%). In the miltefosine group, vomiting and nausea (39%), headache (29%) and dizziness (13%). CL caused by *L. braziliensis* in Brazil has a similar cure rate when treated with antimony or miltefosine. Considering that the oral administration of miltefosine is an advantage compared with the parenteral route used for antimony standard treatment in the rural area, our data suggests that miltefosine may be considered for the treatment of CL caused by L. *braziliensis* in Brazil.

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EVALUATION OF COMPOUNDS FOR CUTANEOUS LEISHMANIAL ACTIVITY IN BALB/C MICE INFECTED WITH *LEISHMANIA MAJOR*

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Cutaneous leishmaniasis (CL) is probably the most neglected disease of all the neglected diseases; a new case of CL happens every 4 seconds. In the United States CL is treated with IV Pentostam (SbV) under IND or Ambisome off-label. A FDA approved topical or oral drug that is safe and easy to use is urgently needed. For this reason, two Cutaneous models of Leishmania major in BALB/c mice are being used to evaluate chemicals for antileishmanial activity as part of the US Army. In one model, the metacyclic forms are injected into the footpads (FP) of mice. In the other model they are injected intradermally in the skin at the base of the tail (BT). Test chemicals are administered starting on the 3rd day after infection for 14 days. Lesions are measured weekly for 4 weeks and expressed as a mean effect of the chemical on footpad thickness or skin lesion size in relation to control infected non-treated mice. AmBisome is used as a positive control. The chemicals are administered either intraperitoneally (IP) or orally (PO) in the footpad test and either IP, SC, transdermally (TD), or PO in the tail base test. Antibiotics, antifungal compounds and 8-aminoquinolines have been evaluated. The most active chemical entities and classes will be presented and discussed.

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A NEW PEDIATRIC TABLET STRENGTH OF BENZNIDAZOLE FOR THE TREATMENT OF CHAGAS DISEASE

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Successful Chagas vector control substantially reduced vector-borne transmission as well as new childhood infections in Latin America in the 1990s. Congenital *T. cruzi* infections have become a key route of transmission and control issue, as vertical transmission remains relatively unaffected by progress in vector control. The main drug for acute Chagas disease, benznidazole (Bz), is only available as an 'adult' tablet formulation, and a pediatric formulation is urgently needed. Bz dose recommendations, dosing practices and patient age and weight profiles were reviewed from 10 centers involved in the treatment of children with *T. cruzi* infections. The priority pediatric target patient population, therapeutic dose range and identified programmatic needs were used to guide a pragmatic decision-making process to determine an appropriate pediatric tablet formulation, strength and associated dosing regimen. In absence of pediatric pharmacokinetic studies, but based on substantial clinical experience, 5-10mg/kg/day Bz split over two doses was considered the appropriate therapeutic intake dose range in children <12 years. Data from 2424 patient records, over 99% from children <18 years and 317 infants, highlighted the challenge for accurate dosing in infants as compared with older children. Prioritizing treatment of congenital Chagas, a dispersible tablet of 12.5mg is proposed to complement the available 100mg tablet and improve dosing accuracy in newborn infants most at risk of over and under-dosing. Use of the proposed pediatric tablet (p) would focus on children <10 kg and consist of 1p tablet (12.5mg) for 2.5-5kg, and 2p (25mg) for 5-10kg per intake dose. In conclusion, a pragmatic review and decision-making process helped to determine an appropriate pediatric tablet strength of Bz, for development as a dispersible tablet by DNDi in partnership with LAFEPE. Such work will help to improve dosing accuracy for infants, an increasingly important patient group.

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EVALUATION OF NEW TESTS FOR EARLY DIAGNOSIS OF VISCERAL LEISHMANIASIS AND ITS COMPLICATIONS AT THE *'POINT-OF-CARE'*

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Bangladesh is one of the endemic countries for visceral leishmaniasis (VL) with reported incidence of 10,000 cases annually with 20 million people considered to be at risk for VL. We conducted a clinical study to assess the cure rate in 200 VL patients treated with 28 intramuscular injections of sodium stibogluconate (20 mg/kg body wt given once daily). The diagnosis of VL in all the subjects with clinical features of the disease was confirmed by splenic aspiration followed by parasitological examination. We evaluated several diagnostic tests for VL including PCR and Loop-Mediated Isothermal Amplification (LAMP) in blood, KATEX and another

novel urine test for detection of anti-rKRP42 in these patients. Preliminary results showed a sensitivity of 90.6% and specificity of 100% for PCR in blood. Sensitivity and specificity of KATEX in urine varied in fresh vs. frozen samples, and the values were 88.2% and 90.2% respectively, for fresh samples versus 64.8% and 90.2% respectively, in frozen samples thawed before the assay. Data on other tests are being analyzed for presentation at the meeting.

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REVERSED AMIDINES AS ANTI LEISHMANIAL AGENTS: IN VIVO EVALUATION OF CANDIDATE COMPOUNDS AGAINST VISCERAL LEISHMANIASIS IN HAMSTER MODEL

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Currently available anti-leishmanial agents are few and have limitations with regard to efficacy, toxicity and development of resistance. Identification and development of newer leismaniacidal drugs need prioritization. We report promising *in vivo* activity of reversed amidines, DB745 and DB766 in *L. donovani* infected hamsters. The Hanson model of *L. donovani* infected hamsters was used to evaluate the efficacy of compounds. DB745 and DB766 were selected for *in vivo* experiment after assessing their antileishmanial activity against intracellular parasites using a new transgenic *L. donovani* with β-lactamase reporter gene developed in our laboratory. Syrian golden hamsters were used for the experiment which is a 11 day protocol, with day 1 as day of infection. Drugs are administered for 5 days from day 4, animals were sacrificed on day 11. Miltefosine was used as standard drug control besides vehicle control. Three doses were used for DB745 viz. 10, 30 and 50 mg/kg and four dose groups were used for DB766 viz. 10, 30, 50 and 100mg/kg. An additional experiment was done with DB766 -50 and 100 mg/kg in chronic model, sacrifice being done on day 39 to see if there was any delayed effect of the compound. Hamsters were intracardially inoculated with late-stage metacyclic promastigotes.Test compounds were administered orally, except for DB745, and where an extra group was administered 10mg/kg of the drug ip. After sacrifice, a part of liver tissue was used to prepare impression smears. Serial dilution culture assay and real time PCR were also employed to determine parasite load. DB745 was remarkably active at all the three doses. IP dose showed better PD results. Probable CNS toxicity is observed at the highest dose. Excellent activity was obtained in all the treatment groups of DB766 including chronic model. DB766 was well tolerated by hamsters. In conclusion, the hamster model has been validated and established for testing of potential anti leishmanial compounds in our lab. All the three methods -LDU, serial dilution and PCR show comparable results. The data also confirms the bioavailiblity of the compounds. Promising activity of reverse amidines has opened doors to use existing reversed amidines or fine tune the leads to improve efficacy and design new more potent analogues as anti leishmanial agents.

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HISTOPATHOLOGIC STUDIES IN DIFFERENT STRAINS OF SEMI IMMUNE MICE INFECTED WITH PBANKA AFTER CHRONIC EXPOSURE

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Due to repeated Plasmodium infections of individuals in holo-endemic areas, adults develop some form of immunity to malaria infection and seldom succumb to the severe forms of malaria such as cerebral malaria (CM) and severe malaria anaemia (SMA). Interestingly variation in responses has also been noted. A recent study has characterized CM in naïve rodent infections but no such characterization is reported in SMA model of the semi-immune. Here we describe a histopathological study of some vital organs that plays a crucial role in the SMA model of different strains (Balb/c, B6, NZW and CBA) of semi immune mice. This model may explain the possible immunity or susceptibility by each semi immune mice strain to the malaria infection, which may mirror similar observations of adults in holo-endemic areas. Parasitaemia was highest in NZW, consequently sequestration was observed in the brain, heart and not in the other strains. Malaria pigment deposition was highest in the spleen, liver, kidney of NZW and low in the other strains. Reticulocyte count was highest in Balb/c (which had high%Hb loss) and minimal in NZW, p=0.0014. This pathology study in the SMA model will be helpful in taking into account different responses to malaria infection when designing therapeutic interventions and vaccine studies.

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ANALYSIS OF *PLASMODIUM* INFECTION IN CULTURED ERYTHROPOIETIC CELLS

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Among *Plasmodium* species that infect humans, *P. vivax* is the most widespread in the world. *P. vivax* infection causes severe illness and significant economic harm in endemic countries. Unlike *P. falciparum*, blood stage *P. vivax* parasites exclusively infect reticulocytes (immature red blood cells) which constitute only a small percentage of human adult blood cells, and there is currently no convenient culture system for the parasite. Recently, the use of cultured erythropoietic cells (cEPCs) produced from hematopoietic stem cells (HSCs) from human cord blood to maintain *P. vivax in vitro* was reported. However, the system has not been widely adapted, partly due to its limited availability and technical complexity. Previously, we reported an improvement in the cEPCs production system by using a mouse stromal cell line that enhances percentage and duration of its reticulocyte production. Here, as part of an effort to develop a more convenient *P. vivax* culture system, we set up a quantitative assay that measures the extent of replication of *P. falciparum* in the cEPCs. By using quantitative PCR of the parasite DNA we determined stages of differentiation optimal for supporting parasite replication. Under static culture conditions, *P. falciparum* replication was supported in cEPCs from day 9 culture, which contains erythroblasts but not detectable reticulocytes. Co-culture with a mouse stromal cell line and agitation during the incubation also enhanced replication of the parasite. The enhancement in reticulocyte production in cEPCs and use of *P. falciparum* as a proof of concept platform within the newly developed assay system will help development of the *P. vivax in vitro* culture system by optimizing production of invasion-competent host cells and fine-tuning cEPCs production. Assays for parasite invasion and replication using *P. vivax* isolated from animal models of infection are planned.

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EUROPEAN CONCERTED RESEARCH ACTION ON LIFE OR DEATH OF PROTOZOAN PARASITES

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Protozoan parasites play a central role in the field of human and animal health causing devastating diseases such as malaria and sleeping sickness. Given the rapid development of drug resistance in these organisms it is crucial that the development of novel drugs or vaccines is based on a detailed understanding of parasite/host biology and interaction. Programmed cell death (PCD), is a keystone of the life or death decision in cells of multicellular organisms and in their interactions with parasites. Evidence is rapidly growing for an important role of PCD in the life history of a range of protozoans, albeit involving molecular mechanisms and functions that may be unique to these parasites. Several European

groups are at the forefront of the rapid growth of research devoted to various aspects of PCD-like processes in a range of protozoan parasites. Networking these teams created a focus for this work and accelerated the European impetus. Interactions will lead to greater dissemination of information on PCD mechanisms, markers and evolutionary concepts. Thereby providing the potential to develop new tools and leading to breakthroughs in the processes involved in PCD, and thus to significant progress in the development of novel treatments. Such cooperation and interaction are essential because a plethora of morphological markers and terminology associated with the process of PCD exist, leading to conflicts regarding the nature of death / survival processes in unicellular versus multicellular organisms. Consensus on methods, markers and terminology must be resolved for this field to progress. Furthermore, a concerted action will enable scientists to strengthen their leading position in this highly attractive field of research and open it up to non-European research groups. This COST action is open for international collaborations and colleagues from lab. and from endemic field areas are wellcomed.

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FLUORESCENCE MULTIPLEXING IMAGING FOR STUDYING PROTEIN TRAFFICKING IN *PLASMODIUM FALCIPARUM* INFECTED HUMAN ERYTHROCYTES USING TETRACYSTEINE-TAGGED KNOB-ASSOCIATED PROTEINS

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In the intraerythrocytic phase of *Plasmodium falciparum (Pf)*, the parasite exports proteins into the erythrocyte cytoplasm and to the plasma membrane forming "knobby" protrusions. These knobs contain knob-associated histidine-rich protein (KAHRP) which interacts with *Pf* erythrocyte membrane protein 1 and 3 (*Pf*EMP). Formation of knob structures is believed to induce a strong cytoadherence of *Pfs* infected erythrocytes to endothelial cell receptors such as CD36, ICAM, and CSA, in the wall of capillary blood vessels and subsequently microvascular sequestration of infected cells, which thus escape elimination by spleen. Although some hypotheses for protein trafficking mechanisms and knobs formation were proposed, dynamic imaging with a higher spatial and temporal resolution is required for a better understanding of mechanisms of knob formation. Common strategies to study protein trafficking often involve green fluorescent protein (GFP) fused to target proteins. However, the protein trafficking may be obscured by the failure of delivering GFP- fusion proteins to the target sites or diffusion rate could be limited by the large size of GFP (27 kD; 238 amino acids) and the perturbation of the conformation of the protein of interest. To improve the study of protein trafficking in *P. falciparum,* we replaced GFP with 6 aminoacids receptor domain tetracysteine (TC) in a total 20 residues moiety (2.2kD) which can interact with organoarsenical ligands FlAsH-EDT2 and ReAsH-EDT2. Here we report on our success in engineering KAHRP-GFP-TC (control) and KAHRP-TC constructs and describes details of experimental strategies to prepare the TC transgenic parasites. TC-tagged transgenic parasites enabled pulse chase labelling of continuously expressed proteins. We successfully identified different distribution of trafficked proteins synthesised at different times.

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METABOLOMIC ANALYSIS OF THE MALARIA PARASITE *PLASMODIUM FALCIPARUM*

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Malaria caused by *Plasmodium falciparum* is one of the most devastating tropical diseases. Annually there are over 500 million infections and over 2 million deaths worldwide. The problem is further compounded by drug resistance to many classes of antimalarials and the unavailability of an effective vaccine. Our efforts to further explore *P. falciparum* biology and discover unique drug targets have enabled us to identify three distinct *in vivo* transcriptional states reflective of novel parasite biology. To understand the functional relevance of these distinct states we are undertaking a large-scale metabolic analysis of the parasite using the accurate-mass time-of-flight mass spectrometry approach. The advantage of this approach is the ability to perform an unsupervised identification of known and novel metabolites that vary during the parasite's life cycle. A combination of metabolic analysis and transcriptional profiling will further enrich our understanding of the basic biology of the parasite. In addition to presenting the analysis of the metabolic profiles of parasites under standard *in vitro* conditions, we will present the results of the parasite's response to different *in vitro* metabolic and stress conditions. Our ultimate goal is to use this combinatorial approach to parasites isolated directly from patient samples, which will provide the most accurate profiling of the physiological state of the parasite in the host, and will better inform our efforts towards disease models and drug and vaccine development.

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DISRUPTION OF LIPOYLATION IN THE *PLASMODIUM FALCIPARUM* MITOCHONDRION AND APICOPLAST IS LETHAL

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Lipoate is a highly conserved cofactor that is covalently bound to four metabolic enzyme complexes. These four complexes are encoded in the *Plasmodium falciparum* genome and are expressed by blood stage parasites. Lipoate metabolism in *P. falciparum* differs from most other organisms in that the lipoylated complexes and lipoylation mechanisms are segregated by organelle. One lipoylated complex, pyruvate dehydrogenase (PDH), has been localized to the apicoplast organelle and is lipoylated exclusively through lipoate synthesis. The three remaining complexes, α-ketoglutarate dehydrogenase (KDH), branched chain α-ketoacid dehydrogenase (BKDH), and the glycine cleavage complex, reside in the mitochondrion, where they are lipoylated through a scavenging mechanism. To address whether lipoate synthesis and lipoate scavenging are essential to parasite survival, we used the unique bacterial enzyme lipoamidase (Lpa) to disrupt lipoylation in the apicoplast and mitochondrion. Lpa cleaves lipoate from lipoylated complexes, producing their inactive, apo forms. We used site-specific chromosomal integration mediated by mycobacteriophage Bxb1 integrase in the *P. falciparum* dd2attB strain, as reported previously, to generate cell lines containing an integrated lipoamidase gene. In order to express Lpa in the apicoplast or mitochondrion, the lipoamidase gene was appended with amino terminal targeting peptides. Active Lpa expressed in the cytoplasm did not affect parasite growth or lipoylation; however, four attempts to generate cell lines expressing active Lpa in the apicoplast and seven attempts to express active Lpa in the mitochondrion were unsuccessful. By comparison, we consistently generated cell lines expressing inactive lipoamidase in the three subcellular compartments of interest. These results indicate that no essential lipoylation occurs in the cytosol and suggest that lipoylation in the apicoplast and mitochondrion may be essential to parasite survival.

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NITRIC OXIDE PROTECTION AGAINST CEREBRAL MALARIA IN MICE IS ASSOCIATED WITH IMPROVED CEREBRAL MICROCIRCULATORY PHYSIOLOGY

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Cerebral malaria (CM) is a major complication of *Plasmodium falciparum* infections, claiming the lives of 1-2 million children every year. In mice infected by Plasmodium berghei ANKA (PbA), the low bioavailability of

nitric oxide (NO) has been shown to be associated with CM pathogenesis, and exogenous NO protects against the development of the syndrome. The mechanisms by which NO protects against CM are unclear, but it may be related to its participation in several processes in vascular physiology. The aim of this study was to determine which of the microcirculatory complications that occur during CM are ameliorated by exogenous NO. Methods: mice infected with PbA were treated with the NO-donor compound DPTA-NO or with saline. NO bioavailability was checked by analysis of plasma nitrite/nitrate and exhaled NO levels. At the time of CM development, blood was collected and the arterial and venous gases profiles and pH were evaluated in a blood chemistry analyzer. The number and location of hemorrhages in the brain was evaluated by histological examination of serial sections. The dynamic cerebral blood flow and leukocyte migration were measured by intravital microscopy of pial vessels in mice with a surgically implanted cranial window. Results: CM incidence in saline-treated mice was 90% against 20% in the DPTA-NO-treated group. DPTA-NO-treated mice increased levels of plasma nitrite/nitrate and of exhaled NO. Saline-treated mice with CM presented a high number of hemorrhages in the brain, particularly in the frontal lobe and olfactory bulb, which were dramatically reduced by DPTA-NO administration. Saline-treated but not DPTA-NO-treated mice showed high arterial and venular pCO2 and low pH, suggesting a state of respiratory acidosis during CM. At the time of CM development, arteriolar and venular blood flows were sharply reduced in saline-treated mice. DPTA-NO-treated mice presented significantly higher pial blood flow as compared to saline-treated mice. DPTA-NO treatment also caused a marked decrease in the number of adherent and rolling leukocytes in pial vessels. Conclusions: NO protects against CM development and this effect is associated with improvements in microcirculatory (increased blood flow) and respiratory (restoration of blood pCO2 and pH levels) physiology and with decreased vascular pathology (protection against cerebral hemorrhages and inflammation).

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ASSOCIATION OF NITRIC OXIDE WITH HEME IN *PLASMODIUM FALCIPARUM* FOOD VACUOLES

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Recent work by the authors has revealed that nitric oxide (NO) is produced in the food vacuole of *Plasmodium falciparum* (*Pf*Fv) parasites both at the trophozoite and gametocyte stage. Accordingly, nitrated tyrosine, indicative of the local generation of reactive nitrogen species (RNS), has been identified in association with the *Pf*Fv. While the mechanistic details of NO biosynthesis in the *Pf*Fv remain to be elucidated, evidence of a role for a putative NADH-cytochrome b_5 reductase and a cytochrome b_5, coded in PF13_0353 and PFL1555w respectively, is emerging from this work. Interestingly, both these protein products are expressed in the *Pf*Fv. Given the many roles of heme nitrosyl complexes in biology and the concurrent presence of heme, NO and/or NO-derived RNS in this organelle, we have undertaken resonance Raman experiments to probe the possible association of endogenous NO with heme in the *Pf*Fv. Current evidence shows that NO generated *in situ* from sodium nitrite ($NaNO_2$) and sodium dithionite reacts with hemozoin contained in a purified food vacuole preparation to yield nitrosyl heme, according to the signature signal detected using high and low frequency resonance Raman spectroscopy. It was observed that the NO generated *in situ* did not disrupt the food vacuole and the resulting heme nitrosyl complex remained within the vacuolar membrane. Biochemical and biophysical evidence that speaks to the physiological relevance of NO chemistry at the site of hemoglobin degradation, heme detoxification and antimalarial drug action will be presented and discussed.

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RELATIONSHIPS BETWEEN HEMOGLOBIN/RED BLOOD CELL POLYMORPHISMS AND HEMOGLOBIN LEVELS IN MALI

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Hemoglobin (Hb) and red blood cell (RBC) polymorphisms, including HbC, HbS, α-thalassemia, hemizygous G6PD deficiency, and type O blood group, protect against severe *Plasmodium falciparum* malaria in Africa. Malaria-associated anemia is multifactorial and in part is due to destruction of non-parasitized RBC. We hypothesized that RBCs under oxidative stress (e.g., G6PD deficiency) might produce a greater degree of anemia during a malaria episode than normal RBC, since the former would be more prone to physiological mechanisms that normally remove senescent RBCs. To test this hypothesis, we first determined whether baseline Hb levels differ between healthy Malian children prior to the annual malaria transmission season. In May 2008, we conducted a survey in 3 Malian villages (Bozokin, Fourda and Kenieroba) located within 3 km of the Niger River. We obtained a finger prick blood sample from 1244 children aged 6 months to 17 years and determined their Hb type (HPLC) and Hb level (Hemocue®), and whether the 3.7-kb deletional determinant of α-thalassemia and the mutational determinant of the A- form of G6PD deficiency were present (PCR). We defined anemia as Hb <11g/dL. The prevalence of anemia varied significantly (P=0.02) among villages: 51%, 33%, and 38% in Bozokin, Fourda, and Kenieroba, respectively Prevalence of anemia also differed significantly between children with normal α-globin (36%) and those with heterozygous (45%, P=0.04) or homozygous (58%, P=0.004) α-thalassemia. Anemia prevalence did not differ significantly between G6PD-normal and G6PD-deficient children or between children with HbA and children with HbS or HbC-trait. However, 88% (7/8) of HbSC children were anemic. Overall, anemia was markedly higher in Bozokin, where perennial transmission has been reported. A-thalassemia is common in Mali and is a significant determinant of baseline Hb level. This Hb trait should therefore be identified and analyzed as a covariate in studies of malaria-associated anemia in Mali and elsewhere in Africa.

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ELEVATED TNF RECEPTOR 2 IS AN INDICATOR OF PLACENTAL MALARIA BUT LEVELS DID NOT CORRELATE WITH MALARIA-ASSOCIATED LOW BIRTH WEIGHT BABIES

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Plasmodium falciparum infections in pregnant women increase the risk of delivering low birth weight (LBW) babies, who will experience health complications during their first year of life. Many studies have described mechanisms leading to the sequestration of infected erythrocytes within the placenta and the resulting stimulation of pro-inflammatory mediators, but few studies have focused on mechanisms the placenta may use to protect itself from inflammation, e.g., deleterious effects of TNF. Two distinct surface receptors, TNFR1 and 2, are differentially expressed on placental tissue and contribute to successful pregnancies. The receptors also modulate the effect of TNF since they are released by shedding; a process where the extracellular portion of the receptor is cleaved in order to neutralize TNF. Receptor shedding results in lower expression of TNFRs 1 and 2 on the surface of placental cells and may be a mechanism where by the placenta protects itself from excessive TNF levels during malarial

infection. In this study, we measured the levels of soluble TNFR1 and 2 in pregnant women infected with malaria during pregnancy and correlated the results with birth outcomes. Results showed that both TNFRs were significantly increased in the peripheral blood of pregnant women when they were infected with malaria compared to when they were not ($p<0.0001$, Mann-Whitney test) and that receptor levels were positively correlated with parasitemia ($r2=0.66$, $p<0.0001$ and $r2=0.23$, $p=0.002$ for TNFR1 and 2, respectively). Interestingly, plasma levels of TNFR2 at delivery were increased in the peripheral blood of women who were blood-smear negative but had placenta malaria ($p=0.0017$, Mann-Whitney test). While TNFR1 and 2 levels in placental blood were associated with low birth weight babies ($p=0.02$ and $p=0.003$, respectively, Mann-Whitney test), the increase was independent of malaria infection. In conclusion, TNFR2 levels can be considered as an indicator of placental malaria infection but higher levels of neither receptors were associated with protection against LBW babies.

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MEROZOITE SURFACE PROTEIN 2 HAS A POTENTIAL ROLE IN THE ATTACHMENT OF *PLASMODIUM FALCIPARUM* MEROZOITES TO ERYTHROCYTES DURING INVASION

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Merozoite surface protein 2 (*Pf*MSP2) is being assessed as a component of a vaccine designed to protect against disease caused by the malaria parasite *Plasmodium falciparum*. The *Pf*MSP2 gene sequence predicts an approximately 26 kDa antigen, which is anchored to the merozoite surface by a glycosyl-phosphatidyl-inositol moiety. *Pf*MSP2 is an intrinsically unstructured protein, which has recently been shown to form ordered oligomeric species, which possess many characteristics of amyloid fibrils. Here we demonstrate that polymeric but not monomeric recombinant MSP2 binds to human erythrocytes. The interaction between MSP2 and the erythrocyte surface is reduced upon treating the cells with trypsin, chymotrypsin, and heparatinase I, a finding consistent with the involvement of a heparan sulphate-containing proteoglycan on the erythrocyte surface. In contrast, treatment of erythrocytes with neuraminidase slightly improved MSP2 binding. The interaction between polymeric MSP2 and erythrocytes is similar to the interaction between Abeta 42 amyloid and erythrocytes. These results raise the possibility that oligomeric complexes of MSP2 related to amyloid fibrils on the merozoite surface, may facilitate merozoite attachment prior to invasion of the host erythrocytes.

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MODIFICATION OF CD47 LEVELS ON *PLASMODIUM FALCIPARUM*-INFECTED ERYTHROCYTES

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Macrophage clearance of senescent red blood cells (RBCs) and apoptotic cells has been shown to be dependent upon CD47 expression on the target cell membrane. CD47 engagement by macrophage SIRPα inhibits phagocytic activity and protects RBC from erythrophagocytosis. Conversely, decreased levels of CD47 expression are associated with an "eat me" signal and increased macrophage clearance. Non-opsonized *Plasmodium falciparum*-parasitized RBCs are phagocytosed by macrophages but CD47 expression on malaria infected RBCs has not been well studied. Based on the hypothesis that *P. falciparum* may modify RBC expression of phagocytic signals such as CD47, here we report, using enzyme immunoassay and flow cytometric assays, that CD47 expression is decreased on falciparum-parasitized RBCs [median (interquartile range), 0.267(0.14-0.325) OD_{450}] versus uninfected RBCs [0.538 (0.387-0.567) OD_{450}]; Mann-Whitney test, $P < 0.001$. These results indicate a potential role for modulation of CD47 in macrophage clearance of malaria-infected erythrocytes.

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SERUM BUT NOT CEREBROSPINAL FLUID ENDOTHELIN-1 LEVELS IN CHILDREN WITH CEREBRAL MALARIA ARE ASSOCIATED WITH NEUROLOGIC DEFICITS

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Activated endothelial cells may play a role in the pathogenesis of cerebral malaria (CM). Endothelial cells release endothelin-1 (ET-1), a potent vasoconstrictor that may also increase permeability of the blood brain barrier. ET-1 is also produced by neurons, and endothelins have been implicated in neurological development. To examine the role of systemic and central nervous system ET-1 in CM, serum and cerebrospinal fluid (CSF) ET-1 levels were measured in children with CM and comparator children. Median [interquartile range] levels in pg/ml are reported. Children with CM had lower serum levels of ET-1 (0.68 [1.07]) than children with uncomplicated malaria (1.0 [2.18], P=0.02) or community children (1.12 [1.51], P=0.07). In contrast, children with CM had higher CSF ET-1 levels than 8 North American control children, though levels in both groups were low (CM, 0.27 [0.17] vs. control 0.10 [0.13], P<0.0001). Serum and CSF ET-1 levels were then compared to neurologic and cognitive outcomes in children with CM. Higher levels of serum ET-1 were seen in children with as compared to without neurological deficits at discharge (1.74 [1.16] vs. 0.52 [0.77], P=0.002) and three months after discharge (1.92 [1.84] vs. 0.67 [1.11], P=0.04). CST ET-1 did not correlate with neurologic deficits, and neither CSF nor serum ET-1 correlated with cognitive impairment. In children with CM, increased levels of serum but not CSF ET-1 are associated with neurologic deficits.

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COMPARATIVE EFFICACY AND SAFETYOF DIHYDROARTEMISININ PLUS AMODIAQUINE OR SULFADOXINE -PYRIMETHAMINE IN TH E TREATMENT OF ACUTE UNCOMPLICATED *FALCIPARUM* MALARIA IN NIGERIAN CHILDREN

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Artemisinin Combination Therapy (ACT) is the drug of choice for the treatment of acute uncomplicated malaria in endemic countries where drug resistance is rampant. We investigated the relative efficacy and safety of dihydroartemisinin plus amodiaquine (DAQ) or sulfadoxine -pyrimethamine (DSP) in the treatment of acute uncomplicated *falciparum* malaria. Children aged 6 months - 12 years with malaria parasite density ≥1000 µL of blood were recruited from the Outpatient Clinic of the University College Hospital, Ibadan, Nigeria. The patients were randomly administered standard doses of DAQ or DSP for 3 days. All patients

were clinically examined from Day 0 - 3, then on Days 7,14, 21, 28 and 42. Thick and thin blood films were prepared from finger prick on each visit for malaria parasite quantification and prevalence of gametocytes. Blood spots were taken on filter paper for PCR to distinguish between re-infection and recrudescence. Fever and parasite clearance times were evaluated in both groups. Haematocrit and blood chemistry were done Adverse events were recorded for each patient. A total of 108 patients (55 DAQ and 53 DSP) were enrolled. Baseline characteristics were comparable between the 2 groups. Fever clearance time (FCT) was significantly shorter among DAQ than DSP group (1.03±1.17 versus 1.22 ± 0.58 days) while parasite clearance time (PCT) was similar in both groups (1.78 ± 0.69 versus 1.69 ± 0.58 days). Haematocrit by day 7 was significantly higher in the DAQ than DSP (31.7± 4.73 versus 28.84 ± 4.51%). Haematocrit normalized by day 28 in all patients. There was no significant difference in gametocyte prevalence between the groups and no adverse event of note in both groups. In conclusion, both DAQ and DSP are efficacious and safe. DAQ has shorter fever clearance time and faster restoration of haematocrit than DSP. Amodiaquine therefore appears to be a more suitable companion drug to dihydroartemisinin.

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AN OPEN RANDOMIZED CLINICAL TRIAL IN COMPARING TWO ARTESUNATE-BASED COMBINATION TREATMENTS [ARTESUNATE/SULFAMETHOXYPYRAZINE/PYRIMETHAMINE (FIXED DOSE OVER 24 HOURS) AND ARTESUNATE/ AMODIAQUINE (FIXED DOSE OVER 48 HOURS)] ON *PLASMODIUM FALCIPARUM* MALARIA IN NIGERIAN CHILDREN

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Patient compliance, tolerance, easiness of administration and efficacy remain key issues for an ideal antimalaria drug. Recently developed fixed dose (FDC) Artemisinin combination therapies (ACT's) offer new opportunities. We evaluated the therapeutic efficacy and effect on gametocyte carriage of two fixed dose therapies (ACTs): [artesunate/ sulfamethoxypyrazine/pyrimethamine (fixed dose over 24 hours (AS/ SMP_{FDC})) and artesunate/amodiaquine (fixed dose over 48 hours(AS/ AQ_{FDC}))] in 500 children aged 1-13 years suffering from uncomplicated falciparum malaria. The children were randomly allocated to receive three doses of AS/SMP_{FDC} 12 hourly over 24 hours or three doses of AS/AQ_{FDC} daily over 48 hours They were followed up for 28 days and had evaluation of hemogram, liver enzymes, serum bilirubin and creatinine levels on days 0, 7 and 14. Polymerase Chain Reaction (PCR) analysis was also done on days 0 and 28. At enrolment,parasite density was similar in the two groups (P= 0.153). Parasite clearance times (1.3799 and 1.3319) (P = 0.435) and the fever clearance times (1.1048 and 1.0756 (P=0.272) for AS/SMP and AS/AQ respectively were similar in the 2 groups. Gametocyte carriage before, during therapy and follow up were also similar (P > 0.05). Gametocyte carriage rate on day 7 were significantly lower than day 0 in the two drugs (P<0.0001).The parasite cure rates on day 28 after PCR correction were 96.5% and 97.9% for AS/SMP_{FDC} and AS/AQ_{FDC} respectively (P=0.360). Proportion of children with anemia at enrolment, day 7 and 14 were not significantly different in the two groups (P=0.568, 0.758 and 0.639). By day 14, 98.7% (AS/SMP_{FDC}) and (AS/AQ_{FDC}) of children with anemia had recovered. Rate of resolution of anemia were similar in the 2 groups. Adverse effects were mild and did not necessitate discontinuation of therapy. The liver enzymes, serum bilirubin and creatinine levels were not adversely affected in the two groups. In conclusion, both fixed dose ACTs are highly effective and safe in the treatment of uncomplicated falciparun malaria in children in an endemic area of southwestern Nigeria.

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A BAYESIAN APPROACH TO REDUCING MISCLASSIFICATION IN ANTIMALARIAL EFFICACY STUDIES

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Defining the true antimalarial cure rate is difficult in falciparum malaria clinical trials because recurrent parasitemias could be due to reinfection or recrudescence. "PCR correction" categorizes recurrences by comparing *msp1, msp2* and *glurp* nested PCR products before and after treatment. However, misclassification may be common due to two sources of error, the presence of undetected minority genotypes before treatment and reinfection by the same genotype (a chance match). We used computer simulations to model the effect of transmission intensity and complexity of infection on the probability of chance matches. Chance matches were more common in areas with less diverse parasite populations and high transmission levels, leading to underestimation of cure rates. Chance matches occurred in more than 50% of patients who had three alleles before and after treatment, a conservative representation of the complexity of infection observed in high transmission areas; this value was under 10% in patients with mono-infections. We developed a simple Bayesian approach for adjusting PCR-corrected cure rates for undetected minority variants and chance matches. Using simulations to inform prior probability distributions for the frequency of chance matches and for the prevalence of minority variants before treatment, and data from real trials, we repeatedly adjusted the observed number of recrudescent infections. The resulting posterior distribution for the cure rate allowed us to generate a 95% posterior probability interval for the cure rate instead of a single estimate likely to be biased by outcome misclassification.

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SAFETY AND EFFICACY OF ARTEMETHER-LUMEFANTRINE (AL; COARTEM®) IN THE TREATMENT OF ACUTE, UNCOMPLICATED FALCIPARUM MALARIA IN ADULTS (>16 YEARS OLD): A POOLED ANALYSIS OF INDIVIDUAL PATIENT DATA

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Safety data from 5 and efficacy data from 4 registration trials were pooled and analyzed to assess the safety and efficacy of a 6-dose regimen of AL (taken as 4 tablets twice daily for 3 days) in the treatment of acute, uncomplicated falciparum malaria (confirmed by blood smear) in adults. These studies were conducted in Thailand and in travelers from non-endemic regions (Europe and Colombia). The primary efficacy endpoint was the 28-day parasitological cure rate, corrected for reinfection by genotyping. Secondary endpoints included clearance time for parasites and fever. Standard safety parameters were evaluated. Adverse events (AEs) were recorded, including those related to the nervous system, hearing loss, electrocardiographic changes. A total of 599 patients were included in the modified intention to treat (mITT) patient set, all with AL standard tablets. The 28-day PCR-corrected cure rate in the evaluable population was 97.1% (495/510). Median parasite clearance time (PCT) in the mITT population was 42.3 hr (95% CI 41.5, 43.2) and median fever clearance time was 28.5 hr (95% CI 22.3, 34.0). The safety analysis (n=647) showed the most frequent (≥ 25%) AEs were nonspecific (headache, anorexia, dizziness, asthenia, arthralgia, myalgia, nausea), consistent with acute malaria. The majority of nervous system and ear AEs were mild, transient, and reversible. No AEs relating to

corrected-QT prolongation occurred. There were no deaths. A total of 22 serious adverse events of which only 7 were related to AL occurred in 1.4% (9/647) of patients. These pooled data show that AL achieved rapid PCT and high cure rates in adults from Southeast Asia and in travelers. AL was also safe and well-tolerated. These results are consistent with recommendations for use of AL as a first-line therapy for acute uncomplicated falciparum malaria in adults in both the developing and developed world.

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SAFETY AND EFFICACY OF ARTEMETHER-LUMEFANTRINE (AL, COARTEM®) IN THE TREATMENT OF ACUTE, UNCOMPLICATED FALCIPARUM MALARIA IN CHILDREN (BELOW 16 YEARS OLD): A POOLED ANALYSIS OF INDIVIDUAL PATIENT DATA

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Data from 5 clinical studies were pooled and analyzed to assess the safety and efficacy of a 6-dose regimen of AL, taken as 6 doses over 3 days (dosed according to body weight) in the treatment of acute, uncomplicated falciparum malaria (confirmed by blood smear) in children. These studies were conducted in malaria-endemic areas of Africa and Asia. The key efficacy endpoint was the 28-day parasitological cure rate, corrected for reinfection by genotyping. Secondary endpoints included parasite clearance time and fever clearance time. Standard safety parameters were evaluated. Adverse events (AEs) of special interest included effects on the nervous system, ear, labyrinth and corrected QT interval (QTc). A total of 877 patients were included in the modified intention to treat (mITT) patient set, all with standard AL tablets. The 28-day PCR-corrected cure rate in the evaluable population was 97.3% (792/814). Median parasite clearance time was 35.3 hr (95% CI 31.7, 35.7), and median fever clearance time was 7.9 hr (95% CI 7.9, 8.0) in the mITT. The safety analysis (n=1332 treated with standard or dispersible AL) showed that the most frequent (>/=10%) AEs were nonspecific (pyrexia, cough, vomiting, anorexia, headache), consistent with acute malaria. AEs leading to AL discontinuation occurred in 1.6% (21/1332) of patients. The majority of nervous system and ear AEs were mild, transient, and reversible. There were no AEs relating to QTc prolongation or labyrinth disorders. Four deaths occurred, 3 from infection and 1 from hemorrhage: all were deemed unrelated to AL. Serious adverse events occurred in 1.3% (17/1332) of patients but only one (urticarial rash) was deemed related to AL. These pooled data show that AL achieves rapid parasite clearance and high cure rate in children with acute, uncomplicated falciparum malaria. AL was also safe and well-tolerated. These results are consistent with recommendations for use of AL as first-line therapy for acute uncomplicated falciparum malaria in children in both the developing and developed world.

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THERAPEUTIC EFFICACY AND EFFECTS OF ARTESUNATE-MEFLOQUINE AND MEFLOQUINE ON GAMETOCYTE CARRIAGE IN CHILDREN WITH UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA IN SOUTHWEST NIGERIA

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The treatment efficacy and effects of artesunate-mefloquine (AMQ) and mefloquine (MQ) on gametocyte carriage were evaluated in 335 children ≤ 10years of age with uncomplicated *P. falciparum* malaria randomized to receive either drug/ drug combination. All children recovered clinically. Fever and parasite clearance were significantly faster with AMQ ($p<0.001$). The rate of *P. falciparum* reappearance (recrudescence or reinfection) between 2 and 6 weeks after start of therapy and the polymerase chain reaction corrected cure rate (96 vs 93%) were similar. Drug attributable fall in hematocrit was significantly lower in AMQ ($p<0.01$), but the rate of resolution of malaria-related anemia 6 weeks after treatment began (28 of 34 vs 27 of 32) was similar for both treatment. Gametocyte carriage rate were significantly lower with AMQ. Both regiments were well tolerated. AMQ clears parasitemia and fever more rapidly than MQ but both regimens are effective in treatment of uncomplicated *P. falciparum* malaria in Nigerian children.

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IMPACT OF THE IMPLEMENTATION OF IPTI ON EPI VACCINES COVERAGE IN THE DISTRICT OF KOLOKANI, MALI: A CLUSTER RANDOMIZED CONTROL TRIAL

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If the efficacy of Intermittent Preventive Treatment in infants (IPTi) with Sulfadoxine-Pyrimethamine (SP) against clinical disease and the absence of its interaction with EPI vaccines have been established, there are concerns that addition of the IPTi will increase the burden and disrupt the routines EPI services especially in Africa where the target EPI vaccines coverage remain to be met. To evaluate the impact of IPTi with SP on coverage of EPI vaccines, the 22 health areas of the district of Kolokani were randomized with the intervention in 11 health areas and the remained serving as control. After one year of implementation of the IPTi within the routine health system, EPI vaccines coverage was assessed in a cross-sectional survey conducted in December 2007. With the exception of Hepatitis B vaccines for which shortages of vaccines occurred through the year, the coverage of the vaccines given with IPTi-SP using information on the vaccination card, was significantly higher for all the antigens in the intervention area compared to the non intervention area, average increase of 15.6% [highest increase with DTP3 (62% versus 82.7%) and lowest with Polio2 (81% versus 91.4%)]. The coverage of other EPI vaccines was slightly or significantly higher in intervention zone than in non-intervention zone with an average of 4.3% (highest with Polio 0 [50.4% versus 56.7%] and lowest with Polio1, [93.8% versus 94.5%]. These differences persisted between the two areas when information from parents or guardian's interview was added to those on the vaccination cards. In summary, twelve months of IPTi implementation within the routine health system resulted in an increase in coverage of EPI vaccines in Kolokani, more marked with the vaccines given with IPTi.

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DIRECT SYNTHESIS OF CYCLOGUANIL AND MISLOCALIZATION OF MITOCHONDRIAL DIHYDROFOLATE REDUCTASE-THYMIDILATE SYNTHASE (DHFR-TS) IN ATOVAQUONE-PROGUANIL TREATED *P. FALCIPARUM*

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Clinically approved drugs and their targets have much to teach about enabling malaria biology and pharmacology. Previous studies have pointed to active-site differences and low expression levels of DHFR-TS in *Plasmodium falciparum,* which helps explain some but not all interesting pharmacology around this target. Proguanil, a prodrug for the DHFR-TS inhibitor cycloguanil, is not effective against *P. falciparum* unless combined with atovaquone, a selective inhibitor of the parasite electron transport system. This has evoked discussions on possible alternate targets of proguanil. Now, sensitive analysis of cycloguanil (after treatment with proguanil alone or in concert with atovaquone) shows low levels of cycloguanil, without a need for host cytochrome P450s. The role of cycloguanil and DHFR-TS in proguanil-atovaquone cytotoxicity is supported by cross-resistance patterns with cycloguanil. Immunocytochemical profiling of *P. falciparum* DHFR-TS shows for the first time that DHFR-TS is normally localized at the mitochondria. Atovaquone-proguanil causes delocalization of DHFR-TS, presumably through degeneration of membrane potential across the mitochondria. The present study reconciles the known action of each partner drug in a clinically useful synergistic process, and offers new paradigms for development of effective antimalarial drug combinations.

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DOSE-DEPENDENT RISK OF NEUTROPENIA FOLLOWING SEVEN-DAY COURSES OF ARTESUNATE MONOTHERAPY IN ADULT CAMBODIAN PATIENTS WITH ACUTE FALCIPARUM MALARIA

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Artemisinin derivatives such as artesunate (AS) remain first-line treatment for *Plasmodium falciparum* malaria. While current treatment guidelines recommend combination with a partner drug, AS monotherapy is being used as a clinical research tool to investigate reports of declining AS sensitivity along Cambodia's western border. In an open-label dose-ranging comparison of three AS monotherapy regimens (2, 4 and 6 mg/kg/day x 7d) complete blood counts (CBC) and plasma ALT were measured at baseline, on Days 3 and 6 of treatment and on Day 14. Analysis of the first 80 cases (39, 18 and 23 patients receiving 2, 4 and 6 mg/kg/day respectively), 78 of who had received all 7 doses of AS, showed no significant differences between groups in baseline or Day 3 CBC parameters. However by Day 6 of treatment, geometric mean WBC and absolute neutrophil counts (ANC) remained significantly depressed in the AS6 arm compared to the other two dosing arms ($p<0.001$ and $p=0.007$ respectively); this difference was even more marked at Day 14 when WBC and ANC had continued to rise in the lower two groups but remained low in the high-dose group ($p<0.001$). There were no major effects of AS dose on other CBC parameters. Plasma ALT values after 6 doses of AS6 were slightly raised though none were outside the normal range. Subsequent safety follow-up of individual subjects with ANC $<1.0 \times 10^9$/L showed rapid recovery of both WBC and ANC values after Day 14. Neutropenia in these individuals was asymptomatic; there were no cases of febrile neutropenia or evidence of increased susceptibility to infection. There is limited high quality safety data available for AS. This study uniquely characterizes its safety without the confounding influence of the partner drug in patients already compromised by acute malaria infection. Seven-day courses of AS when given at high dose have a significant impact on recovery of ANC in patients with malaria; future attempts to optimize dosing must take this into account.

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THE EFFECTS OF CHRONIC ADMINISTRATION OF LOPINAVIR/RITONAVIR ON THE PHARMACOKINETICS OF QUININE IN HEALTHY VOLUNTEERS

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Geographical distribution of falciparum malaria and HIV greatly overlaps, particularly in sub-Saharan Africa where more than two-third of all persons living with HIV reside and where *Plasmodium falciparum* infection claims millions of lives each year. Many of antiretroviral and antimalarial drugs undergo complex and overlapping metabolic pathways, indicating a potential for drug interaction. With greater availability of antiretroviral drugs in malaria endemic countries, clinically important pharmacokinetic drug interaction among these drugs, influencing drug efficacy and/or toxicity has been an emerging concern. We conducted a phase I, single center, open label, multiple dose, sequential design pharmacokinetic study to explore the interaction between antimalarial quinine sulfate and lopinavir/ritonavir (HIV protease inhibitors) in healthy volunteers. A total of 12 eligible healthy male and female volunteers received a single oral dose of quinine sulfate 648 mg (538 mg quinine base) on Day 1; a single oral dose of lopinavir/ritonavir 400/100 mg on Day 4; and twice daily oral doses of lopinavir/ritonavir 400/100 mg on Day 5-17, inclusive. On Day 15, a single oral dose of quinine sulfate 648 mg (538 mg base) was administered, in addition to lopinavir/ritonavir. The study was conducted in both inpatient and outpatient settings and lasted for 29 days for each volunteer. The safety and tolerability of oral quinine sulfate and lopinavir/ritonavir when administered individually, and in combination were evaluated throughout the study. Blood samples were collected at pre-specified time points, and analyzed for the pharmacokinetics of free and bound quinine, major active metabolite 3-hydroxy-quinine, lopinavir and ritonavir. A high-performance liquid chromatography (HPLC) method with appropriate detection techniques were employed for each study compound. A single-dose pharmacokinetics of quinine, lopinavir and ritonavir, alone and in combination will be reported, and clinical findings in association with drug pharmacokinetics will be discussed.

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GLOBAL SEQUENCE VARIATION IN THE HISTIDINE-RICH PROTEIN 2 OF *PLASMODIUM FALCIPARUM*: IMPLICATIONS FOR PERFORMANCE OF RAPID DIAGNOSTIC TESTS FOR MALARIA

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Malaria rapid diagnostic tests (RDTs) offer great potential to improve the diagnosis of malaria, particularly in remote areas. However, RDT sensitivity in operational use has been reported to be highly variable. Detection of falciparum histidine-rich protein 2 (*Pf hrp2*) is the basis for many of these tests, although this protein has previously been shown to be highly variable in its genetic structure. The extent of HRP2 variation on a global scale, and its effect on the performance of RDTs, is unclear. To contribute to understanding the significance of genetic variation in the *hrp2* gene, we have sequenced this gene from 458 isolates originating from African, South American, Pacific and South-East Asian countries. A total of 318 unique sequence types were identified, of which 259 sequences were seen once only, while the remaining 199 sequences combined to give a total 59 sequence types seen in >1 parasite isolate. A selection of sequence features have been compared across geographical regions. The relationships between parasite sequence features and RDT test sensitivity for approximately 20 commercial products involved in the first round of World Health Organisation (WHO) and Foundation for Innovative Diagnostics (FIND) malaria RDT evaluation were assessed. The outcome of the study will help to assess the performance of HRP2-detecting RDTs globally, and has the potential to improve and optimise RDT sensitivities so that they perform with acceptable sensitivity across a broad range of parasites found in geographically distinct regions. This has significant public health implications.

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MALARIA DIAGNOSIS BY POLYMERASE CHAIN REACTION BASED ASSAY USING A POOLING STRATEGY

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Pooling of clinical specimens has been used to reduce the number of assays needed when screening for various infectious diseases. Polymerase chain reaction (PCR) based assays are the most sensitive tests to diagnose malaria, but its use is limited by its high cost. We evaluated if pooling methods using stored serum samples can be used to screen for malaria infection. The pooling platform to detect malaria infection involves arranging 100 individual clinical samples in rows and columns in a 10 x 10 matrix format. Ten individual samples are pooled vertically and horizontally such that each sample is represented in two of the 20 constituted minipools. DNA is then extracted from these pools and used for PCR amplification. Negative PCR of the 20 pools would 'rule out' malaria infection in the 100 samples and preclude further testing. Positive results would require further PCR assays on the individual samples constituting the positive row and column pools. We tested the feasibility of detecting parasite DNA in pools of 10 (1:10 dilution) and 100 (1:100 dilution) serum samples. Serum, cryostored up to 54 months, from 15 microscopy positive malaria cases from Iquitos, Peru was used. 100 µL of serum from malaria positive cases was added to 900 µL of malaria negative to make pools of 10. 100 µL of serum from these pools was added to 900 µL of malaria negative serum to simulate pools of 100. DNA was extracted from 400 µL of pooled sera, in both pools of 10 and 100, and detected by ethidium bromide staining following agarose gel (1.8%) electrophoresis. *Plasmodium vivax* was the predominant infecting species in our study (12/15) with *P. falciparum* accounting for the remainder. The mean parasite counts, as measured by microscopy, were 2,704 parasites/µL for subjects with *P. vivax* malaria and 7,778 parasites/µL for subjects with *P. falciparum* malaria. The PCR-based assay detected parasite DNA in all 15 (100%) undiluted samples as well as in minipools with a 1:10 dilution. In master-pools with a 1:100 dilution, 12/15 (80%) samples had detectable DNA with mean parasite count of 4,083 parasites/µL. In the 3 negative samples, the mean parasitemia was 2,260 parasites/µL. In conclusion, screening for malaria infection using stored serum samples with PCR-based pooling platforms is sensitive and efficient. Field studies with various population prevalences are needed to validate and prove its cost-effectiveness.

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A COMPARISON OF THE SENSITIVITIES OF DETECTION OF *PLASMODIUM FALCIPARUM* GAMETOCYTES BY MAGNETIC FRACTIONATION, THICK BLOOD FILM, AND RT-PCR TECHNIQUES

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Several studies have exploited the magnetic properties of *Plasmodium*-infected erythrocytes for a variety of different purposes. A recent study in a rural clinical setting in Papua New Guinea has demonstrated that *Plasmodium falciparum* gametocyte detection is facilitated by magnetic deposition microscopy. No study has yet determined the sensitivity and limit of detection of a magnetic fractionation technique for *P. falciparum* gametocytes on serial gametocyte dilutions. The present study compares the detection limit and sensitivity of a technique based on the use of commercially available magnetic fractionation columns with those for thick blood film microscopy and reverse transcriptase polymerase chain reaction (RT-PCR) methods. Protocols for the detection of gametocytes in six series of dilutions of parasite culture with known gametocytaemia were conducted using magnetic fractionation, thick blood film and RT-PCR techniques. The preparations obtained by the magnetic fractionation method were of thin film quality allowing easy gametocyte identification by light microscopy. Magnetic fractionation had a higher sensitivity and approximately two orders of magnitude better limit of detection than thick blood film microscopy. Gametocytes were also more readily detectable on the magnetically fractionated preparations. Magnetic fractionation had a similar limit of detection to that of RT-PCR. In conclusion, magnetic fractionation is a highly sensitive and convenient method for gametocyte detection in comparison with the standard thick blood film and RT-PCR methods.

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MALARIA-RELATED MORTALITY IN HOSPITALIZED UGANDAN CHILDREN IN AN AREA OF HIGH MALARIA TRANSMISSION

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In areas of seasonal malaria transmission in sub-Saharan Africa, the presumptive treatment of febrile illnesses as malaria without laboratory confirmation of the diagnosis is associated with high impatient mortality. However this presumptive diagnosis and treatment of malaria and the related inpatient mortality in high malaria endemic areas is not well characterized. A retrospective chart review was conducted of all children up to 15 years of age admitted to Soroti Hospital, Uganda from January 2002 to December 2004, with a diagnosis of malaria and clinical outcome analyzed according to microscopic confirmation of diagnosis. A total of 10,387 children were admitted with a diagnosis of malaria during the study period, 746 (7.2%) of whom died. Severe malarial anemia 3,794 (36.5%) and malaria with convulsions 1,764 (17.0%) were the two common causes of malaria related admissions. Children who did not receive microscopy testing had a higher case fatality rate than those with a positive blood smear (9.0% vs 6.5%, P<0.001). After adjustment for age, malaria complications and co-morbid conditions, children who did not have a smear done had a higher risk of death than those with a positive blood smear, Odds Ratio (OR) 2.08, 95% Confidence Interval (CI) 1.71, 2.53, P< 0.001. In conclusion, in high malaria endemic areas, diagnosis of malaria in the absence of microscopic confirmation is associated with significantly increased mortality in hospitalized Ugandan children. In patient diagnoses of malaria should be supported by laboratory confirmation of diagnosis.

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HEALTH WORKERS' USE OF MALARIA RAPID DIAGNOSTIC TESTS (RDTS) TO GUIDE CLINICAL DECISION-MAKING IN RURAL DISPENSARIES, TANZANIA

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Rapid diagnostic tests (RDTs) were developed to improve the quality of malaria diagnosis in resource-limited settings. RDTs can detect specific malaria parasite antigens in whole blood with high sensitivity and specificity. We assessed health worker prescription practices following the introduction of RDTs in health facilities without laboratory services. From December 2007 to October 2008, we introduced *P. falciparum* HRP-2 based ParaHIT® RDTs for routine use in 12 health facilities in Rufiji District, Tanzania. Health workers received training on how to perform RDTs for patients five years of age and older, with fever or suspected malaria. National guidelines recommended empiric treatment in children less than five years of age with suspected malaria. Among the 30,195 patients seen at these 12 health facilities, 10,737 (35.6%) were tested with an RDT for malaria. 88.3% (9,405/10,648) of tested patients reported fever or history of fever and 2.7% (289/10,677) of all tested individuals were children under five. RDT results were recorded for 10,650 patients (99.2%). Among the 5488 RDT positive patients (51.5%), 5,256 (98.6%) were treated with an appropriate antimalarial per national guidelines (artemether-lumefantrine or quinine). Among the 5162 RDT negative patients, 205 (4.0%) were treated with an antimalarial. Other reported treatments included antibiotics and antipyretics. In conclusion, implementation of RDTs in rural health facilities without other diagnostic services resulted in high adherence to national treatment guidelines. Patients testing negative by RDT were rarely treated with antimalarials. For RDT positive patients, unapproved antimalarials were seldom used. Health workers continued to follow guidelines for the empiric treatment of febrile children.

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COMPARISON OF REAL-TIME PCR AND MICROSCOPY FOR DIAGNOSIS OF MALARIA IN MALAWIAN PREGNANT WOMEN

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In sub-Saharan Africa, pregnancy-associated malaria contributes to pre-term delivery, low birth weight, and fetal growth retardation. Microscopy remains the gold-standard test for parasite detection, but polymerase chain reaction (PCR) may be more sensitive. We compared the diagnostic utility of microscopy to real-time PCR in a cohort of 475 pregnant women in Malawi attending a single rural antenatal clinic. At delivery, thick smears of peripheral blood were prepared and blood spots were collected on filter paper. The blood smears were evaluated for parasitemia by an experienced microscopist in a local research laboratory. Genomic DNA (gDNA) was extracted from the blood spots and amplified in a real-time PCR assay that targets the *Plasmodium falciparum* lactate dehydrogenase gene (pfldh). Subsequently, all samples with discordant microscopy and real-time PCR results were speciated in a real-time PCR assay targeting species-specific sequences of the ribosomal DNA of *P. falciparum*, P. ovale, and *P. malariae*. Of the 475 patients, 11 (2%) were microscopy positive and 51 (11%) were real-time PCR positive for infection with *P. falciparum*; compared to microscopy, the sensitivity of real-time PCR for detection of *P. falciparum* was 90.9% and the specificity 91.2%. Of the samples amplified in the speciation assay, *P. falciparum* alone accounted for all but three infections, with 2 mixed infections with *P. falciparum* and *P. malariae* and one pure *P. malariae* infection. There was good concordance between the pfldh assay and the speciation assay overall. Our study demonstrates that the prevalence of submicroscopic *P. falciparum* infections among pregnant women at delivery in Malawi is high and that, compared with microscopy, real-time PCR can detect substantially more of these infections. We plan to examine the correlation between these submicroscopic parasitemias and clinical birth outcomes.

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USE OF ICT MAL PF RAPID DIAGNOSTIC TEST CASSETTES FOR POLYMERASE CHAIN REACTION (PCR) ANALYSIS OF *PLASMODIUM FALCIPARUM* RNA ANALYSIS IN ZAMBIA

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Malaria diagnostic tools play an essential role in cost-effective malaria case management, especially as malaria parasitemia levels decline. In Zambia, ICT Mal Pf rapid diagnostic tests (RDTs) are widely used in field activities and research studies and offer an opportunity for greater diagnostic confirmation in areas not well served by trained microscopists. Further, under field conditions, where diagnostic confirmation is preferred with PCR-based analyses, we examined the ability of ICT Mal Pf RDT cassettes to perform PCR-based rna extraction. Among 31 children age 0-5 years tested with ICT Mal Pf RDTs, a sensitivity of 96% and specificity of 80% was determined against standard filter paper-based PCR analysis. These results indicate that RDT cassettes are useful and similar in sensitivity