and *Cryptosporidium* showed parasite diversity, as well as evidence of anthroponotic and zoonotic transmission. These findings highlight the importance of molecular tools in public health activities.

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ACANTHAMOEBA KERATITIS OUTBREAK IN CHICAGO, ILLINOIS IS ASSOCIATED WITH THE PRESENCE OF THE PATHOGENIC BACTERIA *LEGIONELLA PNEUMOPHILA*

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Acanthamoeba is a protist which causes a rare sight threatening eye infection, Acanthamoeba keratitis (AK). A dramatic increase of AK in conjunction with discovery that *Acanthamoeba* can harbor pathogenic bacteria as endosymbionts has heightened public health concerns. *Acanthamoeba* may act as a "trojan horse" of many different types of bacteria including Legionella, the causative agent of Legionnaires Disease. In Acanthamoeba, these bacteria multiply and are released into the environment, facilitating transmission to humans. Also, *Acanthamoeba* can survive harsh conditions including most drug, allowing the bacteria to survive within *Acanthamoeba* when it otherwise would have been destroyed. Since 2003, the incidence of Acanthamoeba keratitis has increased dramatically in many metropolitan locations including Chicago, Illinois. These increases have been hypothesized to be a result of recent EPA mandated water treatment changes that has increased the biofilm in the water system and the prevalence of *Acanthamoeba*, which feeds on biofilm. Previous data has confirmed the keratitis-causing *Acanthamoeba* are not a novel or more pathogenic species. We hypothesized that keratitis-causing *Acanthamoeba* in Chicago patients may be associated with *Legionella*, which increased its virulence and therefore its capacity to cause disease. 47 clinical samples of *Acanthamoeba* from keratitis patients from Chicago from 2005-to present were tested for the presence of *Legionella* using *Legionella* specific primers to amplify an internal portion of the 16S ribosomal RNA gene via PCR. Positive samples were confirmed by DNA sequencing. Of 47 clinical samples, 28 tested positive for *Legionella*. Sequence analysis confirmed the presence of Legionella pneumophila in all bacteria-harboring Acanthamoeba. In situ hybridization confirmed the presence of these bacteria intracellularly in the Acanthamoeba. This data shows a surprisingly high amount of bacteria associated with disease causing Acanthamoeba which suggests a roll for pathogenic bacteria in the virulence of *Acanthamoeba*.

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ASSESSMENT OF A NEW PARASITOLOGY SCREENING DIAGNOSTIC ELISA FOR THE DETECTION OF ANTIGENS OF *GIARDIA* SPP., *CRYPTOSPORIDIUM* SPP. AND *ENTAMOEBA HISTOLYTICA* IN FECAL SPECIMENS

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Giardia spp., *Cryptosporidium* spp., and *Entamoeba histolytica* are among the most common protozoan sources of parasite-associated diarrheal disease worldwide. A lack of rapid and cost-effective diagnostic tools is a major challenge to the surveillance of disease caused by these three pathogens. The development of the *TRI-COMBO PARASITE SCREEN* ELISA by TechLab, Inc. that can simultaneously detect antigen for these parasites in clinical stool samples represents a significant advantage in screening for these pathogens. Evaluation of the *TRI-COMBO* test is currently underway at three tropical medicine reference centers, the National Institutes of Infectious Diseases(NIID) in Tokyo, Japan, the International Center for Diarrheal Disease Research, Bangladesh(ICDDR,B), in Dhaka, Bangladesh, and the Bernhard Nocht Institute for Tropical Medicine in Hamburg, Germany. To date, 400 clinical samples have been subjected to analysis by the *TRI-COMBO* test and compared to the *GIARDIA II*, *CRYPTOSPORIDIUM II*, and *E. HISTOLYTICA II* individual stool ELISA tests from TechLab. Out of this panel of samples, the *TRI-COMBO* test detected 161 samples positive for *Giardia* spp., *Cryptosporidium* spp., and/or *E. histolytica*. The *GIARDIA II*, *CRYPTOSPORIDIUM II*, and *E. HISTOLYTICA II* individual stool ELISA tests detected 81 samples positive for *Giardia* spp., 35 samples positive for *Cryptosporidium* spp., and 47 samples positive for *E. histolytica*. 10 samples were positive for more than one parasite, as confirmed by detection with the individual ELISA format tests and 10 samples were found to be positive on the *TRI-COMBO* test but negative on the individual stool ELISA tests. 2 samples were found to be negative on the *TRI-COMBO* test while positive on the individual stool ELISA tests. 237 samples were confirmed negative on all tests. In conclusion, the *TRI-COMBO* test displayed 98.7% sensitivity and 95.95% specificity during screening of a large number of clinical samples for the presence of *Giardia* spp., *Cryptosporidium* spp., and *E. histolytica*.

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EVALUATION OF A NEW RAPID DIAGNOSTIC TEST FOR THE DETETION OF *GIARDIA* SPP. AND *CRYPTOSPORIDIUM* SPP. IN HUMAN FECAL SPECIMENS

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Giardia spp. and *Cryptosporidium* spp. are pathogenic protozoan parasites able to colonize the human intestine and are among the leading causes of traveler's diarrhea. Here, we report the clinical evaluation of the *GIARDIA/CRYPTOSPORIDIUM QUIK CHEK*, a rapid point of care assay capable of simultaneously diagnosing infection of these organisms through the identification of antigen in human fecal specimens. The test involves a membrane-based device with immobilized capture antibodies and a soluble peroxidase-conjugated antibody that is combined with a diluted specimen. Only a simple dilution is necessary, with no filtering or centrifugation required. The diluted sample is then added to the membrane device, with time to result being less than 30 minutes. The assay result is a visible line for a positive result and the absence of a line for a negative result. No equipment is required for the assay or interpretation. The sample panel included 511 samples tested at both LSG & Associates and TechLab, Inc. Specimens tested at LSG & Associates were part of a panel of preserved fecal specimens obtained following routine patient testing. All samples tested were preserved in either 10% formalin or SAF. Specimens tested at TechLab, Inc. were originally submitted to a local clinical diagnostic laboratory for routine microbiology testing. These samples were fresh (undiluted) or preserved in either 10% formalin or sodium acetate formalin (SAF). All rapid test results were compared to microscopy using a direct immunofluorescent detection procedure (MERIFLUOR *Cryptosporidium/Giardia*). The evaluation included 431 preserved (215 10% formalin and 216 SAF) and 80 unpreserved fecal specimens. The *Giardia* line compared to IFA had sensitivity = 98.6%, specificity = 100%, and correlation = 99.6%. The *Cryptosporidium* line compared to IFA had sensitivity = 100%, specificity = 99.7%, and correlation = 99.8%. The simple format and rapid detection ability of this test makes it ideal for a variety of uses: small or large-scale screenings, field diagnostics, and use in developing countries.

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TRNA GENE STUDIES FOR GENOTYPING OF *ENTAMOEBA HISTOLYTICA* IN STOOL SAMPLES

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Entamoeba histolytica tRNA-linked STR (short tandem region) can be useful to establish a correlation between genotype of the parasite and the outcome of infection. A total of 662 stool samples from asymptomatic subjects (n:488) and suffering from diarrhea (n:174) in Diyarbakir, Turkey, were examined for the presence of *E. histolytica* using microscopy, stool antigen ELISA, conventional PCR, and real-time PCR from July 2008 through June 2010. *E. histolytica* was detected in 3.3% (22/662) of the stool samples by real-time PCR. Clinically, diarrhea was mostly prevalent in patients with positive testing by *E. histolytica* real-time PCR assay (20 associated with diarrhea/dysentery, but 2 associated with no symptoms). Parasite load can be correlated with clinical outcome in *E. histolytica* infected patients, since a parasite load of 10^3 copy/µl and than higher was detected mostly in patients with diarrhea. We investigated the tRNA - linked STR gene polymorphism in clinical isolates of *E. histolytica*. In the present study, we identified three different genotypes among 22 isolates when two loci (SD and SQ) were used. PCR amplification was not observed at the other loci (A-L, N-K, D-A, and R-R) in genotyping. We observed a limited degree of STR polymorphism among *E. histolytica* strains obtained from stool samples, even in the strains isolated from a restricted area. It was found that SD and SQ loci seem to be suitable in the study of tRNA-based genotyping of *E. histolytica.*

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DIAGNOSIS OF *GIARDIA* AND *CRYPTOSPORIDIUM* ENTERIC INFECTIONS WITH A NEW POINT-OF-CARE RAPID ASSAY

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Giardia spp. and *Cryptosporidium* spp. are pathogenic protozoan parasites able to colonize the human intestine and are among the leading causes of traveler's diarrhea. Infection can result in chronic debilitating diarrhea, nutrient malabsorption, and human-to-human transmission, making diagnosis a high priority for fecal testing laboratories. Here, we report the clinical evaluation of the *GIARDIA/CRYPTOSPORIDIUM QUIK CHEK*, a rapid membrane-based assay capable of detecting *Giardia* cyst antigen and *Cryptosporidium* oocyst antigen in human fecal specimens. Specimens tested were obtained by the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) from a cohort of children in an area where *Giardia* and *Cryptosporidium* infections are prevalent. The test utilizes immobilized capture antibodies and a soluble peroxidase-conjugated antibody that is combined with a diluted specimen. Only a simple dilution is necessary, with no filtering or centrifugation required. The diluted sample is then added to the membrane device, with time to result being less than 30 minutes. A visible line is required for a positive result and the absence of a line for a negative result. No equipment is required for the assay or interpretation. Results from the rapid test were compared to two FDA-cleared ELISA tests specific for *Giardia* and *Cryptosporidium*. The evaluation utilized 129 fresh and 180 frozen specimens (including 89 *Giardia* positives and 44 *Cryptosporidium* positives). The *Giardia* line compared to ELISA had 100% positive agreement, 100% negative agreement, 100% overall agreement. The *Cryptosporidium* line compared to ELISA had 100% positive agreement, 100% negative agreement, 100% overall agreement. The ease of use and rapid detection ability of this test makes it desirable to use in a variety of settings: small or large-scale screenings, field diagnostics, and use in developing countries. The data from this clinical evaluation indicate that the assay is a reliable method for the identification of *Giardia* and *Cryptosporidium* in unpreserved human fecal specimens.

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EVALUATION OF A POINT-OF-CARE SEROLOGY ASSAY SPECIFIC FOR THE DETECTION OF *ENTAMOEBA HISTOLYTICA*

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Entameoba histolytica is a protozoan parasite that infects over 50 million people annually resulting in approximately 100,000 deaths. Ingested cysts cause diarrhea and colitis; infection may lead to extra-intestinal symptoms such as brain and liver abscesses (ALA - amebic liver abscess). Serological tests for *E. histolytica* can be used as a marker of current and past infection. Here, we evaluate an *E. histolytica* rapid test and ELISA; both capable of detecting anti-*E. histolytica* adherence lectin antibodies in human serum. The membrane-based rapid test utilizes immobilized rLecA, a recombinant fragment of adherence lectin, on the membrane and a soluble peroxidase-conjugated anti-human IgG detection antibody. Similarly, the ELISA uses immobilized rLecA and the same detection antibody. Specimens were collected and screened at the International Centre for Diarrheal Disease Research (Dhaka, Bangladesh) and the Bernhard Nocht Institute (Hamburg, Germany). Results were compared to physician assessment of ALA, RT-PCR, and/or two laboratory assays previously described in the reviewed literature as specific for identifying human anti-*E. histolytica* antibodies in serum ("laboratory assay"). The rapid test was evaluated using 186 serum specimens resulting in 96.9% sensitivity and 100% specificity. Of the 186 specimens, 88 indicated negative serum titers with the laboratory assay, while 43 specimens indicated positive serum titers. ALA- and PCR-confirmed positive patients comprised the remaining 55 specimens. The ELISA was evaluated using 140 specimens resulting in 95.7% sensitivity and 87.1% specificity. Of the 140 specimens, 70 indicated negative titers with the laboratory assay, while 30 specimens indicated positive serum titers. ALA- and PCR-confirmed patients comprised the remaining 40 specimens. Results indicate that the *E. histolytica* serum rapid test and ELISA correlate with established clinical diagnosis. The simple format of the rapid test and large scale screening capability of the ELISA make these assays ideal for field diagnostics and use in developing countries.

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BURDEN AND FACTORS ASSOCIATED WITH *GIARDIA* INFECTION IN INFANTS OF SOUTH INDIA

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Giardia intestinalis is a common gastrointestinal protozoan worldwide. Infection varies from asymptomatic episodes to acute diarrhea, and it may persist as chronic diarrhea leading to malnutrition and growth failure in early infancy. In this study, we estimated the incidence rates and investigated risk factors for *Giardia* infection and their influence on growth at one year of age in a birth cohort of children (N=340) from an urban slum community in Vellore, South India. Intensive bi-weekly field surveillance visits were made for collection of morbidity data, diarrheal and surveillance samples and monthly anthropometric measurements. There were 107 episodes of giardial infection in 60 children. Twelve of these children had *Giardia* associated diarrhea, 33 had only asymptomatic infection and 15 children had both. Children were more likely to have

asymptomatic infection than symptomatic infection (Ratio=2:1). The median age (IQR) for first *Giardia* diarrhea was 10 (7-11) months. The median age (IQR) for the first *Giardia* asymptomatic infection was 8 (7-10). Overall incidence of *Giardia* infection was 33.02 episodes /100 child years (95% CI=27.32-39.91), with symptomatic infections at11.42 episodes/100 child years (8.27-15.76), and asymptomatic *Giardia* with a rate of 21.60 episodes/100 child years (17.09-27.30). In Poisson regression model the factors significantly associated with *Giardia* infection were presence of one or more siblings (RR=2.82, 95% CI 1.78-4.84), lower socio economic status (1.51, 0.98-2.33) and wasting (weight-for-height Z score <2) (2.53, 1.71-3.76). There was no association between stunting (RR=0.86, 95% CI 0.54-1.39) and giardiasis. The overall incidence suggests a high burden of giardiasis during infancy in this community. There is also evidence of association between giardiasis and acute malnutrition, although there was no apparent effect on chronic malnutrition.

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EVALUATION OF A DIAGNOSTIC SCREENING ELISA FOR THE DETECTION OF *GIARDIA* SPP., *CRYPTOSPORIDIUM* SPP. AND *ENTAMOEBA HISTOLYTICA* IN HUMAN FECAL SAMPLES

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The *TRI-COMBO PARASITE SCREEN* test is a diagnostic ELISA for the detection of *Giardia* spp, *Cryptosporidium* spp. and *Entamoeba histolytica* in human fecal specimens to aid in diagnosis of giardiasis, cryptosporidiosis and/or amebiasis. Identification of these parasites, the three most common enteric protozoan parasites worldwide, often involves microscopy which is labor-intensive, time-consuming and requires advanced training. Here we report results of a clinical evaluation of the *TRI-COMBO PARASITE SCREEN* test, a qualitative ELISA which offers a simple, highly sensitive and specific method of screening fecal specimens to identify those specimens positive for one or more of these parasites, eliminating the need for expensive microscopy methods on the majority of specimens. Positive results are indicated by the presence of a yellow color in the wells that can be interpreted visually or analyzed spectrophotometrically. A positive result indicates the presence of cysts or antigen from *Giardia* spp., *Cryptosporidium* spp., and/or *E. histolytica*. Clinical evaluations are currently underway for this test in order to seek FDA clearance. Fecal specimens are being tested on the *TRI-COMBO* test and individual commercial ELISAs specific for *Giardia* spp., *Cryptosporidium* spp. and *E. histolytica*. 75 specimens have been tested thus far in this study (35 positive for one or more parasites and 40 negative for all three parasites), and the sensitivity and specificity to date are 100%. The study goal is to test 250 specimens. Previous studies with the TRI-COMBO test have been conducted in our laboratory and in a birth cohort study at the ICDDR in Dhaka, Bangladesh. Results with 950 specimens demonstrated assay sensitivity and specificity in excess of 98% compared to individual ELISAs. The *TRI-COMBO* test can be used as a cost-effective screening assay to eliminate negative specimens and identify *Giardia*-positive, *Cryptosporidium*-positive and *E. histolytica*-positive specimens requiring further parasitological analysis.

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DETECTION AND GENOTYPING OF *CRYPTOSPORIDIUM* AND *GIARDIA* SPECIES IN PUBLIC PLACES IN SOUTHEASTERN OHIO

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Cryptosporidiosis and giardiasis are one of the most common causes of protozoal diarrhoea worldwide, and cause of significant morbidity and mortality in both the developing and developed world. The aim of this study is to investigate contamination of public places by *Cryptosporidium* and *Giardia* species. A total of 170 soil samples were collected from four school playgrounds and two public parks in Zanesville OH in 2008 and 2009. These samples were screened for *Cryptosporidium* and *Giardia* species cysts using a modified direct immunofluorescence assay. *Cryptosporidium* species oocysts were seen in 15 samples (9%) while 7 samples (4%) were positive for *Giardia* species cysts. Several molecular markers are being used for genotyping of these samples. Our results underlie the significance of public places in the transmission of these emerging protozoan infections and will add to our understanding of the contributions of humans and other reservoirs of infection to the epidemiology and risk assessment of the transmission of these diseases.

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MAPPING THE 2010 CHOLERA EPIDEMIC IN THE FAR NORTH REGION OF CAMEROON

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Cholera is caused by consuming water and food contaminated by *Vibrio cholera* and often spreads as a result of poor sanitation and hygiene. Cholera has long been, and continues to be, a world health issue. In 2010, a major cholera outbreak occurred in Cameroon where a total of 10,759 cases with 657 deaths were reported. The Far North Region of Cameroon was the epicenter of the outbreak during which almost 9,500 cases with 600 deaths were reported, the most severe outbreaks of cholera during the last fifteen years. This study seeks to understand the spatial and temporal dynamics of the 2010 epidemic in the Far North Region. A Geographic Information System (GIS) and spatial statistics are used to map and explore the spatial and temporal pattern of reported cases in 2010. The results show that the attack rate varied from 4.2 per 100 000 inhabitants in Kaélé health district to 1293.1 per 100 000 inhabitants in Kolofata health district, while the case fatality rate varied from zero in Bourha health district to 66.7 % in Mindif health district. We found that the epidemic broke out with the first rainfall in early May. The epidemic developed mainly during the months of high rainfall, in particular August, that had a monthly mean of 300 mm and 14 days of rain. The epidemic ended with the end of the rainy season in December. With analysis of additional years of data, positive correlation could be further understood between the attack rate and the rainy season. This study reveals that almost all the affected areas lacked access to a good potable water supply.

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HAS IMPROVED WATER AND SANITATION CHANGED THE PREVALENCE OF SCHISTOSOMIASIS AND SOIL TRANSMITTED HELMINTHS (STH) AMONGST PRIMARY SCHOOL AGED CHILDREN IN UGU DISTRICT OF KWAZULU - NATAL

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In South Africa, it is estimated that 2.5 million people are infected with schistosomiasis and a much larger but unknown number with geohelminths, as reported previously. This study is to be conducted in the south coast region of Ugu district in female primary school aged children from schools that are located in rural and semi-rural areas below 3000 meters attitude from sea level. Two common soil transmitted helminths are targeted i.e. *Ascaris lumbricoides* and *Trichuris trichiura* as well as *Schistosomiasis haematobium* (urinary bilharzia). Schools will be selected

randomly from across the region and consent will be obtained from parents and assent from young girls aged 10-12 years. Three urines and one stool sample will be collected per participating individual at the school after demographic information collected from each subject investigated. Samples will be safely transported to the laboratory for microscopic quality controlled analysis by the study PI hence prevalence and intensity will be determined and also whether improved delivery of basic resources to the community has impacted the prevalence and intensity of the targeted parasites. The findings from the study will also be compared with published findings from the piloted study of Parasite Control Programme which was designed in 1997 and targeted three common STHs i.e. *Ascariasis lumbricoides*, *Trichuris trichiura* and hookworm as well as urinary Schistosomiasis haematobium.

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BARRIERS AND MOTIVATORS FOR HANDWASHING AMONG MOTHERS OF NEONATES IN RURAL BANGLADESH

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Infections are important causes of neonatal mortality in Bangladesh. One study observed that maternal handwashing was associated with reduced neonatal mortality. To design a maternal handwashing intervention, we conducted a qualitative study to identify barriers and motivators to handwashing among primiparous mothers of neonates and infants in Matlab, a rural area of Bangladesh. We observed 20 mothers of neonates to understand the contextual factors that facilitated or hampered their handwashing behavior. We conducted indepth interviews with 32 mothers of neonates and infants to explore perceptions, beliefs, and practices related to handwashing. Mothers perceived the need to wash hands with or without soap before eating, or before feeding a child by hand. Mothers reported that elders advised new mothers to wash hands if eating after breastfeeding; mothers believed their child could die if they did not wash their hands after breastfeeding and then ingested their own breastmilk from their hands. Although mothers expressed the importance of washing hands before holding a baby to prevent skin problems and diarrhea, we only occasionally observed them to wash hands before holding their own baby. They prioritized using soap if there was any visible dirt or feces. Otherwise, washing hands with water alone was deemed sufficient. Mothers perceived that infrequent handwashing is a social norm in this rural area. The new responsibilities of nurturing a newborn, who eats and defecates frequently, was cited by mothers as a barrier to washing hands, often because they simply forgot. Sometimes mothers avoided handwashing because they believed neonates could catch a cold if mothers frequently touched water. Reinforcement by family members during the neonatal period and perceived risk of getting diarrheal illness were cited as important motivators for washing hands. Reminders from family members and risk perception are important motivators to maternal handwashing behavior during the neonatal period in rural Bangladesh. Enhancing external cues, by engaging family members and providing visual reminders of critical times for handwashing, leveraging existing cultural beliefs, and clarifying neonatal health threats, may improve maternal handwashing behavior in the neonatal period.

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ISOLATION OF *ESCHERICHIA COLI* 0157:H7 AND OTHER AEROBIC PATHOGENS FROM HAWKED FOODS IN EKPOMA, EDO STATE, NIGERIA

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Food-borne diseases present public health challenges related to food-handling practices. Prompt and thorough laboratory evaluation of cases and suspected foods is essential. This study therefore designed to determine the laboratory evaluation of pathogens from Hawked Foods in Ekpoma, Nigeria This experimental study was carried out at the diagnostic and research laboratory, Ambrose Alli University, Ekpoma. A total of 50 samples were collected randomly from hawked edible foods from different locations in Ekpoma area. The samples were cultured on Eosin Methylene Blue and Sorbitol MacConkey Agar as well as selenite F-Broth, Nutrient Broth and Deoxycholate Citrate Agar. Plates were incubated overnight at 37°C, and bacterial isolates were identified based on Morphological, biochemical and serological characteristics. The samples obtained were placed in a clean polythene bag and immediately transferred to the laboratory and inoculated into appropriate media Glasswares were sterilized in a hot air oven at 160° C for 1 hour before use. Out of 50 samples, 36 (72%) shown a bacterial growth and 14 (28%) shown no bacteria growth. Of this 36 samples showing bacterial growth after 24 hours incubation, 1 (2.8%) yielded *E. coli* 0157:H7, while other strains of *E. coli* accounted for 11 (30.6%), *Klebsiella aerogenes*, 4 (11.1%), *Salmonella* sp, 5 (13.9%), *Proteus* sp, 6 (16.7%), *Shigella* sp, 4 (11.1%), Coliforms 4 (11.1%) and 1(2.8%) yield *Staphylococcus aureus*. The bacterial isolates revealed low prevalence of *E.coli* 0157:H7 in hawked food samples (2.8%) in comparism with other enteropathogens *E. coli* strains (30.6%)), *Klebsiella aerogenes* (11.1%), *Salmonena* sp, (13.9%), *Proteus* sp (16.7%), *Shigella* sp (11.1%), *Staphylococcus aureus* (2.8%) isolated (P<0.05) The study confirmed the presence of *E. coli* 0157:H7 among other enteropathogens isolated from hawked foods items in Ekpoma, Nigeria. Burns, carrot, peas and meat had the highest frequency of isolated organisms while cassava, egg roll, and locus beans has low frequency.

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PERSISTENT OPEN DEFECATION IN BANGLADESH COMMUNITIES DESPITE HIGH PROPORTION OF HOUSEHOLDS WITH LATRINES

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Defecation in an open environment is a risk factor for diarrheal disease. Interventions aimed at reducing environmental fecal contamination prioritize latrine coverage. We analyzed baseline data from a rural population to assess the relationship between the presence of human feces in the environment and access to a latrine. Field workers conducted a cross sectional survey and environmental spot check to collect data on latrine ownership, sanitation practices and observations of visible feces in a sample of 1,431 households in 506 compounds in rural Bangladesh. Even though no latrine facility was reported for 25 (2%) households, human feces were observed inside 174 (12%) households; within 338 (24%) courtyards in the compounds, which serve as a social gathering place for household members; and in 658 (46%) areas surrounding the compounds. Latrines were privately owned in 662 (47%) households. Among these, 81 (12%) had visible feces in the household and 147 (22%) in their courtyards. There was a latrine available for use in 235 (97%) households that had visible feces in the courtyard. Whether or not the family owned or only shared a latrine, they were equally likely to have visible feces at both household and courtyard levels. Thirty two percent of fathers in households who owned a latrine reported not always using the latrine. Visible feces at the compound level was seen less commonly among households with a latrine with indications of regular use, such as a well worn path (OR: 0.53, 95% CI: 0.34-0.71). When asked about the last site of defecation for a child < 3, 69% of mothers reported that they defecated in the courtyard and 91% of these feces were disposed in bushes, drains or left in the open. Mothers from compounds where we observed visible feces reported more often that their children > 5 sometimes used the latrine compared with compounds where mothers reported their children > 5 always used the latrine (OR: 6.1, 95% CI: 3.9-

9.5). Fecal contamination of the rural household environment remains common despite the presence of latrines. Further research to explore why people do not use their latrines could help to develop interventions to encourage consistent use of available latrines for defecation and sanitary child feces disposal to reduce environmental contamination.

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A NEW STRATEGY OF EMERGENCY TREATMENT FOR *SCHISTOSOMA JAPONICUM*-INFESTED WATER

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The purpose of the present study was to investigate the effect of suspension concentrate of niclosamide (SCN) on killing cercaria of *Schistosoma japonicum* that floats on water surface, and its toxicity to fish, so as to establish an emergency-treatment intervention for rapidly killing cercaria and eliminate water infectivity. SCN was formulated into different concentrations of solutions, and then were sprayed on surface of the *S. japonicum* cercaria-infested water. The water infectivity was determined using mice at 0, 10, 30 min after spraying. SCN was formulated into a solution of 100 mg/L, and then were sprayed on surface of the water with niclosamide dosages of 0.01, 0.02, 0.03 and 0.04 g/m2. At 30 and 60 min after spraying, the water infectivity was determined using mice. Zebra fish were transferred into the static water, then 100 mg/L SCN, with niclosamide dosages of 0.01, 0.02, 0.03 and 0.04 g/m^2, were sprayed on water surface. At 0, 10, 30, 60 min after spraying, water samples were collected at water depths of 0, 10, 20, 30, 40 cm, and niclosamide was determined using high-performance liquid chromatography. And the death of zebra fish was continually observed for 96 h after spraying SCN. At 0, 10, 30 min after spraying 1 000, 100, 10, 1, 0.1 mg/L SCN on water surface, the infectivity of water all significantly decreased. Among them, at 30 min after spraying 1 000 mg/L and 100 mg/L SCN, no *S. japonicum* infectivity was detected in water. At 30 min after spraying 100 mg/L SCN, with niclosamide dosages of 0.01, 0.02, 0.03, 0.04 g/m^2, the water infectivity reduced significantly, and no infectivity was found at 60 min after spraying SCN. The surface of static water was sprayed with 100 mg/L SCN, the peak concentration was found at 0 min, and the solution diffused to site with a water depth of 10 cm after 10 min. 30 min later, SCN diffused to the whole water body, and distributed evenly. After spraying 100 mg/L SCN on surface of water with a volume of(3.14×202×50)cm^3, with niclosamide dosage of 0.02 g/m2, 96 h later, no death of zebra fish was found. It is concluded that spraying 100 mg/L SCN, with niclosamide dosage of 0.02 g/m^2 on surface of *S. japonicum*-infested water, the water infectivity can be eliminated after 30-60 min, and there is no evident toxicity to fish. This cercaria-killing method, as an emergency-treatment intervention for infested water, can be applied in those surveillance and forecast system for schistosomiasis.

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BOILING DRINKING WATER IN PERI-URBAN ZAMBIA: A COSTLY AND INEFFECTIVE APPROACH TO IMPROVE MICROBIOLOGICAL QUALITY

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Unsafe drinking water is a leading cause of preventable diarrheal disease, particularly among children in developing countries. Waterborne pathogens contribute to an estimated 4 billion cases and 2.5 million deaths from diarrheal disease each year. It is estimated that almost 900 million people lack access to improved drinking water worldwide; over 5 million of those live in Zambia. For those without access to reticulated water supplies, boiling is the most common method of disinfecting water in the home and the benchmark against which other point-of-use water treatment is compared. In a five-week study in peri-urban Zambia, we assessed the microbiological effectiveness and cost of boiling among 49 households without a water connection who reported "always" or "almost always" boiling their water before drinking it. Source and household drinking water samples were compared weekly for thermotolerant coliforms (TTC), an indicator of fecal contamination. Demographics, costs and other information were collected through surveys and structured observations. Drinking water samples (geometric mean 7.2 TTC/100ml, 95%CI 5.4-9.7) were actually worse in microbiological quality than source water (geometric mean 4.0 TTC/100ml, 95%CI 3.1-5.1) ($p<0.001$), although both are relatively low levels of contamination. Evidence suggests that water quality deteriorated after boiling due to lack of residual protection and unsafe storage and handling. We found that using a drinking cup to transfer freshly boiled water into the storage container was strongly associated with a decline in drinking water quality ($p<0.01$). The constructed cost of solid fuel or electricity used for boiling was estimated to represent a median 5-7% of income. In this setting where microbiological water quality was relatively good at the source, safe-storage practices that minimize recontamination may be more effective in managing the risk of disease from drinking water at a fraction of the cost of boiling.

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MICROBIOLOGICAL EFFECTIVENESS OF TREATING AND SAFELY STORING SHALLOW TUBE WELL WATER IN RURAL BANGLADESH: A PILOT STUDY

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Chlorine treatment and safe storage can improve microbiological quality of drinking water and reduce diarrhea. In Bangladesh, where the population mostly uses tubewells, the effectiveness of chlorine may be compromised by groundwater constituents that exert chlorine demand, leading to low chlorine residual. Moreover, fecal contamination in tubewells is sporadic and at moderate levels, suggesting safe storage to prevent contamination at the point-of-use may suffice to ensure microbiologically safe drinking water. We conducted a pilot study in rural Bangladesh to assess the microbiological field effectiveness of sodium dichloroisocyanurate (NaDCC) tablets and two types of storage containers. We enrolled 80 households using tubewell water with no self-reported presence of iron, as chlorine demand by iron adversely affects disinfection. Half of the households were randomized to receive a 10-liter jerry can or storage jar with lid and tap. In addition, half were randomized to receive NaDCC tablets and the rest received no water treatment. We assessed the microbiological quality of tubewell and stored water by H2S testing for all 80 households six weeks after we distributed the products, as well as by membrane filtration for Escherichia coli in 24 households three and six weeks after product distribution. At the same two time points, we measured free chlorine in stored water by digital colorimeter in the 40 households receiving tablets. Of 24 tubewells tested by membrane filtration, E. coli in moderate concentrations (range of 1 to 31 CFU/100 mL) was detected in 12.5% and 25% of the wells at the two subsequent testing points. H2S test was positive in 30% of 80 wells. Among households not receiving tablets, 7% of jerry can samples and 19% of storage jar samples coming from uncontaminated tubewells had a positive H2S test, suggesting within-household contamination. H2S testing showed no contamination in stored water treated with chlorine. Free chlorine was above the CDC recommendation of 0.2 mg/L in 85% of samples after three weeks and 90% after six weeks. NaDCC tablets provided sufficient chlorine residual and improved microbiological water quality. Chlorine treatment coupled

with safe storage was more effective at reducing contamination than safe storage alone, while of the two containers, the jerry can was less frequently contaminated.

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IMPACT OF A SAFE WATER AND HYGIENE PROGRAM IN RURAL HEALTH FACILITIES, ZAMBIA, 2010

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In developing countries, many health facilities lack access to safe water for drinking and handwashing, putting patients and health workers at risk for health facility-acquired infections. In Zambia, where only 48% of the rural population has access to improved water supplies, we installed handwashing and drinking water stations (40-liter plastic buckets with spigots and lids, metal stands, chlorine solution, and soap) in eight rural health facilities, conducted training in water treatment and storage, and hand washing, and evaluated the impact on health worker and patient knowledge and practices. In February 2010, we conducted baseline surveys of health worker and patient knowledge and practices regarding handwashing, safe water storage, and water treatment in eight health facilities, tested stored water in clinics and households for residual chlorine as an objective measure of water treatment, and observed hand washing technique among patients. In March 2010, we installed handwashing and drinking water stations in the health facilities and trained health facility staff. In July 2010, we conducted a follow-up evaluation using the baseline survey instruments. Chlorination of stored water was observed in 0 clinics at baseline and four (50%) at follow-up; seven (88%) clinics were using the installed water stations at follow-up. Compared to baseline, a higher percentage of patients at follow-up used improved water storage containers at home (24% vs. 61% [p<0.001]), had detectable residual chlorine in stored water (3% vs. 15%, p=0.025), and demonstrated correct handwashing procedure (42% vs. 65% [p=0.016]). Installation of water stations combined with training in rural Zambian clinics resulted in improved water treatment and storage practices in health clinics and patients, and increased ability to demonstrate proper handwashing technique among patients. This intervention has now expanded to 150 additional health facilities in Zambia.

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RESPONDING TO AN OUTBREAK OF TYPHOID FEVER: ASSESSMENT OF WATER, SANITATION, AND HYGIENE INTERVENTIONS IN NENO DISTRICT, MALAWI

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Typhoid fever, caused by *Salmonella enterica* serovar Typhi, results in an estimated 21 million cases and 200,000 deaths annually worldwide. On May 2, 2009 an outbreak of typhoid fever began in rural villages along the Malawi-Mozambique border. Despite numerous interventions, including distribution of WaterGuard (WG) for in-home water treatment, cases of typhoid fever continued, resulting in 748 illnesses and 44 deaths by September 2010. To better understand knowledge, attitudes, and practices surrounding typhoid fever, safe water, and hygiene, and to inform future interventions, a survey was administered in September, 2010, to female heads of randomly selected households in 17 villages in Neno District, Malawi and stored household drinking water was tested for free residual chlorine. Among 202 households, primary sources of drinking water were boreholes (48%), unimproved wells (46%) and rivers (4%). Households who previously attended a community-wide typhoid talk were more likely to report that typhoid fever is caused by poor hygiene and (85% vs. 64%, P = <0.01) drinking unsafe water (55% vs. 42%, P = 0.02) compared to households not attending a talk. WG was present in 53% of households; only 33% of those households reported treatment of currently stored water. Residual free chlorine levels were adequate in stored water samples from only 15% of all households surveyed, but were adequate in 63% of households that reported water treatment and had WG present. Seventy-seven percent reported soap in the home, but only 46% reported use of soap for hand washing. Knowledge regarding the association between unsafe drinking water and typhoid fever improved after community-wide education, but remains low. Despite the presence of WG in over half of all households, less than one-third were using this method to protect their drinking water. Therefore, many households remain without safe drinking water. Future interventions should focus on increasing use of WG and improving hand washing and hygiene practices to prevent waterborne illnesses, including typhoid fever.

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DETECTION OF *ANAPLASMA PHAGOCYTOPHILUM* INFECTIONS: A CASE SERIES FROM A SUBURBAN COMMUNITY HOSPITAL IN MASSACHUSETTS

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Human granulocytic anaplasmosis (HGA) can range in presentation from subclinical disease to severe febrile illness and death. We examined medical records for patients diagnosed with HGA at Newton-Wellesley Hospital in 2009, and compared rates of infection to confirmed cases of HGA in Massachusetts. Epidemiologic case confirmation requires both laboratory confirmation of disease and a corresponding clinical history obtained from a clinician or the patient. A PCR tick-associated pathogen panel that included assays for *Anaplasma phagocytophilum, Ehrlichia chaffeensis, E. ewingii*, *Borrelia burgdorferi* and *Babesia microti* was used for laboratory diagnosis. Thirty-three cases of *A. phagocytophilum* were confirmed during 2009 at our hospital. Thrombocytopenia and/or leukopenia were observed at the time of presentation in 30/33 (91%) patients, and 28/33 (85%) reported fever. Cases were geographically distributed diffusely throughout the hospital catchment area in 21 zip code regions, with the exception of one cluster of seven cases in a single zip code area. Because few clinicians indicated suspicion for non-Lyme tick-borne disease, the use of a tick panel that includes PCR testing for several organisms could improve disease detection of underrecognized tick-borne diseases. In Massachusetts, the Department of Public Health estimates of confirmed HGA cases demonstrate a steady increase since 2005 (23 cases compared to 61 in 2010). Of the 33 cases our hospital reported to the MDPH in 2009, only 20 (62%) were confirmed due to a lack of accompanying clinical data. Statewide in 2009, only 61/146 (42%) of reported PCR positive HGA results became confirmed cases, primarily due to absence of reporting clinical data to the MDPH. Lack of communication between clinicians and public health authorities, in addition to scarce resources for epidemiologic follow up, prevent accurate case reporting and assessment of the true HGA burden.

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IMPACT OF CONFIRMED INVASIVE BACTERIAL INFECTION IN THE CHILDREN MORTALITY IN PEDIATRIC CHU-GABRIEL TOURÉ

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The new tendencies and characteristic of children mortality are function of the sanitary arrangements, environmental and socio-economic conditions which prevail in a population. In the world, the frequency of various pathologies varies according to countries. It's in developing countries such as African's that the high probability of death is notified. Invasive bacterial infections are responsible of serious pathologies such as meningitis, bronchopneumonia, septicemias, peritonitis, typhoid fevers and especially diarrheas which constitute a handicap for the infantile population in developing countries. To study the etiologies of children mortality, systematic blood smears for malaria parasites were integrated into ongoing hospital-based surveillance for invasive bacterial infection at CHU Gabriel Touré in Bamako, Mali. Children aged 0-15 years with fever ≥ 39° C or suspicion of invasive bacterial infection (SIBI) admitted to HGT were eligible. Blood and relevant body fluids were collected and cultured after obtaining informed consent. Blood smears for malaria were performed. Cases are define as children included in the study and died during follow up. From January 2008 to December 2010, 15278 children were included. Among this, 1169 died (7, 65%). Pathogens have been isolated in 433 samples among whom 270 (62%) was invasive bacterial infection and 163 (38%)was malaria. The mean age was 30,51 ±40,36. The most frequent pathogens in blood culture was *Streptococcus pneumoniae* (80/232), *Escherichia coli* (22/232), *Staphylococcus aureus* (23/232). Children less than 1 year was the most affected (115/232, $p = 10^{-3}$). In Cerebrospinal fluid, the isolated pathogens were respectively: *S. pneumoniae* (61/101), *Hæmophilus influenzae* b (11/101) and *Neisseria meningitidis* (10/101). No significant differences have been observed between the three years but we noticed two peaks in April and October every year. In conclusion, children less than 1 year are highly affected by childhood mortality. In most of the case any pathogen is incriminated. Gram negative bacilli and S. pneumoniae are responsible of the greatest rate of mortality. Efforts should be done to improve the identification and prevention of pathogens responsible of child mortality.

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A NEW APPROACH TO THE MANAGEMENT OF SEVERE ANAEMIA IN *PLASMODIUM FALCIPARUM* INFECTION

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Rupture of invaded Red blood cells as they release merozoites into the blood circulation, is a cause of the anaemia in *Plasmodium falciparum* Infection. There appears to be yet another and, perhaps, more serious mechanism that contributes to the severe anaemia seen in *P. falciparum* infection. Some patients on blood transfusion (whole blood or packed cells) for severe anaemia in *P. falciparum* infection have been observed to return to square one (became pale again) within 24 to 72 hours of such transfusion. Giving more blood never changed the situation as they always returned to square one. The issue of jaundice seen in some of these cases tends to start when the spleen began to enlarge and did not correspond to the degree of anaemia as it was usually mild. In some of these patients, there was no jaundice, the level of bilirubin in the blood was normal and there was no urobilirubin in the urine. Perhaps the severe anaemia in *P. falciparum* infection is due to a phenomenon of massive pooling of un-invaded red blood cells from the peripheral circulation into some capillary beds ?the liver and/or the intestine. This may be an auto-protective mechanism to prevent these cells from being invaded by the *P. falciparum* merocites as they are released into the circulation from the liver. The anaemia in all these cases of severe anaemia that returned to square one after blood transfusion was corrected by adequately treating the malaria and reversing the Auto-Protective massive pooling of un-invaded red blood cells from the peripheral circulation, without further blood transfusion. Perhaps the solution to the management of severe anaemia in *P. falciparum* Infection is not Blood Transfusion but adequate treatment of the malaria fever {total clearance of malaria parasites} and the reversal of the auto-protective massive pooling of un-invaded red blood cells from the peripheral circulation phenomenon.

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HELMINTH ROLE IN LOWERING THE ATHEROSCLEROSIS RISK FACTORS: EVIDENCE IN A POPULATION AT SECONDARY EPIDEMIOLOGICAL TRANSITION

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Atherosclerosis is characterized by chronic local inflammation of the vascular wall, involving both innate and adaptive arms of immunity and is largely due to combination of inflammation and dyslipidemia. It is known that chronic helminth infections are associated with lower nutritional status and anti-inflammatory response and a reduction of helminth burden might therefore play a role in the development of cardiovascular disease in developed societies. We aim to investigate the association of helminthes and atherosclerosis in a population in transition. In Flores, Indonesia, an area highly endemic for geohelminthes, a cross-sectional study was performed in 1040 participants. Stool samples were collected and tested for *Ascaris lumbricoides* and *Necator americanus* by PCR and *Trichuris trichiura* by microscopy. A subset of 528 adults aged 40-80 (male/female) was included for intima media thickness (IMT) measurement. In addition we also measured information on atherosclerosis risk factors such as blood pressure, body mass index (BMI), waist hip ratio (WHR), lipid level, blood glucose (FBG), CRP and whole blood stimulated cytokine production. The classical cardiovascular risk factors such as WHR, BP, FBG, CRP, triglyceride, and ratio of TC/HDL-c were positively associated with IMT while innate stimulation of IL-10 by LPS were negatively associated with IMT. Helminth infection was negatively associated with BMI, WHR, lipid levels, blood glucose, and CRP, but we found no direct association of helminths and IMT. The atherosclerosis is a process that progresses over a lifetime. We have shown that current helminth infections were not directly reducing the risk of atherosclerosis, but were involved in lowering its risk factors. Therefore, we conclude that a reduction of helminth burden might play a role in the rise of cardiovascular disease in developed societies.

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DESCRIPTIVE STUDY OF IRON BIOMARKERS IN ETHIOPIAN VISCERAL LEISHMANIASIS PATIENTS

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Visceral leishmaniasis (VL) is a neglected systemic parasitic disease caused by the *Leishmania donovani* complex species. It commonly affects poor populations in the tropics and sub-tropical endemic areas, causing 500,000 morbidities and more than 50,000 deaths annually. Although anaemia is a common sequel of VL, use of iron status biomarkers in

these patients is not well studied. This study was undertaken to describe the clinical characteristics, and changes in iron status biomarkers (ferritin, sTfR, and hepcidin) at admission and during a month following commencement of anti-leishmanial treatment in newly diagnosed VL patients. A prospective longitudinal descriptive study was conducted in a newly diagnosed, HIV negative VL patients admitted to Arba Minch Hospital-Leishmaniasis Research and Treatment Centre, South-West Ethiopia, between April and May 2010. A total of 20 confirmed VL cases, 2 female and 18 male, with a median age of 18 years were included in the study. While fever was the initial presenting symptom, with mean duration of 4.4±3.7 months, 6 (30%) patients had no measurable fever despite repeated follow-ups. Splenomegaly was present in all patients with 12 (60%) of them being malnourished. Pancytopenia was a common hematologic manifestation. The Mean±SD of haemoglobin at admission was 7.2± 1.99g/dl with 9(45%) of patients being iron deficient (ID). Ferritin was elevated at baseline, 1373.13±1191.19µg/l, which significantly decreased following anti-leishmanial treatment. sTfR was increased in ID patients; and serum hepcidin concentration was higher in non-ID (NID) patients. Significant correlation ($p<0.05$) observed between sTfR and haemoglobin, hepcidin and ferritin, ferritin and body mass index, and sTfR and sTfR-F index. With treatment, significant improvement was observed in both clinical and laboratory parameters. In conclusion, TfR-F index was a useful biomarker in differentiating ID and NID patients. Iron deficiency contributed to development of anaemia in about half of the patients. A future study is recommended to evaluate utility of serum sTfR and hepcidin against bone marrow staining for iron, and consideration of iron intervention efficacy in ID patients

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UTILIZATION OF ARTEMISININ-BASED COMBINATION THERAPY AMONG CHILDREN UNDER FIVE YEARS OF AGE WITH SUSPECTED MALARIA IN JINJA DISTRICT, UGANDA

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In 2004, the Ugandan Ministry of Health (MoH) adopted artemisinin-based combination therapy (ACTs) for treatment of uncomplicated malaria. Artemether-lumefantrine (AL) and artesunate-amodiaquine were recommended as first line and alternative treatment, respectively. However, policy implementation has been slowly translated into practice. This study sought to establish the proportion of children under five with suspected malaria treated with ACT and factors associated with ACT use in Jinja district, a peri-urban area with mesoendemic malaria transmission. Two-stage cluster sampling was used to identify eligible households. Caretakers (n=380) of children under five who had suspected malaria four weeks preceding the study date were interviewed by trained study personnel using pretested standardized questionnaires. Suspected malaria was defined as any illness in a child < 5 years of age perceived as malaria by the caretaker irrespective of whether this was confirmed or not. Antimalarials administered were determined by the caretakers' report. If an ACT was reported, the regimen was verified using sample drugs and packages. A child was considered to have received an ACT if they had received any ACT regimen, at any dose, for any duration. Out of 380 children studied, 207 (54.5%) had received an ACT; AL was the only ACT administered. There was significant association between utilization of AL and source of treatment (OR=16.35, 95% CI 9.00-29.71), caretaker's knowledge about first line treatment for uncomplicated malaria (OR=2.15, 95% CI 1.23-3.76) and whether the caretaker had heard about ACTs before (OR=3.07, 95% CI 1.40-6.70). Out of 207 children that had received AL, most (79.7%) had acquired it from public health facilities. Children who did not receive an ACT had most commonly visited drug shops (52.6%) and private clinics (27.2%). Out of 173 caretakers whose children had not received ACT, the reasons reported included: not prescribed by the health worker (49.7%), out-of-stock (17.3%), and unknown (16.2%). Only 2.9% of caretakers reported that AL was too expensive. ACT utilization among children with malaria in Jinja, Uganda is far below the national malaria control target of 85%. Source of treatment and caretaker knowledge about ACTs impact on the utilization of ACTs. There is need for subsidized ACTs to be made available through private sector, including drug shops, where many children access care but are not receiving ACTs.

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PANDEMIC H1N1 2009 SEVERITY IS ASSOCIATED WITH NK AND T CELLS COUNT AND DIFFERENTIATION STATUS

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An antigenically novel H1N1 virus was transmitted to humans from pigs in 2009. It is now known that while infection rates reached pandemic proportions, most cases were mild or asymptomatic. However, at least 50% of severe patients were previously healthy young adults. It is not clear whether this reflects intrinsic viral factors or host immunity. To investigate the contribution of the host immune response we compared lymphocyte counts and phenotypes during the course of acute H1N1 2009 infection in patients with mild and severe illness. Patients with pandemic H1N1 illness confirmed by RT-PCR were enrolled. Fresh peripheral blood samples were assessed by 6 colour flow cytometry using Trucount tubes with panels of monoclonal antibodies to determine absolute counts for lymphocyte subsets and the percentage of CD4, CD8 T cells expressing activation and differentiation markers. Patients were categorized as having severe or mild illness on the basis of clinical findings.62 patients were enrolled including 51 with mild and 11 with severe illness, of which 2 died. The age and sex of severe and mild patients was similar but severe patients admitted later and were hospitalized longer. Lymphopenia was more common in severe cases with CD4 lymphopenia in 60% of severe versus 15% of mild patients ($p=0.007$). CD4:CD8 ratios were also decreased. NK cell counts were lower in severe patients on all illness days and this was significant on day 7 ($p = 0.002$). B cells counts were similar. CD8 T cell activation and differentiation tended to be rapid in patient with mild illness but delayed and exacerbated in those with severe illness with accummulation of the CD27+CD28- subset.This study shows that pandemic H1N1 severity is associated with low NK and CD4 T cell counts. NK cells and other pre-existing effector mechanisms are likely to be important for preventing severe influenza because virus titers generally peak early after infection. Lymphopenia and inverted CD4:CD8 ratios are also common in highly pathogenic H5N1 infection indicating that lymphopenia is associated with severe illness irrespective of the virus strain. We suggest that CD4 lymphopenia and aberrant CD8 activation are consequences of excessive virus growth which can occur when infected with a highly virulent virus or with a low virulence virus when early immune responses are inadequate.

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IN-HOSPITAL PREVALENCE AND PROGNOSIS OF NEONATAL TETANUS IN MBUJIMAYI, DEMOCRATIC REPUBLIC OF CONGO

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Most of 1 million yearly deaths from tetanus occur in Sub-saharian Africa and 90% of them corcern newborns(WHO report).In our circle, 3 previous studies on neonatal tetanus were undertaken and reported the prevalence of 0.79% in 2003; 0.85% in 2005 and 2.10% in 2007.In

order to determine the prevalence of neonatal tetanus and epidemiologic-clinical factors that influence its evolutionary prognosis in term of mortality,a retrospective study based on analysis of hospital documents and spread out over 36 months was conducted last year(2010) in the Pediatric Departement of Bonzola Hospital at Mbujimayi and related to 114 tetanic newborns admitted during the period of 2007-2009. The prevalence of neonatal tetanus was 2.18% with about 3 new cases by month.Of overall 114 cases, 77 newborns(67.54%) died and the majority of them died in 1-3 days after admission.There was no significant correlation between sex and mortality(sex ratio was 1.5).At admission, 22 newborns(19.30%) had a tetanus of class II and 92(80.70%) a tetanus of class III according to the International Classification of Dakar.The majority of tetanic newborns(70.17%) came from the vicinity of Mbujimayi and all of them were born at home.Only 19 mothers (16.67%) respected calendar vaccination.The mortality rate was influenced by:absence of pregnent women's vaccination(83.33%);entry door of tetanus(umbilical in 82.46% of the cases);method of umbilical cord section(razor blade with 80.70% against pair of scissors with 12.42%) and the severity of the disease at admission(tetanus of class III with 80.70%).Neonatal tetanus is still a serious problem of Public Health in Mbujmayi,so that measures active on factors above-mentionned are required in order to improve this situation.

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DIFFERENT PHARMACOKINETIC APPROACHES WHEN ANALYSING ARTEMETHER AND DIHYDROARTEMISININ IN PREGNANT WOMEN WITH UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA IN UGANDA

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Pregnancy alters the pharmacokinetic (PK) properties of many antimalarial compounds what might result in lower drug exposure and increased risk of treatment failure. Therefore, the pharmacokinetic properties of antimalarial compounds need to be characterized in specific subpopulations. The objective of this study was to evaluate the PK properties of artemether (ARM) and its metabolite dihydroartemisinin (DHA) in pregnant women with uncomplicated *Plasmodium falciparum* malaria in Uganda. Non compartmental analysis (NCA) was performed using WinNonlin and compared to parameter estimates obtained by population PK modeling using NONMEM. A sequential and a simultaneous 1 compartment (CMT) ARM, 1 CMT DHA with 1st order absorption and elimination population PK model was used in the comparison. The simultaneous ARM-DHA model was further developed by the evaluation of different distribution, absorption, error and covariate models. A simultaneous model with sequential zero-order and transit-absorption and a 1 CMT distribution model for ARM and DHA provided the best fit to the data. Using NCA or sequential drug-metabolite modeling, the metabolite was assumed to be absorbed from the gut into the systemic circulation however this is mechanistically incorrect. In a simultaneous population PK drug-metabolite model the absorption and the *in vivo* conversion of the parent drug into the metabolite dictates the appearance rate of the metabolite. This enables an understanding and a PK interpretation of different drug exposure obtained in different populations. The different PK approaches resulted in large differences (5 to 7-fold) in the estimated apparent volume of distribution, which emphasize the need for using an adequate analysis approach. This has to be considered when comparing different methodologies used in available published literature. In conclusion, the population PK properties of ARM and DHA were described by a simultaneous model in pregnant women with malaria. Simultaneous population PK models should be used in the analysis of drug-metabolite data to be able to obtain parameter estimates that reflect physiological values.

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NEW VACCINATION DELIVERY REGIMEN DRIVES ENHANCED VACCINE-SPECIFIC IMMUNE RESPONSES

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The goal of vaccine delivery is to present vaccine immunogens/organisms in manner that enhances antigen presenting cell activation, uptake of antigen and processing. We focused on use of a gel slurry delivery method to drive pro-inflammatory, vaccine-specific responses with or without the use of CpGs as adjuvant. The gel slurry hardens at body temperature, forming a gel matrix depot that releases CpGs and antigen, attracting antigen presenting cells. Using recombinant hepatitis B antigen, we evaluated 8 different vaccine delivery schemes ability to induce antibodies and cytokines to recHepB ag. Mice were vaccinated with recHepB ag in two different gel slurrys, with Alhydrogel (Alum) or with Complete Freunds adjuvant (CFA). The slurry and alhydrogel delivery methods were +/- CpGs. Initial results showed mice vaccinated with either gel slurry plus CpGs had significantly higher vaccine-specific IgG2a 14 days after the prime, and IgA, IgM at 28 days post inoculation than mice vaccinated with alum or CFA. One gel slurry delivery drove significantly higher vaccine-specific IgG titers 14 days post-prime, than the other delivery methods did post-boost. Suggesting that the boost was unnecessary. Recall assays showed upregulated IL-10 and IL-4 from splenocytes of mice vaccinated with Alhydrogel or CFA compared to cells from gel-slurry + CpG vaccinated mice. CpG use reduced levels of IL-5 to background in all groups compared to elevated levels in CFA. No differences in levels of IFNγ or TNF were seen. Based on the data that showed a Th1 driven immune response after prime inoculation, we are currently assaying for T cells activation with flow cytometry at 28 days. The use of gel slurry plus CpGs, or other pro-inflammatory adjuvants to deliver vaccine antigens/organisms could have great utility for enhancing vaccines such as influenza, hepatitis A and B, as well as in development of vaccines for parasitic diseases. Ultimately, this type of vaccine delivery system may facilitate development of therapeutic vaccines as well as prophylactic.

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UNDERSTANDING THE CHALLENGES FOR ELIMINATING TUBERCULOSIS FROM TROPICAL COUNTRIES

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Tuberculosis (TB) poses a serious threat to public health in low-income tropical countries. Substantial variability in the response to antituberculous therapy and prolonged duration of infectivity in many of these patients provide challenges for elimination of tuberculosis in tropics. Within this background we conducted a hospital based descriptive study over the period of 2 years (Jan 2009 to Dec 2010) in the department of internal medicine at B.P Koirala Institute of Health Sciences, a university teaching hospital in eastern Nepal to characterize the factors responsible for prolonged infectivity in smear positive pulmonary tuberculosis patients. 150 consecutive patients with smear positive pulmonary tuberculosis were included in our study. Patients that remained smear positive even after 2 month of Antitubercular treatment were considered as having prolonged infectivity. All the socio-demographic predictors for tubercular infection, disease and affecting prolonged infectivity based upon clinical experience, published literature and biological plausibility were recorded and subjected to univariate and multivariate analysis for significance testing. Our study revealed striking dose-response relationship between tobacco smoking as well as exposure to indoor air pollution and prolonged infectivity. Past history of pulmonary tuberculosis, HIV seropositivity, diabetes, high bacteriological burden at treatment initiation and radiological abnormalities especially bilateral infiltrations on initial CXR were also independent predictor of prolonged infectivity. Progress towards

elimination of tuberculosis will remain elusive in tropical countries unless these critical issues are identified; understood and targeted interventions aiming them are formulated. This mandates further discussion of the current TB treatment, control and elimination strategies in tropical countries.

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INCIDENCE OF AND MORTALITY DUE TO SEPSIS AMONG CHILDREN OF 0-5 YEARS HOSPITALIZED AT DIPUMBA GENERAL HOSPITAL

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In order to understand and improve case management of pediatric sepsis, we explored the patterns of incidence and mortality due to sepsis in a pediatric population in Eastern Kasai province (Congo). We reviewed medical records of all 0-5 year children hospitalized at Dipumba General Hospital in Mbuji Mayi over a one-year period. To be included, a case had to be a 0-5 year old child hospitalized and then discharged any time between July 2009 and June 2010, with a documented positive sepsis diagnosis. We reviewed 1482 medical charts and collected data on diagnosis, gender, age, disease onset, admission timing and treatment outcomes. We found 469 cases of sepsis (31.6% incidence rate); these included 263 boys and 206 girls (1 to 1.3 sex ratio). There was no significant difference according to gender. However sepsis cases were significantly more frequent (65.03%) among toddlers (< 3 years) than among older children. We recorded 260 deaths (55,4%). This is significantly higher level of mortality, particularly among cases that were not more promptly brought to the hospital. There is a need to increase the survival rate of children affected with sepsis, notably by improving access to medical care.

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IMMUNOLOGICAL AND VIRAL DETERMINANTS OF DENGUE SEVERITY IN HOSPITALIZED ADULTS IN HANOI, VIETNAM

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Dengue is estimated to affect 50 million people each year and can occur as explosive outbreaks that overwhelm health systems. The emerging picture is that multiple factors including prior immunity, viral load, age of the patient and infecting serotype and genotype may contribute to the severity of dengue infection. This cross-sectional study examined these interactions in adults hospitalized with dengue in low transmission settings, to better identify factors associated with severity across serotype and immunity groups. Patients admitted to the National Hospital of Tropical Disease, Vietnam with a clinical diagnosis of dengue according to the WHO criteria. A patient is considered to have confirmed dengue if either RT-PCR or NS1 is positive, if there is an increase in the level of IgM detected by ELISA or an IgG ELISA conversion in the presence of a positive IgM ELISA. Patients were then categorized as having primary or secondary infection on the basis of serology results. 158 adult patients were enrolled with 130 (82%) laboratory-confirmed cases. Serology was indicative of secondary and primary infection in 61% and 34%, respectively. The infecting serotype was DENV-1 in 42 (32%), DENV-2 in 38 (30%) and unknown in 49 (38%). Secondary infection was significantly more common in DENV-2 patients (79%) compared to DENV-1 patients (36%, $p < 0.001$). This could reflect viral loads which were lower for DENV-2 than for DENV-1 infections but higher in secondary than primary infection. The time until NS1 and plasma viral RNA were undetectable was shorter for DENV-2 compared to DENV-1 ($p \leq 0.001$) and plasma viral RNA concentration on day 5 was higher for DENV-1 (p=0.03). Dengue is emerging as a major public health problem in Hanoi with high rates of primary infection compared to Southern Vietnam and other hyper-endemic regions. DENV-1 and DENV-2 were the prevalent serotypes with similar numbers and clinical presentation but secondary infection may be more common amongst DENV-2 than DENV-1, indicate an association between secondary infection and clinically overt DENV-2 infection. Our study also suggests that primary DENV-2 infections may be less virulent than DENV-1 during primary infection. The situation in Ha Noi provides an opportunity to further examine the roles of serotype and infection sequence in dengue severity and emergence.

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EPIDEMIOLOGICAL VIGILANCE OF *LEPTOSPIRA INTERROGANS* IN PORCINE FARMS THE MEDIO SINÚ IN THE DEPARTMENT OF C'ORDOBA (COLOMBIA)

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Leptospirosis is a re-emerging world wide zoonosis caused for pathogenic spirochetes of the genus *Leptospira*. This disease has been traditionally catalogued as an occupational disease; its presentation is related to a series of epidemiological factors that highlight the presence of animals as canine, rodents and other species of domestic animals or with that one coexists in porcine farms, being of importance the sanitary aspect, the quality of the water and the hygienic of porcine farms conditions. This study was undertaken to realize an epidemiological vigilance in farm workers, canine, porcine and water associated with pig farms in the department of Córdoba. By convenience sampling, in 18 farms, samples of canine and porcine serum and urine, farm worker serum and served and not served water were taken. MAT using 14 serovars of pathogenic *Leptospira* was carried out. Urine and water samples were cultivated in EMJH enriched with 1% rabbit serum. The isolations were confirmed for pathogenic strains by PCR. An inquiry was realized to register general, epidemiological information and pathological precedents. A seroprevalence of 46,66% in pigs, 37% in canine and 77,04% in human beings of these farms was established. Seven *Leptospira* pathogenic strains were isolated from 3 samples of pig urine, 2 samples of canine urine, 1 sample of served water and 1 sample of non served water. In one farm was isolated pathogenic *Leptospira* from samples of porks, canine and water. In conclusion, the maintenance-host role of porcine and canine was demonstrated by leptospiruria, the served waters as propagators of this etiologic agent, and its contribution to the permanency in the environment (non served waters), factors that contribute to the high prevalence of the disease in the workers. The absence of symptomatology, in the human population, compatible with a classic picture of Leptospirosis (jaundice, renal and hepatic failure) confirms that the zone is highly endemic, associated with asymptomatic infections or non-jaundice forms, characterized by general and unspecific symptoms.

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RELATIONSHIP BETWEEN BUILDING MATERIALS AND THE PRESENCE OF VECTORS IN CAJAMARCA, PERU

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We conducted a cross sectional entomological survey for Chagas disease vectors in five communities in the Cajamarca region of northern Peru. Intestinal contents of triatomines were examined for the presence of *Trypanosoma cruzi*. A total of 213 houses were searched for the triatomine vector for one person hour. Eighty-five of 213 household (39.9%) were found to be infested with triatomine insects; all insects were identified as *Panstrongylus herreri* (aka *P. lignarius*). In 38.5 % of infested households at least one insect was found to be carrying the parasite *T. cruzi*. Vector infestation was strongly associated with the housing materials, especially adobe(ODD ratio 5.37, and p-value<0.001) as well as the presence of guinea pigs. In contemporaneous cross-sectional serological surveys 83 of 529 (15.7%) people were found to be seropositive for *T. cruzi* infestation. Although it is possible that additional

vectors are involved in the transmission, the predominance of domiciliated *P. herreri* suggests that this vector is the mediating transmission resulting in some of the highest rates of *T. cruzi* infection in Peru.

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AGGREGATE ORGAN DYSFUNCTION PREDICTS IN-HOSPITAL MORTALITY FROM SEPSIS IN UGANDA

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Sepsis syndrome is not fully characterized in sub-Saharan Africa. We evaluated the association between severity of sepsis and in-hospital mortality in 150 patients with non-surgical sepsis at a regional referral hospital in Mbarara, Uganda. Patients were included if they were ≥ 18 years of age, admitted to the medical ward and had: 1) a suspected infection, and 2) ≥ 2 of the systemic inflammatory response syndrome (SIRS) criteria (temperature >38°C or <36°C; heart rate >90 beats/min; or respiratory rate >20 breaths/min). The patients were predominantly young men (63%) of Nkole ethnicity who were HIV-infected (74%). The mean (± SD) age was 35 ± 14 years. A majority of patients, 120 of 148 (81%), met 3 or 4 SIRS criteria. Sepsis, severe sepsis, and septic shock was diagnosed in 52 (35%), 71 (47%), and 27 (18%) of 150 patients respectively. Of the 98 patients with end-organ dysfunction, 47 (31%) had single organ dysfunction, 36 (24%) had 2 organ dysfunction, and 15 (10%) had 3 or 4 organ dysfunction. In-hospital mortality occurred in 45 of 150 (30%) enrolled patients and in 5 of 52 (9.6%) patients with sepsis, 24 of 71 (33.8%) patients with severe sepsis, and 16 of 27 (59.3%) patients with septic shock. In the multivariate analysis, the identification of severe sepsis (adjusted hazard ratio [AHR] 2.9, 95 % confidence interval [CI] 1.0-8.2, $p = 0.04$), septic shock (AHR 5.7, 95% CI 1.6-20.3, $p = 0.007$) and the dysfunction of 3 or more organs (AHR 2.9, 95% CI 1.1-7.3, $p = 0.03$) increased the risk of in-hospital mortality. Adding aggregate organ dysfunction to the multivariate equation that included sepsis category statistically significantly improved the model but the converse did not (change from previous step, $\chi^2 = 9.7$, $p = 0.008$ vs. $\chi^2 = 5.4$, $p = 0.07$). In conclusion, predictors of mortality were easily measurable and could be used to risk-stratify critically ill patients in resource constrained settings.

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DYNAMIC CHANGES OF 14-3-3ß IN PATIENTS AND MICE INFECTED WITH EOSINOPHILIC MENINGITIS CAUSED BY *ANGIOSTRONGYLUS CANTONENSIS*

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The 14-3-3β protein is a CSF marker of neuronal damage during the development of Creutzfeldt-Jakob disease. Increased 14-3-3β protein is also found in CSF from patients with a variety of neurological disorders. The goal of this study is to determine whether the levels of serum/CSF14-3-3β protein in patients with eosinophilic meningitis correlates with other CSF parameters and the patients clinical course. An in-house 14-3-3β ELISA was established to determine the dynamic changes of 14-3-3β expressions in mice and patients infected with *Angiostrongylus cantonensis.* In a cohort study among nine Thai laborers with eosinophilic meningitis from eating raw snails, we examined the CSF weekly while patients were hospitalized and followed up the serum for 6 months. Forty BALB/c mice were randomly allocated to five groups: control, D7, D14, D21, and D21+dex (10ug dexamethasone was given daily via intra-peritoneal route from D7 to D21). The mice in the infection groups were given 50 *A. cantonensis* infective larvae by oral inoculation on day zero and sacrificed on days 7, 14, and 21 post-infection (PI). In each group, serum and CSF were obtained for 14-3-3β concentrations measurement by in house ELISA. All of the nine patients with eosinophilic meningitis underwent a total of 23 lumbar punctures. The elevated 14-3-3β level was detected in the CSF of eight out of nine (81%) patients during initial hospitalization. After 2 weeks of treatment, all patients showed a declined level or clearance of 14-3-3β protein in the CSF. By developing an in-house ELISA for measurement of 14-3-3β protein, it was found that the serum 14-3-3β level was significantly increased in patients during initial visit. After treatment, the serum 14-3-3β level in meningitis patients rapidly returned to normal levels. There was a trend of correlation between initial CSF 14-3-3β level with pleocytosis or eosinophilia in the CSF of patients with eosinophilic meningitis (Spearman's correlation test, r = 0.6, *P = 0.089)*. In the mice infected with *A. cantonensis*, we identified that there was a significant increase in the CSF levels of 14-3-3β 3 weeks after infection (p=0.027) and steroid treatment could reduce the expression of 14-3-3β (p=0.023). The serum 14-3-3β levels were not changed with the infection. In conclusion,14-3-3β concentrations may constitute a useful marker for disease severity and follow up in patients and mice with eosinophilic meningitis caused by *A.cantonensis*.

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TRAVMIL: TRAVEL-RELATED INFECTIOUS DISEASE RISK ASSESSMENT, OUTCOMES AND PREVENTION STRATEGIES AMONG DEPARTMENT OF DEFENSE BENEFICIARIES

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Large prospective studies describing risk factors for and location specific frequencies of infectious diseases in travelers are needed to improve guidelines for preventive advice, vaccinations, self-treatment strategies and prophylactic medications. The TRAVMIL study applies a novel approach to prospectively describe the epidemiology of travel related infectious diseases in US Department of Defense personnel and their beneficiaries and evaluates compliance rates and effectiveness of risk reduction and self treatment strategies. The study focuses on traveler's diarrhea (TD), febrile illness, and influenza like illness (ILI), combining clinical and laboratory data. Adults and children seen at two naval hospital travel clinics have been enrolled since 2010. Clinical information is obtained by medical record extraction, pre- and post travel surveys and illness diaries completed during travel. Laboratory specimens obtained include paired pre- and post-travel blood samples for serologic and immunologic testing, self-collected stool samples smeared on Whatman FTA® cards during travel from participants with and without TD, self-collected finger stick blood samples transferred to Whatman FTA® cards from those who develop a febrile illness during travel and oropharyngeal swabs from those who develop an ILI during travel. Serologic testing for pathogens associated with TD and febrile illness is performed on pre- and post-travel serum samples. Polymerase Chain Reaction (PCR) assays for viral pathogens are performed on oropharyngeal swabs. PCR is employed to detect *Plasmodium* sp., Dengue virus, *Leptospira* sp., chikungunya, and *Rickettsia* from blood blots. A multiplex PCR test to detect common diarrheal pathogens (*Salmonella*, *Shigella*, *Campylobacter*, Enterotoxigenic *E. coli,* Enteroaggregative *E. coli*, *Giardia*, *Cyclospora*, *Cryptosporidium*, Norovirus) in stool smears is under development. Preliminary data regarding assay development and frequency of infectious diseases among travelers will be presented.

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ANTIMALARIAL PRESCRIBING PRACTICES AMONG INFECTIOUS DISEASES PHYSICIANS AT A SINGLE DOD TRAVELER'S HEALTH CLINIC

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Factors associated with antimalarial prescribing practices among physicians are not well described. We performed a retrospective chart review to examine antimalarial prescribing practices among four infectious disease physicians at a military traveler's health clinic between April 2007 and October 2009. A total of 1,052 travel clinic visits were evaluated. Adults aged 18-65 accounted for 65% of travelers. Twelve percent of patients were < 12 years old. The most common regional destinations were Africa (29%), Southeast Asia/Pacific (21%), Central America/Caribbean (20%), Central Asia (11%) and South America (8%). Six hundred and sixty two (59%) travelers were given antimalarial prescriptions, the majority for atovaquone/proguanil (50%) followed by mefloquine (28%) and off-label primaquine primary prophylaxis (15%). Chloroquine (4%) and doxycycline (3%) were rarely used. Atovaquone/proguanil was the most often prescribed for all regional destinations and was prescribed frequently for travelers to Central America/Caribbean (17%). Duration of travel did not influence whether chloroquine or atovaquone/proguanil was used in chloroquine sensitive areas. However, in chloroquine resistant regions, duration of travel was associated with antimalarial choice. The mean duration of travel was 3.6 weeks for those prescribed atovaquone/proguanil and 7.6 weeks for mefloquine. The mean duration difference (4 weeks) was significant (95% CI = 1.3 - 6.7). Physician 1 was more likely to prescribe atovaquone/proguanil than all other physicians combined (OR 2.65, 95% CI 1.87 - 3.77, p <.0001). Physician 2 was more likely to prescribe off-label primaquine for primary prophylaxis than all other physicians combined (OR 7.49, 95% CI 4.08 - 13.74, p <.0001) primarily for *P. vivax* predominant areas. This study suggests that factors such as duration of travel, chloroquine resistance, prevalence of *P. vivax*, and individual prescriber preferences account for differences in antimalarial prescribing practices. Additional data, including prescriber resource utilization will be presented.

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ARTEMETHER, DIHYDROARTEMISININ AND LUMEFANTRINE DO NOT INDUCE *IN VITRO* DRUG METABOLIZING ENZYMES AND METABOLISM OF ORAL CONTRACEPTIVES

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The goal of this study was to evaluate *in vitro* the components of Coartem/Riamet (artemether and lumefantrine) and the active metabolite dihydroartemisinin (DHA) for their potential to induce drug-metabolizing CYP enzymes and the metabolism of oral contraceptives. The experiments were conducted according to the FDA drug drug interaction guidance. The assessment was done *in vitro* in cryopreserved primary human hepatocytes of at least three individual donors. Induction of mRNA, relative to the vehicle control, was determined by real-time PCR and evaluation of changes in cytochrome P450 (CYP) enzyme activities were assessed after 48-h induction periods by LC/MS/MS analysis of CYP-selective probe substrate metabolism. Metabolism of the oral contraceptives was tested by HPLC analysis. Human hepatocytes were incubated with the three test substances up to concentrations which exceeded their therapeutic concentrations by a factor of 10. Ethinyl estradiol and levonorgestrel were selected as active ingredients of oral contraceptives and were tested at their therapeutic concentrations of 1 nM and 20 nM, respectively. Rifampicin at 0.1, 1, and 20 µM, and phenobarbital at 1000 µM were used as positive controls for induction of genes regulated by PXR and/or CAR like CYP2B6, CYP2C, and CYP3A; β-napthoflavone at 10 µM was included as positive control for AhR-mediated induction of genes like CYP1A. Artemether, DHA, and lumefantrine were determined not to be inducers of CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, or CYP3A enzyme activity in hepatocytes or CYP1A1, CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP3A4, or CYP3A5 mRNA. Metabolism of ethinyl estradiol and levonorgestrel was determined not to be induced by artemether, DHA, and lumefantrine. As per FDA criteria, these conclusions were based upon the levels of mRNA or activity at least less than 2-fold, with respect to the vehicle control, and/or less than 40% of the maximal positive control induction response, indicative of a non-inducer *in vitro*.

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ASSESSMENT OF THE THERAPEUTIC EFFICACY AND TOLERABILITY OF THE ARTESUNATE/AMODIAQUINE COMBINATION AND ARTEMETHER/LUMEFANTRINE COMBINATION, FOR THE TREATMENT OF UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA IN THE DEPARTMENT OF CHOCÓ (COLOMBIA)

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Malaria due to *Plasmodium falciparum* is a public health problem in more than 100 municipalities of Colombia. The country is using artemether + lumefantrine (AL) fixed-dose combination for uncomplicated *P. falciparum* malaria. One alternative treatment is the WHO prequalified fixed-dose combination of artesunate and amodiaquine (ASAQ), which can be given in a simpler dosing regimen. This open controlled clinical trial, comparing AL and ASAQ efficacy and safety profiles was carried out from August 2008 to September 2009, in Chocó, a highly endemic area for *P. falciparum* malaria,. Adult patients diagnosed with uncomplicated malaria (n=210) were randomized into two arms, one receiving ASAQ (n=105) and the other AL (n=105). Clinical and parasitological parameters were monitored over a 28-day follow-up period to evaluate drug efficacy and safety. There were no losses to follow up. The mean age of the enrolled patients was 37.5 years without differences between study arms. Both therapies were similarly well tolerated, with the exception of epigastric burning sensation, which occurred in 1 patient during ASAQ treatment and 14 patients during AL treatment (p<0.001). D28 efficacy of ASAQ was 100%, and that of AL was 99% (NS). On average, blood smears became negative after one day of ASAQ treatment and after two days of AL treatment; gametocytes disappeared after 2 days of treatment in the ASAQ arm compared to 4 days in the AL arm. In this study, the efficacy and safety profile of the ASAQ combination was similar to that of AL. These findings support the use of ASAQ as an alternative treatment for uncomplicated *P. falciparum* malaria in Colombia.

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SOLUTION FOR THE POWER SUPPLY OF PORTABLE ULTRASOUND MACHINES IN THE DEVELOPING WORLD IN AREAS WITHOUT ELECTRICITY

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Portable ultrasound has been shown to be an important adjunct to clinical diagnosis and patient management in resource limited areas in the developing world. There have been several impediments to its further expansion in remote settings. One impediment has been the lack of a reliable and stable electrical source to power the machines or to recharge the batteries. Formerly scanning has been limited to one battery discharge- 45 minutes to 1.5 hours, dependant on the machine and

ambient temperature. We demonstrated the reliability and effectiveness of a commercially available solar charging unit to provide power for 3 different ultrasound machines in areas lacking electricity. The study was a retrospective observational study of four different locations. Locations included Mt. Everest, Bangladesh, Haiti, and Mali. Ultrasound machines used for the study included a SonoSite Nanomaxx and Micromaxx, and GE Logiqbook. The power source evaluated was a Brunton brand 26 Watt folding solar panel, lithium ion polymer battery and power inverter. Scans were preformed from 2 to 8 hours per day with no down time experienced secondary to electrical failure or mechanical dysfunction. Environmental extremes included ambient temperatures from -10 degrees to 113 degrees Fahrenheit. The combination of the Brunton battery, solar panel and inverter is a reliable, commercially available solution for powering portable ultrasound machines in developing areas lacking electricity.

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HYPERTENSION IN RURAL CENTRAL INDIA: A STUDY OF PREVALENCE AND POTENTIAL RISK FACTORS

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The villages in the area surrounding Jamkhed, India have undergone a dramatic epidemiological transition with a shift from communicable disease to more chronic non-communicable conditions in the past two decades and due to the work of the Comprehensive Rural Health Project, a non-governmental organization operating in the area since 1970. To develop a sound prevention and management strategy we collected data on the prevalence of hypertension and its potential risk factors. In summer 2010, we randomly surveyed households in six villages surrounding Jamkhed, a township of 40,000 in rural central India. Using an oral questionnaire, we evaluated 226 subjects above the age of 40 for risk factors for hypertension. We measured blood pressure in both arms and the abdominal girth. Mean age was 56 years old (40-85). 80% were farmers and 56% female. 30% met criteria for high blood pressure (as defined by systolic BP greater than/equal to 140 mm Hg or diastolic BP greater than/equal to 90 mm Hg) with higher rate in men. 6% were at risk of Hypertension. Increased abdominal girdle was associated with high blood pressure. Diet was carbohydrate-based with high salt intake. The most common risk factors for hypertension were tobacco use, increased abdominal girth, increased age, and family history. Prevalence of high blood pressure in this rural area with subsistence farming is alarming and warrants further investigation. This study helps to raise awareness for the public and healthcare providers about hypertension. Strategies to prevent and manage hypertension should be considered.

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PRE-TRAVEL PREPARATION OF U.S. TRAVELERS PROVIDING HUMANITARIAN SERVICE, GLOBAL TRAVEPINET, 2009-2010

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Humanitarian service workers (HSW) face risks different from those faced by persons traveling for other purposes. HSW are at higher risk due to close contact with local communities in countries high-risk for disease. We describe characteristics of HSW visiting clinics pre-deployment from January 2009_January 2011 in Global TravEpiNet (GTEN), a national network of clinics providing care to International travelers. HSW were categorized as those who traveled for work involving missionary, nonmedical, medical service or a combination therein. Vaccination specifications and holoendemnicity were defined according to CDC recommendations. We also performed a subanalysis involving HSW traveling to Haiti in 2010. Fifteen percent (1946/13235) of GTEN travelers indicated they were HSW; 59% of these were aged 18_35 years, and 64% were female. HSW were stratified as missionary (26%), nonmedical (39%), and medical service workers (26%); 9% reported >1 HSW category. The most common destinations were Haiti (15%), Uganda (6%), Tanzania (5%), Kenya (5%), and Ghana (5%). Of HSW going to countries holoendemic for malaria, 95% received antimalarial chemoprophylaxis. Of HSW going to countries holoendemic for yellow fever, 96% of HSW were already immune or received yellow fever vaccine at the clinic visit. However, 20% of medical workers either did not have self-reported pre-existing varicella immunity or did not receive varicella vaccine. Further, 25% of missionaries either did not have self-reported pre-existing Hepatitis B immunity or did not receive vaccine. The number of HSW deploying to Haiti increased markedly following the 2010 earthquake; the median duration of stay in Haiti for 2010 HSW was 8 days. In summary, the majority of GTEN HSW are under 35, more likely female, and often travel for short durations. Some HSW may not be fully immune to illnesses common in destination countries. Travel medicine clinicians should utilize the pre-travel consultation as a platform to immunize for routine and travel-related vaccinations, especially when the traveler might be at high risk.

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EPIDEMIOLOGY OF *CANDIDA* AMONG WOMEN VAGINAL TRACT IN QATAR

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Vaginal symptoms are a leading cause for women patients to visit her gynecologist. Since the incidence of candidiasis has increased dramatically during the last decade, thus risk factors and symptoms must be considered in order to deal with this disease and avoid its complication. The aim of this study was to determine the frequency and distribution of *Candida* spp. in women with vulvovaginal symptoms. High vaginal swabs were collected from women patients refer to Women Hospital in Doha, Qatar in summer 2010 suffering from abnormal vaginal discharge, leaking, itching. Samples were identified using culture method and Vitiek II system for species conformation to record the species types. For each patient, age, nationalities, pregnancy, vaginal discharge, leaking were recorded as risk factors for vulvovaginal candididas. During the period of the current study (June-November 2010), a total number of 222 women were found to be infected with at least one of the following *Candida* species: the most predominant species was *C. albicans* (77.5%) followed by *C. parapsilosis* (13.5%), *C. tropicalis* (7.2%), with less detected species for *C. kruzie* and *C. glabrata* (2.7%). However these results indicate that factors associated with age and pregnancy may influence the occurrence of *Candida* spp. in women with vulvovaginal symptoms. The frequency distribution of *C. albicans* in pregnant women was (30.6%) and (32.4%) with women complains from vaginal leaking. The incidence of vulvovaginal candidiasis was found to be 57.65% in women less than or equal to 30 years old compared with 42.34%in women greater than 30 years of age. With relation to patients nationalities, (52.52%) of non- Qatari women were suffering from candidal vaginitis compared to (46.74%)of Qatari women. In conclusion, the limitations of the present study, it has not documented the history of recurrent infection and antimicrobial use. The current clinical data listed in patients were limited. As a result, sufficient data were unavailable to evaluate the risk of contraceptive practices and antimicrobial use of vaginal candidiasis.

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SEVERE DENGUE VIRUS INFECTION IN PEDIATRIC TRAVELERS VISITING FRIENDS AND RELATIVES IN THE BRONX, NEW YORK

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Dengue fever (DF) has been recognized to be the most frequent cause of a systemic febrile illness in travelers returning from tropical regions other than Africa. Hitherto travel-associated severe dengue infections have been mostly described in adult international travelers. The objective of this report is to analyze the travel, clinical and laboratory characteristics of children who were diagnosed with DF after return from international travel. Data was abstracted from charts of pediatric patients who were diagnosed with DF at the Bronx-Lebanon Hospital Center betweeen May 2007 and December 2010. A commercial dengue virus IgM capture ELISA and a dengue virus IgG indirect ELISA was used for diagnostic testing. An IgM/IgG index ratio ≤1.07 was applied to distinguish secondary from primary dengue virus infection. The WHO criteria were used to diagnose dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). We identified 8 children with acute dengue virus infection (3 children (38%) with severe dengue infection [DHF, n=2] and [DSS, n=1]). All had traveled to visit friends or relatives (VFR) for the median duration (range) of 32 days (10 days -4.3 years) in the Dominican Republic (88%) or Puerto Rico (12%), and presented ill after the median time since return (range) of 6 days (1-11), (63% females, 75% U.S. born, median age [range] of 13.6 [0.3-17.6 years). All presented with an acute febrile illness accompanied by gastrointestinal complaints (63%), myalgia (50%), petechial rash (38%), dehydration (25%), and headache (13%). Relevant laboratory findings included leukopenia (63%), thrombocytopenia (75%), elevated serum alanine aminotransferase (38%), low serum albumin (38%), and elevated hematocrit (25%). Sonogram revealed ascites (50%), pleural effusion (38%), gallbladder thickening (38%), and heterogenous liver parenchyma (25%). Of the children with severe dengue virus infection, 2 teenagers (DHF) had a secondary immune response, and one infant (DSS) had a primary immune response. Due to increasing global migration a growing proportion of children traveling to tropical and subtropical regions are at risk of exposure to dengue virus infection. Therefore children of immigrant families originally from dengue-endemic countries may benefit from competent pre-travel advice, and may represent candidates for a future dengue vaccine.

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RECOMBINANT SINDBIS/VENEZUELAN EQUINE ENCEPHALITIS VIRUS FOR THE DETECTION OF SEROTYPE-SPECIFIC IMMUNOGLOBULIN M ANTIBODIES IN MEXICO

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Venezuelan equine encephalitis virus (VEEV) is an important arboviral pathogen in Central and South America and has caused numerous outbreaks in both equids and humans since the 1920s. Subtype I VEEVs comprise 5 varieties, including subtypes IAB, IC, ID, IE and IF, four of which are classified as HHS select agents. To avoid the risk and regulatory difficulties of working in high containment under select agent regulations, vaccine strain TC-83, which belongs to the IAB variety, is commonly used as an antigen for detecting immunoglobulin M (IgM) antibodies in response to all subtype I VEEV strains. However, it is less sensitive for detecting IgM antibodies induced by other VEEV varieties, and therefore, it limits the accuracy of etiologic diagnostics. To explore the potential application of a recombinant Sindbis virus (SIN)/VEEV for clinical diagnosis, we compared the sensitivity of SIN/VEEV that was derived from subtype IE VEEV (SIN/VEEV-IE) to the vaccine strain TC-83 for IgM detection in sera from either naturally exposed humans in Mexico or experimentally infected equids. When using goat anti-human IgM to test human VEEV-IE positive sera in an IgM capture ELISA, we observed a higher sensitivity of detection when using SIN/VEEV-IE (80%) than TC-83 (60%). When VEEV-IE-specific monoclonal antibody 1A1B-9 was used to in a sandwich ELISA, SIN/VEEV-IE generated 100% positivity in both human and equine sera, whereas TC-83 failed to detect positive sera, confirming the enhanced antigenic accuracy with recombinant virus. Endpoint IgM antibody titers using SIN/VEEV-IE were only slightly lower (2-fold) when compared to a wild-type VEEV IE strain. Our results indicate that recombinant SIN/VEEV-IE provides a better target than TC-83 for IgM detection of a recent infection by subtype IE VEEV, potentially replacing the use of wild-type VEEV in serological tests.

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FACTORS ASSOCIATED WITH APPROPRIATE HOUSEHOLD DIARRHEA CASE MANAGEMENT IN HOSPITALIZED CHILDREN, NYANZA PROVINCE, KENYA, 2007

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Diarrhea is a major cause of morbidity and mortality in children under five in Nyanza Province, Kenya. Oral rehydration therapy (ORT), defined here as initiating or increasing oral rehydration solution (ORS), breast milk, watery porridge, homemade sugar-salt solution, and/or other fluids, is an affordable and effective treatment for childhood diarrhea. The goal of this study was to identify modifiable factors associated with ORT use in children with diarrhea requiring hospitalization. In 2007, we conducted a cross-sectional study of caregivers of children under five who were hospitalized for diarrhea at two district hospitals in Nyanza Province. We developed a behavioral model that included constructs hypothesized to be associated with ORT use, including perceived positive and negative attributes of ORT, self-efficacy, beliefs regarding diarrhea and treatment, and demographic factors. Using logistic regression, we identified factors associated with ORT use *within* each behavioral construct. We tested those variables associated with ORT use within each construct in a final cross-construct logistic regression model. The median age of the 119 respondents was 21 years (range 15-40 years) and median age of the children was 10 months (range 1 month to 3 years). Twenty one (18%) respondents had less than primary school education, 73 (61%) completed primary school, and 26 (22%) completed some secondary school. ORT use was reported by 93 (78%) respondents, with 73 (61%) reporting use of ORS specifically. The following factors were independently and significantly associated with ORT use in the final cross-construct mode: a caregiver believing a child could die (OR_{adj} 4.2 95% CI 1.4-12.8, p = 0.01), a caregiver feeling she knows how to prepare ORS (OR_{adj} 3.4, 95% CI 1.2-9.8, p = 0.03), and advisors to the caregiver recommending ORS and/ or increased fluids (OR_{adj} 3.8 95% CI 1.3-11.1, p = 0.01). ORT use was relatively common among children requiring hospitalization for diarrhea in rural western Kenya. Our results suggest that risk perception, self efficacy, and social support are important determinants of ORT use. Interventions to improve diarrhea case management should educate caregivers to appreciate the risk of dehydration from diarrhea, emphasize ORT's ability to prevent and treat dehydration, and enhance self efficacy of caregivers by encouraging them to initiate ORT at home based on its ease of use, affordability, and effectiveness.

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DYING WITH THEIR BOOTS OFF: NON-TRAUMATIC DEATHS AMONG AMERICAN TROOPS IN VIETNAM, 1960-1975

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The medical literature, ranging from ancient to modern sources, describes a staggering number of causes of combat-related mortality and morbidity. Moreover, the extent of such casualties broadened with the development of new modes of military technology. Traumatic injuries due to lead shot, rifle bullets, and high explosives undoubtedly lead the list among the aetiology of these events. Such categorization of causes of wartime deaths, however, has expanded even more significantly in the post-WWII era. The Southeast Asia Combat Area Casualty File, for example, contains a rich array of mortality data concerning American troops who died between 1960 and 1975 in Vietnam. The purpose of this study is to analyze cases in this dataset that died as a result of non-traumatic injuries (*e.g.,* heart attack, suicide, etc.), aetiologies more often associated with civilian rather than military life. Among the twenty-one official death categories listed in the Southeastern Asian Combat Casualty File about half of them relate directly to traditional military operations, *e.g.*, gunshot wound, grenade shrapnel, etc. The remainder, however, include deaths from suicide, heart attack, "misadventure" etc, the epidemiology of which will comprise the focus of this study.

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INTESTINAL BACTERIAL/VIRAL AND PARASITE CO-INFECTION IN INFANTS WITH ACUTE DIARRHEA

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The estimated worldwide death rate from diarrheal diseases is about 2.2 million deaths per year as reposted by the WHO. Diarrheal infections may be caused by an array of bacterial, viral, or parasitic pathogens. Some cases have 1 single defined cause, others do not have any defined cause, and a substantial number (one third) are caused by multiple pathogens. It's very little known about confection of bacteria and parasite and how it reflects on clinical course of acute diarrhea, especially in infants. Totally data of 168 children cases with acute diarrhea were analyzed. The data collected included census data, history of disease (onset, severity, duration of disease), common blood count, stool bacteriological (culture for intestinal bacterial pathogens following world wide accepted technique), O&P (ova and parasites microscopy) and ELISA for rotavirus antigen (IDEIA, Dako Ltd., United Kingdom) examination. The data was analyzed with "R" software (http://www.R-project.org/). The average age of children was 13 months (±8,2 months) *Salmonella typhimurium* was isolated in 14 patients (8.34%). Rotavirus was detected in 47 patients (27.97%). The parasites detected in the following sequence: 26 Blastocyst (15.48%), 25 Pinworms (Enterobius vermicularis) (14.88), 3 Pinworms (*Enterobius vermicularis*) + Blastocyst (1.79%), 1 Pinworms (*Enterobius vermicularis*) + *Giardia intestinalis* (0.6%), 15 *G. intestinalis* (8.93%), 2 *G. intestinalis* + Blastocyst (1.19%) and no parasite detected in 96 patients (57.14%). There was significant difference in duration of hospitalization in children with bacterial and/or rotavirus diarrhea and children with co-infection of bacteria/virus and parasite ($p<0.001$). The parasites like Blastocyst, pinworms and *G. intestinalis* are very common in children with diarrhea. Due to poor control of antimicrobial utilization the bacterial pathogens is rarely isolated. Though rotavirus is the predominant viral diarrheal agent among children the intestinal parasites burden like *G. intestinalis* is underestimated and must be considered. In general the children with bacteria/virus and parasite co-infection have longer staying in hospital comparing the ones with bacterial and/or viral diarrhea. We recommend performing O&A stool examination in all children with acute diarrhea.

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LABORATORY DIAGNOSTIC DETERMINATION OF C-REACTIVE PROTEIN IN AIDS POPULATION IN ETHIOPIA

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The aim of the study was assess of CRP testing as an important indicator of bacterial infection in the diagnostic of inflammation and monitoring the effectiveness of antibiotic treatment in terms of Ethiopia. In admission we were willing to extend the basic laboratory in Kibre Mengist with assessment of clinical outcome and correlation with test results provided the diagnosis only on the basis of clinical signs of disease. The diagnostic of hospitalized patients and outpatients in a hospital in Kibre Mengist based on clinical signs of disease, using rapid diagnostics tests (RDT) and quantitative determination of the value of CRP marker. The data were statistical analyses were carried out using SPSS software. The results clearly show that it is important to first obtain the consent to use RDT for screening in developing countries and accelerate reliable diagnostic and the treatment itself. The introduction of CRP examination is a clear contribution, especially in case of patients with ambiguous clinical picture of disease and especially where it is not possible to wait for the typical clinical signs, that means in case of young children and HIV/AIDS patients. Although the clinical diagnostic is cheap, wrong diagnosis leads to unnecessary prescriptions, which in turn supports the growth of resistance to drugs in the world and increases the cost of a new, effective treatment.

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ACUTE UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA IN INFANTS <5 KG BODY WEIGHT IN FOUR SUB-SAHARAN AFRICAN COUNTRIES - A DESCRIPTIVE STUDY

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Artemisinin-based combination therapy (ACT) is recommended as first-line treatment for infants ≥5 kg of body weight (BW) with uncomplicated *falciparum* malaria, but no ACTs are indicated in the population <5 kg. Published reports on malaria in younger infants are scanty, leaving a significant knowledge gap about the pattern and outcome of malaria in this sub-population. Hospital charts from 4 countries from Sub-Saharan Africa (Bénin, Democratic Republic of Congo, Nigeria, and Togo) were retrospectively reviewed for the period between 2006-2010 for inpatient neonates and infants <5 kg BW with a confirmed diagnosis of uncomplicated *Plasmodium falciparum* malaria. Clinical features, age group, treatment, and outcome were collected. The annual incidence ranged from <20 to >90 cases across hospitals and calendar years. The proportion of cases varied by age (≤28 days vs. >28 days): the proportion of infants in the older group was generally higher, but the younger group represented from <2% at one hospital in the Democratic Republic of Congo to >70% at another in Togo. The most frequent clinical presentation was fever, followed by dyspnea, crying, or vomiting. Whenever results were available, parasite load was generally low; <10%

of the infants presented with parasitemia >5000/µL. The majority of the infants were treated with oral quinine, except at two hospitals in Bénin and Togo, where AL and intramuscular artemether were administered, respectively. Although infrequent, malaria in neonates and infants <5 kg of BW does exist in certain endemic countries and calls for appropriate treatment. Further clinical evidence regarding the use of ACTs in this population is warranted.

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MATURATION OF DENGUE VIRUS NONSTRUCTURAL PROTEIN 4B IN MONOCYTES ENHANCES PRODUCTION OF DENGUE HEMORRHAGIC FEVER-ASSOCIATED CHEMOKINES AND CYTOKINES

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Of the 50-100 million dengue virus (DENV) infections worldwide each year, approximately half-million hospitalizations occur. Mechanisms explaining why some individuals progress to severe disease are unclear. However, DENV preferentially infects peripheral blood monocytes, which secrete elevated levels of chemokines and cytokines in patients progressing to severe disease. Of the ten DENV proteins, several nonstructural proteins (NS) including NS4B and NS5 are capable of inhibiting interferon signaling. For the first time, we report that NS4B and NS5 expressed in monocytes are potent inducers of immunomediators associated with severe disease. Further, we demonstrate that cleavage of the NS4AB polyprotein by the viral protease NS2B3(pro), via the intermediate 2KNS4B, is significantly more potent than NS4B or NS5 alone, inducing immunomediators to levels similar to DENV infection. The 2K-signal peptide is not required for the induction of immunomediators yet it plays a synergistic role with NS4B. These data suggest that maturation of NS4B is primarily responsible for the induction of immunomediators associated with severe disease. Given that NS4B structures are conserved across flaviviruses, NS4B may be an attractive target for the development of Flavivirus-wide therapeutic interventions.

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RISKS FACTORS AND INCIDENCE OF DENGUE IN A PROSPECTIVE COHORT STUDY IN MARACAY, VENEZUELA

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Dengue has become the most important vector-borne viral disease in the Americas. Dengue in Maracay, Venezuela, is hyperendemic with co-circulation of the 4 viral serotypes. The increment of dengue transmission in Venezuela has coincided with an increase in the incidence of severe disease which in 2010 reached nearly 10% of all cases. A cohort study of 2000 individuals aged 5-30 years was established between August and December 2010 in Maracay city. Baseline epidemiological data and blood samples were obtained. Annual cross-sectional sampling will take place. Febrile cases are identified through passive and active house-to-house surveillance. Blood samples will be analysed using hemagglutination inhibition test and plaque reduction neutralization test to determine the incidence of symptomatic and inapparent dengue infection and the possible association with primary or secondary infection. Epidemiological data will be used to identify potential risk factors for dengue infection. Preliminary results will be presented and discussed.

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VISUALIZATION OF DENGUE RNA REPLICATION IN LIVING CELLS

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Dengue virus (DENV) is an enveloped virus with a single-stranded, 10.7 kb positive-sense RNA genome. There are two complementary sequences in the 5′ and 3′ untranslated regions that interact with each other to circularize the viral genome. Following circularization, the viral RNA-dependent RNA polymerase, known as NS5, plays a crucial role in the initiation and regulation of RNA synthesis. However, it still remains controversial whether the DENV genome exists primarily as single or double stranded RNAs in host cells. Because of the lack of a direct method to investigate each form of RNA in living cells, localization of the viral RNA genome has also to be elucidated. Here, we introduced two fluorophore specific RNA aptamers; Malachite green RNA aptamer and Hoechst RNA aptamer to directly visualize the viral RNA replication process and differentiate each strand of RNA *in vivo*. Both RNA aptamers were constructed into a new reporter system for investigate the mechanism of DENV RNA replication, transportation and localization process in human cell lines in real-time. We will further discuss the use of the system to trace the life time of non-coding RNAs in living cells.

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VECTOR CONTROL AND SURVEILLANCE DURING A MAJOR OUTBREAK OF DENGUE IN A COASTAL RED SEA AREA: PORT SUDAN CITY

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An unprecedented dengue outbreak occurred on the coastal Red Sea in Port Sudan during the period (27 February - 25 June)2010. A vector control response plan to the outbreak had mainly entailed house inspection and insecticide space spraying against the dengue vector, as well as integration of vector surveillance work to evaluate the response plan. A total of 3223 enrolled dengue cases in Port Sudan was reported over 17 epidemiological weeks. A total of 3048 houses were inspected during the vector surveillance work resulting in collection of 19,794 larvae and 3,240 pupae of *Aedes aegypti*. A significant decrease in the entomological indices was shown during the observed period: House Index (HI) has declined from 100% to 16% ($F= 57.8$, $p<0.001$), while pupal/demographic (P/D) index has decreased from 0.77 to 0.10 ($F= 3.06$, $p<0.01$), on the 9th and 21st weeks, respectively. Accordingly, this decline has been accompanied by a decrease in the numbers of dengue cases from a peak observed on the 13th week (341 cases) to the lowest on the 25th week (49 cases) per week. Using regression line analysis, a significant relationship exists between the measured entomological parameters and the trend of dengue cases on the next weeks ($R2=0.83$; $F= 23.9$, $p<0.001$). This study clearly shows entomological surveillance is sensitive to evaluate vector control and monitor dengue epidemics in such coastal area. Integration of epidemiological and entomological indicators should be the basis of a surveillance system for the emerging dengue on the coastlines of Red sea.

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DUAL ROLE OF INTERFERON RESPONSE IN DENGUE INFECTION

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Over the past quarter century, risk for infection with one of four serotypes of dengue virus (DENV) has markedly increased. Currently, 40% of the world's population lives in endemic regions. Understanding the mechanisms of DENV emergence has been complicated by the lack of animal models of transmission, where susceptibility to infection establishment by non-adapted virus and natural routes of infection are critical. *In vivo* studies have also shown the importance of the type I interferon and JAK/STAT pathways, and specifically, the IRF family in dengue and flavivirus infection. We introduce a novel model for transmission, an IRF3/7 double knockout (DKO) mouse strain (C57BL/6 background) which has a delayed and significantly blunted systemic type I interferon response. We hypothesized that early type I interferon only, and not type II, is critical for the inhibition of dengue virus infection establishment, and thus successful transmission. We further evaluated this hypothesis via a natural transmission route to determine whether the relative importance of types I and II interferon is changed by exposure route. We were able to established 100% infection rate in these DKO mouse with three different, non-adapted strains of dengue virus of two serotypes. Type II interferon appears necessary for viral clearance, but is apparently not as critical for the initial establishment of dengue infection *in vivo*. We conclude, then, that type I interferon, and not type II, is the essential mediator of successful transmission of dengue virus.

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AEDES AEGYPTI IN CAPE VERDE: STATUS AND RESEARCH PERSPECTIVE

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Aedes aegypti the most competent dengue vector is present in the all islands of Cape Verde. In 2008 were the first dengue cases in the country and in September 2009, Cape Verde was ravaged by an epidemic of dengue. During this first outbreak of dengue in Cape Verde, were registered 25 071 suspected cases nationwide, 6 747 confirmed cases, 174 cases of Dengue hemorrhagic fever/Dengue shock syndrome and 4 deaths (MOH data)caused by serotype DEN-3, and occurred from September to November 2009. Several factors may be responsible for this epidemic, the rapid and disorganized urban growth and consequent negative impact on sanitation, a relatively long rainy season with rainfall and the multiplication of air links with regions of the world where presence dengue is circulating. Sensibility tests on *Ae. aegypti* with larval sampling of populations from five areas of Santiago Island and São Filipe, using the diagnostic dose of temephos recommended by WHO, showed mortality rate between 95 to 100%. With these experiences, we can confirm that the population of *Aedes* in Cape Verde is sensible to temephos. This could be explained by the low use of insecticides in vector control. So is necessary to have a constant surveillance of mosquitoes susceptibility against insecticides, as well their effectiveness in the field in order to avoid high level of resistance, that facility the management of resistance case if detected. Results of dissemination and transmission rates of *Ae. aegypti* orally exposed to DEN-3, Chikungunya (CHIK) and Yellow Fever (YF) viruses show that population of *Ae. aegypti* collected in Praia (Cape Verde) has high vectorial competence to CHIK and YF. In the case of introduction of those viruses in the country can be cause major epidemics cases such as occurred with dengue. So, with the increasing incidence of dengue, yellow fever and Chikungunya worldwide, especially in tropical countries, it is crucial to develop studies on ecology, mapping of insecticides susceptibility/resistance, vectorial competence and genetic populations of *Ae. aegypti* in Cape Verde Islands. For those studies, we will create partnerships between Local Institutions and International Agencies. All entomological data will be associated with epidemiological, social and environmental data in order to reinforce the national program activities and to develop some new approaches in vector control.

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THE SEROPREVALENCE OF DENGUE FEVER IN SOLOMON ISLANDS

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There is lacking in the prevalence data of dengue fever in Solomon Islands in recent years. During 2009 and 2010, we investigated the seroprevalence of dengue fever infections of school students in Solomon Islands. Under IRB approval, we conducted this survey and used the Panbio Dengue IgG Indirect ELISA to detect IgG antibodies to dengue antigen serotypes (1, 2, 3 and 4) in serum, as an aid in the clinical laboratory diagnosis of patients with clinical symptoms and past exposure consistent with dengue fever. We then used the PRNT (plaque-reduction neutralization test) of Japanese encephalitis (JE) to exclude the cross reaction of positive dengue specimens with Panbio Dengue IgG Indirect ELISA. At the same time, we examined the stool parasites for every enrolled subject using MIF (Merthiolate-iodine-formaldehyde) method. From the 588 serum specimens of study subjects under 20 years old, the seroprevalence rate of dengue is 62.2 % (366/588). The distribution of dengue in rural citizens (64.2%, 235/366) is significantly higher than that in urban citizens (35.8%, 131/366) ($p<0.001$). We analyzed the variables with multiple logistic regression method to find out the independent risk factors for seropositive dengue subjects, which indicated two important factors-- the rural citizens(OR 2.229, 95% CI 1.586-3.132), and the patients with hook worm infections(OR 1.686, 95% CI 1.095-2.596). The JE seropositive rate is 9.8% (38/386). On the contrary, the distribution of JE in urban citizens (17%) is significantly higher than that in rural citizens (5.2%)($p<0.001$). From this study, we did find the flaviviral infections existed in this community and dengue is prevalent in Solomon Islands which might be overlooked before. More intensive surveillance and control should be implemented.

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10 KDA INTERFERONΓ-INDUCED PROTEIN (IP-10) IS SPECIFICALLY ELEVATED IN DENGUE FEVER IN SUBJECTS WITH ACUTE FEBRILE SYNDROME AND PREDICTS THE DEVELOPMENT OF DENGUE HEMORRHAGIC FEVER: A CASE-CONTROL STUDY FROM COLOMBIA

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Causes of fever are often non-specific. Acute febrile syndromes like dengue fever, influenza and leptospirosis can have overlapping geographic distribution and clinical presentations but may require different treatments. This study was undertaken to identify biomarkers that can improve clinical discrimination of dengue fever, influenza and leptospirosis in individuals

with acute febrile syndrome in Bucaramanga, Colombia. Between 2003-2008, outpatients with an acute febrile syndrome were enrolled in a prospective cohort study. Serum levels of biomarkers from pathways of immune activation were measured in a random subset of subjects with dengue fever (n=113), leptospirosis (n=47), influenza (n=37) and healthy controls (n=14). 10 kDa interferonγ-induced protein (IP-10) was elevated in subjects with dengue fever compared to influenza, leptospirosis or healthy controls ($p<0.01$ for each). We evaluated the ability of IP-10 to discriminate between the three clinically similar syndromes by receiver operating characteristic curve (ROC) analysis. IP-10 was able to discriminate between dengue fever and leptospirosis or influenza with good diagnostic accuracy (area under the ROC curve (95% CI), p-value: 0.84 (0.77-0.89), $p<0.0001$ and 0.84 (0.77-0.90), $p<0.0001$ respectively). IP-10 levels at enrolment were elevated in individuals who developed dengue hemorrhagic fever (n=46, $p=0.014$). We used classification analysis (CRT) to integrate the clinical, laboratory and biomarker data into a single decision tree to discriminate between the three groups. A tree was generated where IP-10 was able to identify the majority of dengue cases and then cough, dizziness, and leukocyte count were able to further differentiate between dengue fever, leptospirosis and influenza. The model with IP-10 had a sensitivity of 80.4% and specificity of 94.6% to identify dengue fever compared to a sensitivity of 81.2% and specificity of 71.4% using clinical and laboratory parameters alone. In conclusion, IP-10 is a promising biomarker of dengue fever and may predict the development of dengue hemorrhagic fever.

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THE HISTORY OF DENGUE OUTBREAKS IN THE AMERICAS

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Dengue disease is caused by dengue virus serotypes 1-4 usually transmitted by *Aedes aegypti* mosquitoes. Currently, dengue is the most common endemo-epidemic viral arthropod-borne disease worldwide. We report the epidemic patterns of outbreaks in the Region of the Americas from 1600 to 2010. Dengue outbreaks reported in the literature and to the Pan American Health Organization (PAHO) were reviewed. Outbreaks were analyzed in 4 periods: A) Introduction of dengue in the Americas (1600-1940), B) Plan for the eradication of the *Ae, aegypti* (1947-1970), C) *Ae. aegypti r*e-infestation (1971-2000), D) Increased dispersion of *Ae. aegypti* and dengue virus circulation (2001-10). A) The first dengue epidemics occurred in 1635 in Martinique and Guadalupe. In 1818, an outbreak was reported in Peru (n~ 50,000 cases). In 1827 the first multi-country outbreak occurred (Virgin Islands, Cuba, Jamaica, Venezuela and many cities in the US). In 1912 a dengue epidemics was reported in Panama, Puerto Rico and northern Chile and Argentina. B) Successful control efforts resulted in a decrease in the number of outbreaks during 1948-1972 when the complete eradication of *Aedes* mosquitoes was certified in 21 countries. C) Outbreaks increased as the eradication program deteriorated. Approximately 702,000 dengue cases most DEN-1 were reported during 1977-80. In 1981 Cuba reported an outbreak with 344,203 cases, 10,312 cases of dengue hemorrhagic fever (DHF) and 158 deaths. Other epidemics occurred in Northern Brazil in 1982 and Rio de Janeiro in 1986, extending to Bolivia, Paraguay and Ecuador in 1988 and Peru in 1990. D) Outbreaks were reported in 2002 in Brazil [n=780,644; incidence/100,000 (I)= 452]; in 2007 in Paraguay (n=28,182; I= 500); in 2008 in Brazil (n=734,384; I=426); in 2009 in Bolivia (n=84,047; I= 864) and Argentina (n=26,612; I=71); and in 2010 in Honduras (n=66,814; I=1,016), Colombia (n=157,152; I= 685), and Brazil (n=1,004,392; I=525). An increasing number of outbreaks have been reported in the Americas during the last decades. Urgent global actions addressing integrated public policies for effective prevention and control are currently needed to avoid further spread of the disease.

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IDENTIFICATION AND CHARACTERIZATION OF NEUTRALIZING AND ENHANCING EPITOPES ON DENGUE VIRUS ENVELOPE PROTEIN

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Shotgun Mutagenesis technology was employed to identify detailed epitope maps for human MAbs derived from vaccine recipients and naturally-infected patients against the immunodominant envelope protein (prM/E) of Dengue virus (DENV). A comprehensive plasmid mutation library for DENV-3 prM/E was created in which every prM/E residue was individually mutated to a defined substitution, expressed in human cells, and analyzed for its effect on antibody reactivity and viral infectivity. For each MAb, we identified amino acids on prM/E that are required for antibody binding, and these residues were mapped onto the prM/E crystal structure to visualize epitopes. Our goals are to map and compare epitopes of antibodies against all 4 DENV prM/E serotypes and determine their role in viral protection and pathogenesis. The molecular and functional mechanisms by which MAb-epitope interactions contribute to the humoral immune response were characterized by measuring neutralization and antibody-dependent enhancement titers, MAb binding affinities, timing of inhibition (pre- or post-viral attachment), and the ability to support complement fixation. We expect that this approach will help define the range of immunodominant structures on prM/E and identify novel enhancing and neutralizing antibody epitopes that can be used for therapeutics, diagnostics, and vaccine development.

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DEVELOPMENT AND CHARACTERIZATION OF A STABLE REVERSE GENETIC SYSTEM OF A MALAYSIAN SYLVATIC DENGUE VIRUS TYPE 2 STRAIN (P8-1407)

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Dengue viruses (DENV) exist in two ecologically and evolutionarily distinct transmission cycles: 1) an urban cycle, which involves human reservoir and amplification hosts and peridomestic *Aedes* vectors, primarily *Aedes aegypti* and *Ae. albopictus* mosquitoes, and 2) a sylvatic (enzootic) cycle that involves transmission most likely among nonhuman primates and arboreal Aedes spp. Full-length infectious clones (FLIC) are powerful tools for the experimental investigation of the mechanisms that lead to viral emergence from the enzootic cycle. Here, we describe construction and characterization of the FLIC of the prototype Southeast Asian sylvatic DENV-2 strain P8-1407, isolated in 1970 from a Sentinel monkey in Malaysia. Viral cDNA was cloned under the control of a CMV promoter into the low-copy-number plasmid pACNR. To circumvent the inherent instability of the plasmid during its propagation in E. coli, an intron sequence encoding several stop-codons was introduced between the structural and non-structural protein genes of the viral genome. The resultant plasmid (pAC-P8-1407) was fully sequenced to verify its genetic integrity. Infectious virus was rescued by transfection of the pAC-P8-1407 FLIC DNA into Vero cells, where transfected cells yielded a productive virus infection as determined by an infectious center assay. Maximum titers of rescued virus were obtained 5 days post-transfection, which is consistent with the replication kinetics of the parental virus in Vero cells. Detailed genetic and phenotypic characterization of the rescued virus in cell culture and *Ae. albopictus* mosquitoes is currently ongoing. A subgenomic replicon of P8-1407 expressing the mKate2 fluorescent protein was also constructed and its application will be discussed.

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FAILURE OF HIGH TITER DENV-2 NEUTRALIZING ANTIBODIES TO PROTECT AGAINST SYMPTOMATIC AMERICAN/ASIAN DENV-2 INFECTION

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Following the introduction of a new lineage of American/Asian genotype of dengue virus serotype 2 (DENV-2), a large epidemic of dengue hemorrhagic fever (DHF) occurred in December 2010 in the Amazon basin city of Iquitos, Peru. While American genotype DENV-2 had been the cause of large outbreaks of dengue fever (DF) in the mid-1990s, little DENV-2 circulation had been detected in Iquitos since 2000. As part of on-going community-based longitudinal studies of DENV transmission in Iquitos, we used a plaque reduction neutralization test (PRNT) to determine the prevalence of serotype-specific neutralizing antibodies among residents of Iquitos immediately prior to the 2010 DHF epidemic. We found that 73.5% (95% confidence interval [CI]: 71.5%_75.4%) of the population had DENV-2 neutralizing antibodies, with a much higher prevalence among individuals 15 years of age or older (81.6%; 95% CI: 79.9%_83.3%) than children under the age of 15 (21.5%; 95% CI: 17.0% -- 25.0%). Among participants born after 2000, DENV-2 antibody prevalence was 4.8% (95% CI: 2.0%_7.6%). We found that many of the individuals who experienced symptomatic DENV-2 infection, as identified through door-to-door community-based surveillance, had robust DENV-2-specific antibody titers prior to infection, at a proportion similar to the general population. Greater than 50% of symptomatic infections occurred among individuals older than 15 years, despite the high prevalence of DENV-2 antibodies. These data suggest that antibodies generated against American DENV-2 strains do not provide robust protection against American/Asian strains. We plan to address this hypothesis by challenging pre-infection sera with American/Asian DENV-2 strains collected during the 2010 epidemic. Alternative hypotheses, including cross-reactive antibodies that do not accurately reflect past infection history, will also be explored. These studies have significant implications for understanding the immune response to DENV serotypes and for dengue vaccine development.

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INVESTIGATING THE HUMAN ANTIBODY RESPONSE TO PRIMARY DENGUE INFECTIONS

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Dengue viruses (DENV) are considered a major global health concern, infecting over 50 million individuals across the globe each year. DENV are mosquito-borne flaviviruses, existing as four serotypes (named DENV1 through 4). Following a primary DENV infection, individuals produce a complex mixture of serotype-specific and cross-reactive antibodies. This pre-existing immunity is thought to be sufficient to protect against re-infection with the same serotype, but may enhance infection and increase disease severity during a second infection with one of the other three DENV serotypes. Due to the complexity of the human humoral immune response, the binding, neutralization and enhancing properties of human polyclonal sera against DENV are not well understood. The goal of the current study was to fractionate antibodies in DENV-immune human sera using viral antigen or recombinant protein, and to investigate the role of specific antibody populations in DENV neutralization or enhancement. Depleted sera were tested for the ability to neutralize or enhance DENV in cell culture and in the AG129 mouse model of infection and disease. Both cell culture and animal studies demonstrated that after a primary infection, humans produce two distinct subpopulations of antibodies that are 1) type-specific and strongly neutralizing, and 2) cross-reactive, weakly neutralizing and enhancing. Further efforts to characterize neutralizing epitopes using recombinant envelope (rE) protein demonstrated that strongly neutralizing type-specific antibodies bound whole virus, but not the rE protein. We hypothesize that after a natural primary infection, neutralizing antibodies recognize epitopes that are only preserved on the whole virus, and not on rE dimers.

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VALIDATION OF THE RAPID DIAGNOSTIC TEST FOR DENGUE FEVER/DENGUE HEMORRHAGIC FEVER WHICH DETECTING EITHER ANTIGEN OR ANTIBODY

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Dengue fever (DF) is endemic in Thailand and dengue hemorrhagic fever (DHF) has been reported in 2010 with double increase to 2009. The objective of this study was to evaluate the rapid diagnostic test for Dengue and Dengue haemorrhagic fever (DF/DHF) which detecting RDT NS1 and RDT IgM/IgG to assess their performance in a diagnostic laboratory. Sera from 110 patients collected during a febrile outbreak at Chumpae Community Hospital, Khon Kean Province, Thailand during August-December 2010 were studied. The results showed this rapid diagnostic test detected 40 and 23 primary and secondary infective patients respectively. The sensitivity was 63.48 % and specificity was 85.91 % in acute phrase of illness while convalescence phase, the sensitivity and specificity were 83.3 % and 85.81 % respectively. However, the RDT's sensitivity was deviated by the duration after onset of fever. This rapid diagnostic test showed rather low sensitivity because of hospital's delay visit of the patient but showed high specificity in both acute and convalescence phase of illness. The sensitivity was the highest in the early phase of illness (the 3rd - 4th day after onset of illness). By the way, this rapid test would give some sort of benefit by facilitating clinicians to discriminate between primary and secondary infections without the need for expensive equipment or highly trained personnel. In this study, Among the 23 secondary infected child were intensively cared to prevent them from hemorrhagic manifestations.

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APPLICATIONS OF THE ESSENCE DESKTOP SOFTWARE IN THE ANALYSIS OF PHILIPPINE NATIONAL DENGUE DATA

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The recent decades highlight emerging and re-emerging infectious diseases as serious public health threats. There is a need to develop disease surveillance systems able to provide early detection of health threats posing significant risk. The Philippine National Epidemiology Center and the Philippines-AFRIMS Virology Research Unit, Armed Forces Research Institute of Medical Sciences used the open source analysis program, Essence Desktop Edition (EDE) (http://www.jhuapl.edu/Sages/pages/tools/tools.html) developed by Johns Hopkins University-Applied Physics Lab) to analyze CY2003 - 2010 national dengue data collected by the Philippine Integrated Disease Surveillance and Response System. The detection

algorithm in EDE automatically selects linear regression, exponentially-weighted moving average or Poisson regression analysis depending on which algorithm best fits the data. Dengue cases that might signal an outbreak are categorized as "alerts". At the start of May 2010, clusters of red (p value<0.01) and yellow (p value<0.05) alerts were detected, with 134 cases triggering a red alert on May 2. Clusters of alerts were triggered as cases increased to 186 on May 19. From May 11-19, 7 out of 9 days triggered red alerts followed by a steady surge in cases, peaking on August 23 at 1,330 cases. Analysis of dengue cases from the Philippine National Capital Region closely mirrored the 2010 national dengue data time series trend and alerts. A similar predictive trend was seen for the 2009 national dengue data with consecutive red alerts triggered on 9 out of 10 days from May 14-23, followed by a sharp increase in dengue cases. Analysis of 2003-2008 annual dengue data also showed good prediction with cluster of alerts seen approximately 4-6 weeks prior to rapid increase of cases. EDE shows promising applications in early warning alert capability to impending increases in dengue cases in the Philippines. EDE applications are not just limited to disease data but may also have potential applications particularly in analysis of syndromic data.

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EVIDENCE FOR SPATIALLY AND TEMPORALLY CLUSTERED TRANSMISSION AND IMMUNITY OF DENGUE VIRUS FROM HOSPITAL BASED SURVEILLANCE

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Hospital-based surveillance systems are used to characterize the changing patterns of dengue disease incidence over time. These systems often focus on identifying temporal peaks in incidence or spatial hotspots of elevated risk. We demonstrate that by analyzing the distribution of geocoded home addresses of cases over time, these systems can also help us understand the impact of immunity on future case distribution at small spatial scales. All four serotypes of dengue have co-circulated in Bangkok, Thailand, for decades. We characterize the degree to which the homes of dengue cases presenting at a Bangkok hospital between 1995 and 1999 are near each other in space and time. We have found evidence for clustering of cases infected by the same serotype in the same month at distances up to 0.9 km relative to that expected given the overall distribution of cases. This evidence supports the focal nature of dengue dispersal even in a large urban setting. Further, we demonstrate a reduction of homotypic cases with the introduction of temporal lags of greater than 4 months, a reduction of heterotypic cases at lags between 3 and 10 months and an increase in heterotypic cases after 22 months. These clustering patterns are consistent with short term cross-protection between serotypes. At longer time frames, the patterns suggest an increased risk of severe disease from sequential heterotypic infections.

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FOLLOW-UP OF *TRYPANOSOMA CRUZI* RESISTANCE INDUCED BY THE COMPOUNDS BENZNIDAZOLE AND THIOSEMICARBAZONE AND ITS ASSOCIATION WITH P-GLYCOPROTEIN EFFLUX PUMP

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Chagas disease specific treatment is up to now not efficient and presents high toxicity, besides the resistance to the reference drug benznidazole (Bz) for a variety of *T. cruzi* strains. Our group has been focused on the study of the synthetic compound (2-methoxy-styryl)-thiosemicarbazone (2-MEOTIO), a compound that shows trypanocidal activity at concentrations non toxic for the mammal cells. One of the mechanisms related to drug resistance in different pathogenic protozoa is the transport of drugs across the membrane by ATP-binding cassette (ABC) transporters involving P-glycoprotein (Pgp), an efflux pump implicated in multidrug resistance. In the present study, we followed-up the induction of resistance in *Trypanosoma cruzi* epimastigotes to the compounds Bz and 2-MEOTIO and evaluate the possible participation of Pgp in this process. We also investigate the persistence of resistance after morphological transformation to the metacyclic trypomastigote stage. For the trypanocidal activity assay epimastigotes (Y strain) were incubated with Bz and 2-MEOTIO at different concentrations. The IC50 was determined and both drugs were then used to induce resistance in epimastigotes. After 15 passages under drug pressure, it was obtained resistant parasites as demonstrated by a significant increase of the $IC_{s_{50}}$ for both compounds. In order to verify the influence of Pgp, in the mechanism of drug resistance in *T. cruzi,* it was analyzed the efflux of the fluorescent probe Rhodamine 123 (Rho-123) by resistant and wild-type epimastigotes. The assay was realized in the presence/absence of verapamil or cyclosporine A (Pgp inhibitors) and the Rho-123 fluorescence was analyzed on a FACScan flow cytometer. It was observed a significant concentration-dependent reduction of Rho-123 fluorescence in resistant parasites in comparison with wild-type and also the reversion of Rho-123 efflux in the presence of Pgp inhibitors. Metacyclic trypomastigotes were obtained by keeping epimastigotes for 20 days in LIT medium without reposition. The IC50 values for resistant and wild-type trypomastigotes treated with Bz and 2-MEOTIO was then calculated and compared and it was observed an increase of those values. In this work it was demonstrate the participation of Pgp in *T. cruzi* epimastigote resistance induced by Bz and 2-MEOTIO, as well as the persistence of resistance after the differentiation to the metacyclic trypomastigote stage.

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SAFETY AND EFFECTIVENESS OF MEGLUMINE ANTIMONIATE IN THE TREATMENT OF ETHIOPIAN VISCERAL LEISHMANIASIS PATIENTS WITH AND WITHOUT HIV CO-INFECTION

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In sub-Saharan Africa, visceral leishmaniasis (VL) is treated with either PentostamTM (sodium antimony gluconate) or generic sodium stibogluconate (SSG), except in Uganda where Glucantime® (meglumine antimoniate) has been in use for at least a decade. Between January 2008 and February 2009, 54 Ethiopian VL patients were treated with

Glucantime. The medical charts of these patients were reviewed to assess the effectiveness and safety profile of Glucantime in a routine healthcare setting. None of the patients from south Ethiopia (n=24) and 46.4% of the patients from north Ethiopia (n=30) were HIV co-infected. At completion of treatment (Day 31), cure rates were 78.6% (95% CI 59.0-91.7%) in north Ethiopia and 100% (95% CI 85.8-100%) in south Ethiopia. Thirty-three non-serious and six serious adverse events (two pancreatitis, one renal failure and three deaths) were observed in 26 patients. One-third of the non-serious adverse events were due to biochemical pancreatitis. During treatment, a case-fatality rate of 10.0% in north Ethiopia and 0.0% in south Ethiopia was noted. These data show that Glucantime can be as effective as Pentostam or SSG in HIV-negative patients. The data also point to clinical pancreatitis as a safety concern, especially in patients with HIV co-infection.

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THIOSEMICARBAZONES AND SEMICARBAZONES AS POTENT TRYPANOCIDAL AGENTS

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A specific treatment, with more efficiency and less toxicity for Chagas'disease, is the main objective of this study. Thiosemicarbazones and semicarbazones are classes of compounds with medical interest because of their capacity to inhibit the growth of several pathogens. As part of our research program on chemotherapy against diseases caused by trypanosomatids, five thiosemicarbazones and semicarbazones were synthesized, in order to reach a high trypanocidal activity with low toxicity. *In vitro* experiments using *T. cruzi* were carried out to evaluate the effect of those compounds against culture trypomastigotes and amastigotes lodged in both mouse and human macrophages. The enzymatic activity of the nitric oxide synthase (NOS) of the parasite was also evaluated considering that it would be a potential target for those compounds. Besides, the *in vitro* toxicity of those derivatives was evaluated on murine macrophages. In general, thiosemicarbazone derivatives were most effective and among them the 4-N-(2'-methoxy styryl)-thiosemicarbazone was chosen, to compare its *in vitro* effect against amastigotes lodged in both mouse peritoneal and human macrophages. A potent trypanocidal effect of this molecule was observed, more pronounced against parasites interiorized in human macrophages. A potential target in the parasite for this compound was also evaluated by measuring the NOS through NADPH consumption. A significant decrease in the enzyme activity was observed. No macrophage toxicity was observed by any of the compounds, indicating that their activity was specific for the parasite forms investigated. It is important to note the use of human macrophages once these results would be closer to the *in vivo* effect. The significant inhibition of NOS activity is important, considering that this enzyme is a defense mechanism for the parasite allowing its survival within the host macrophage. These data are very promising and a challenge for further studies with these classes of compounds.

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DEVELOPMENT OF NOVEL CHEMOTHERAPY FOR CHAGAS' DISEASE

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Chagas' disease, caused by the parasite *Trypanosoma cruzi*, is a neglected parasitic disease that affects millions of people worldwide mostly residing in Latin America. As a consequence of massive immigration and eco-tourism, numerous patients now live in developed countries including an estimated 1-300,000 in the US. Our group has focused on developing new chemotherapy for Chagas' disease, without the significant toxicity and severe side effects associated to current therapy with benznidazole or nifurtimox. Cruzain (a.k.a cruzipain) and CYP51 are two validated drug targets in the infectious agent *T. cruzi*. Effective irreversible and reversible inhibitors of the major cysteine protease cruzain are available. We identified K11777 as a potent protease inhibitor that rescued animals form lethal infection and characterized its mechanism of action. Briefly, K11777 blocked the autocatalytic processing of the cruzain prodomain inducing accumulation in the Golgi compartment and parasite death. Moreover, K11777 treatment also induced activation of macrophages infected with *T. cruzi* and was cidal for the pathogenic intracellular parasite. We have now identified new derivatives with up to 10-fold more potency than K11777 that are being characterized further and target both cruzain and CYP51 in *T. cruzi*.

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A PROMASTIGOTES RESCUE ASSAY FOR ANTILEISHMANIAL SCREENING OF COMPOUNDS AGAINST INTRACELLULAR *LEISHMANIA DONOVANI* AMASTIGOTES IN THP1 HUMAN ACUTE MONOCYTIC LEUKEMIA CELL LINE

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Leishmania donovani is the causative agents for visceral leishmaniasis (VL), the most fetal form of the disease. The choice of drugs available to treat leishmaniasis is already limited, and even these suffer from limited efficacy and toxicities at therapeutic doses. Most of the first line treatment drugs have already lost their utility due to increasing multiple drug resistance. The current pipeline of antileishmanial drugs is also severely depleted. Sustained efforts are needed to enrich new antileishmanial drug discovery pipeline, which primarily rely on the availability of suitable *in vitro* screening models. The *in vitro* promastigotes and axenic amastigotes assays primarily used for antileishmania drug screening, may not be appropriate due to significant biochemical and molecular differences among these parasite stages compared to intracellular amastigotes. The assays with macrophage- amastigotes models are considered closest to the pathophysiological conditions of VL and are the most appropriate for *in vitro* screening. A promastigotes-rescue assay with transformed THP1 cells infected *in vitro* with *Leishmania donovani* has been developed for screening the pure compounds and natural products extracts and determination of efficacy against the intracellular Leishmania amastigotes. The assay involves controlled lysis of infected macrophages, release of amastigotes and transformation of live amastigotes to promastigotes. The assay compares well with currently used microscopic method, transgenic reporter gene and digital image analysis assays. The assay is highly robust and better compared to microscopic method and measures only the live intracellular amastigotes compared to reporter gene and image analysis assays, which measure both live and dead amastigotes. The assay has

been validated with current battery of antileishmania drugs and has been successful applied for large-scale screening of pure compounds and a library of natural products fractions.

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EVALUATION OF ANTIBODY RESPONSE AGAINST *GLOSSINA* SALIVA IN CATTLE: AN ALTERNATIVE APPROACH TO ASSESS EXPOSURE OF TSETSE BITES

Martin Bienvenu Somda[1], Zakaria Bengaly[1], Emilie Thérèse Dama[1], Anne Poinsignon[2], Sylvie Cornelie[2], Françoise Mathieu-Daude[2], Franck Remoue[2], Antoine Sanon[3], Bruno Bucheton[4]

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Tsetse flies are the notorious transmitters of African Animal Trypanosomosis, the main livestock disease caused by the *Trypanosoma* parasite on the sub-Saharan Africa. Currently, there are not efficient methods to estimate zones at risk or the impact of *Glossina* control campaigns. Therefore, it is important to develop effective tools in order to target the areas where the populations are exposed to a high risk of transmission. The saliva from hematophagous arthropods contains bioactive compounds which play a role in pathogen transmission and induce an immune response into their hosts. Several studies have shown that the antibodies (Ab) against salivary antigens could be used as biomakers of exposure to vector-borne diseases. Our study aims to develop a sero-epidemiological tool to evaluate the cattle exposure to tsetse bites. IgG response against *Glossina* saliva was assessed by ELISA on (i) 101 bovine sera from Burkina Faso of which 48 were sedentary cattle from a tsetse free area and 53 were from a tsetse infested area and (ii) on bovine that were experimentally exposed to tsetse flies and other bloodsucking arthropods. High anti-saliva responses were detected in cows from the tsetse infested area and showed a significantly higher response during the hot dry season. Furthermore, there was a positive association between the anti-saliva response and the risk of being infected by trypanosomes (p=0.03). We have assessed cross-reactions between *Glossina* saliva and other hematophagous vectors. Only the saliva of *Tabanidae spp* induces cross-reactions. In any case, Ab response to *Glossina spp* saliva is transient and decreases within 4 weeks after the stop of experimental exposure. This character is a major advantage to design a biomarker of exposure based on Ab response to tsetse saliva. Immunoproteomic analysis was performed and several specific salivary antigens of *Glossina* were identified. Mass spectrometry is underway. In perspectives, synthetic peptides will be design so as to develop an easy and reproducible test with higher sensitivity and specificity to *Glossina*.

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VALIDATION STUDY OF PCR-MINIEXON FOR DIAGNOSIS OF CUTANEOUS LEISHMANIASIS IN COLOMBIAN PATIENTS

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We evaluated the polymerase chain reaction (PCR) accuracy from Giemsa-stained and methanol-fixed slides from 228 Colombian patients, clinically suspected of having cutaneous leishmaniasis comparing it with a composite reference standard. This reference standard included clinical, histopathological, epidemiological and laboratory criteria. 115 were cases and 113 were non cases according to it. Two samples from cases were excluded from the statistical analysis due to the presence of PCR inhibitors. Patient classification and test application were carried out independently by two blind observers. Miniexon primers were used to amplify a 226-230 bp fragment for *Viannia* subgenus or a 308 bp fragment for *Leishmania amazonensis*; 340 bp fragment for *L. mexicana* or a 418 bp fragment for *L. chagasi*. PCR was positive for 124 samples and negative for 102 samples. All positive samples belonged to *Viannia* subgenus. PCR showed 89.4% (95% CI: 82.4-93.8%) sensitivity and 79.6% specificity (95% CI: 71.3-86%), the positive likelihood ratio was 4.391 (95%CI: 3.03-6.36) and the negative likelihood ratio was 0.133 (95% CI: 0.077-0.229%). The area under the curve in ROC analysis was 0.845 (95% CI: 0.791-0.890) (p<0.0001). 19 of the 23 non cases that showed a positive PCR had an infectious disease (8 gram positive skin infections, 5 allergic reaction to insect bite, 4 sporotrichosis, 1 chromomycosis and 1 myasis) considered in the differential diagnosis. We believe that these false positives may be due to those pathogens and *Leishmania* share miniexon sequences or those patients had mixed infections or these results are mistakes of this assay that impact the specificity. In conclusion, ROC analysis showed that PCR-miniexon from Giemsa-stained and methanol-fixed slides has a good sensitivity and an acceptable specificity in the diagnosis of cutaneous leishmaniasis.

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GENOTYPING OF AMERICAN TEGUMENTARY LEISHMANIASIS USING NON-INVASIVE SAMPLES: PRELIMINARY RESULTS

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Current WHO guidelines recommend genotyping of *Leishmania* as part of the standard management and treatment of American Tegumentary Leishmaniasis (ATL). In Peru, ATL control programs have reduced resources to perform genotyping of *Leishmania* species due to the rural condition of this disease and the limited number of centers enabled to perform the process. Cultures or biopsies have been the most accepted samples to perform genotyping; however, those are invasive, time-consuming and they are not necessarily the best alternatives in terms of tolerability, cost, efficacy and simplification of process. We want to evaluate the usefulness of PCR amplification in lymphatic secretion as a non-invasive sample for the genotyping of ATL. As part of the IDRI-LCVTC-202 LEISH-F2+MPL-SE vaccine trial, genotyping of *Leishmania* by PCR was evaluated in patients coming from endemic areas of *L. (V) peruviana*. Samples were obtained using three methods: biopsies, dermal scrapings and lymphatic secretion. Lymphatic secretion was collected with microhematocrit capillary tubes without any kind of cleaning except debriding of crusty material. Cleaning with Isopropyl pads was performed before dermal scrapings and biopsies were taken with sterile lancets and 2-mm punches. For *L. (V.) peruviana* genotyping, the PCR target was mannose phosphate isomerase gene (MPI), using allele specific primers that distinguish easily between *L. (V.) peruviana* and *L. (V.) braziliensis* or *L. (V.) guyanensis*. Seventeen patients were confirmed as ATL by PCR, scraping or culture of which 14 were identified as *L. (V). peruviana*. The 3 non-identifiable lesions did not have enough DNA to genotype the species. The proportion of samples genotyped by PCR based on each sampling method was as follows: 13 of 17 (76.5%) using biopsies, 11 of 17 (64.7%) using dermal scrapings and 14 of 17 (82.3%) using lymphatic secretion; however, it was not possible to identify statistical differences between methods. In conclusion, the proportion of samples genotypable by PCR using lymphatic secretion may be similar to or even higher than that achieved using invasive samples such as biopsies. We aim to show that genotyping of ATL can be done without invasive procedures while achieving a high performance. These promising results encourage us to conduct better studies to identify differences in diagnostic performance.

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TRANSPORT OF GLUCANTIME® IN HUMAN MACROPHAGES AND ITS INVOLVEMENT IN ANTILEISHMANIAL TREATMENT OUTCOME

Maria Adelaida Gomez, Adriana Navas, Laura Jimena Rojas, Nancy Saravia

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Failure of antileishmanial chemotherapy and the capacity of species of the *Leishmania Viannia* subgenus to cause chronic and persistent infection set the stage for drug tolerant/resistant parasite populations. Host factors are prominently involved in treatment outcome, yet the mechanisms that regulate drug traffiking and metabolism in host cells, and their variation among individuals are unknown. Gene expression profiles of 84 drug transporters were examined in human macrophages following *in vitro Leishmania panamensis* infection and/or exposure to Glucantime®. Ten candidate transporters, including ABC, SLC and AQP family members, whose expression was differentially regulated in response to infection or drug treatment were identified. Transporter gene expression profiles in macrophages from patients who failed or responded to Glucantime® treatment suggest that events of active efflux, reduced uptake, intra-vesicular sequestration and cytoplasmic accumulation, may differentially operate in both patient groups. Downregulation of MRP-1 (GSH-Sb efflux pump) and AQP-9 (an Sb-influx channel) in macrophages from drug responder (n=5) and non-responder (n=7) patients, respectively, strongly suggest a mechanism of transporter-mediated modulation of drug accumulation in host cells from these patient groups. Conversely, ABCB6 and SLC7A11 were upregulated by drug treatment in macrophages from both patient groups suggesting a novel and basic role in intracellular drug mobilization and/or accumulation. Our data shows that *Leishmania* infection and/or drug treatment modulates macrophage transporters for GSH and GSH conjugates, heavy metals and cysteine, strongly suggesting their involvement in transport and detoxification of the drug. These findings provide insights into the mechanisms of drug uptake, efflux and accumulation by the host cells, and variations among individuals that could determine the outcome of treatment.

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SYBR GREEN-BASED QUANTITATION OF *LEISHMANIA* PARASITE LOAD IN LESION BIOPSIES FROM PERUVIAN PATIENTS WITH TEGUMENTARY LEISHMANIASIS

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American tegumentary leishmaniasis (ATL) is a major public health problem in several areas of Latin America. It is characterized by a significant clinical pleomorphism, which has been related to both the infecting *Leishmania* species and the human immune response. Cutaneous leishmaniasis (CL) and mucocutaneous leishmaniasis (MCL) are the main clinical forms of ATL. Real-time quantitative PCR techniques have been utilized for the detection, identification and quantification of New World *Leishmania* species; however, the available techniques generally use expensive labeled probes or lack adequate sensitivity, depending on the target and amplification method used. In this study, we developed a SYBR Green-based real-time quantitative PCR (qPCR) assay to evaluate the *Leishmania* parasite load in Peruvian patients with CL and MCL. Our assay targets the *L. (Viannia)* minicircle kinetoplast DNA (kDNA) that is present at about 10000 copies per parasite. The assay has a linear detection range of 50000 to 0.005 parasite DNA equivalents per reaction. Thirty four lesion biopsies from confirmed CL (n=14) and MCL (n=20) patients were analyzed, among which the parasite numbers ranged from 1 to 144000 per µg of DNA. Patients with CL had significantly higher parasite loads (median 648 parasites/µg DNA) than patients with MCL (median 31 parasites/µg DNA) (P=0.02, Mann-Whitney test). This finding is consistent with earlier observations reported by others, based on histopathology and microscopy of stained tissues. Differences in parasite loads between CL and MCL could reflect the distinct immune responses reported for these clinical forms and warrant further investigation. Our kDNA qPCR assay is highly sensitive and affordable for its implementation in resource-limited settings. It promises to be a useful tool in ATL for studying host-parasite interactions and could be used to guide chemotherapy follow-up and prognosis of disease outcome.

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DEVELOPMENT AND VALIDATION OF A SPECIFIC PCR DIAGNOSTIC PROTOCOL FOR *LEISHMANIA AETHIOPICA*

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Leishmania aethiopica is the main cause of cutaneous leishmaniasis in the Ethiopian highlands, and a growing health burden as more endemic areas

are reported. While the fact that *L. aethiopica* is a clear health threat in Ethiopia, the true endemic extent of the parasite requires further study. Molecular methods, particularly PCR, are commonly used for the detection of *Leishmania* parasites, but species specificity and sufficient sensitivity has often been an issue. Existing protocols specific for *L. aethiopica* required PCR-RFLP, or were not sensitive enough to be clinically applicable. We have developed and validated primers (V5F/V10R) based on the cysteine protease B gene, which is multi copy and polymorphic in *Leishmania*. It is able to differentiate *L.aethiopica* from *L. tropica, L. major, L. donovani, L. infantum*, and *L. chagasi*, and is sensitive enough to detect *L. aethiopica* parasites in biopsy samples alone. These primers have the potential to be extremely useful in a clinical setting for rapid diagnosis of cutaneous leishmaniasis. Additionally, their use in epidemiological studies may aid in better knowledge of the true prevalence and impact of *L. aethiopica* in East Africa.

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A COMBINED DNA VACCINE AGAINST *TRYPANOSOMA CRUZI* REDUCES CARDIAC INFLAMMATION IN DOGS

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American trypanosomiasis is a major neglected public health problem in America. No vaccines are available for the disease. DNA vaccine represents an alternative technology to evaluate, as they induce mainly a Th1 immune response; Th1 responses are needed to control *Trypanosoma cruzi* infections. In this study we tested a DNA vaccines encoding TSA-1 and Tc24; two plasmid construction -that have proven its potential in murine models- in dogs during the acute phase (60 days post-infection). 6 healthy stray dogs were vaccined intramuscularlly with 250µg of each plasmid at days -28 and -14 of infection, while 5 received 500µg of empty plasmid (PcDNA3) in the same regimen. Dogs were experimentally infected with 2000 metacyclic trypomastigotes per kg via intraperitoneal, then, clinical, immunological and pathological features were followed the next 60 days. 5 of 5 dogs of control group developed cardiac arrythmias while only 2 of 6 vaccinated dogs did, but differences did not reach significance (p=0.065; log rank test). Dogs developed a very low parasitemia and no significant differences were found between groups along the observation time. No differences were found for IgG, and isotypes IgG2a and IgG1 between groups either. Cardiac inflammation was significantly lower in vaccinated dogs (p=0.0303; Mann-Whitney test). Lymphocyte counts were significantly higher for vaccinated dogs at day 28 post-infection (p=0.0215; T). Hematocrit was significantly lower in control dogs by day 28 post-infection (p=0.0149; T). Although this DNA vaccine could not avoid infection, and did not alter parasitemia its beneficial effects were observed reducing cardiac inflammation, this effect might be due to a control of the immune system of *T. cruzi* infection, this also led to a minor development of cardiac arrhythmias. Lymphocyte counts may be evidence of an induction of immunity as vaccinated dogs showed higher counts and developed lower degree of inflammation. Control of chronic inflammation was also reflected by higher hematocrit in vaccinated dogs by day 28. Parasitic load is to be measured by qPCR. This results strongly indicate that DNA vaccination has improved the immune response against *T. cruzi* infection during the acute phase in dogs.

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THE NATURAL PRODUCT CYNAROPICRIN INHIBITS *TRYPANOSOMA BRUCEI RHODESIENSE* IN THE ACUTE MOUSE MODEL

Stefanie Zimmermann[1], Michael Adams[1], Reto Brun[2], Matthias Hamburger[1]

[1]University Basel, Basel, Switzerland, [2]Swiss Tropical and Public Health Institute, Basel, Switzerland

In a medium throughput screen of 1800 plant and fungal extracts for antiplasmodial, antitrypanosomal and leishmanicidal activity, a dichloromethane extract of *Centaurea salmantica* L. (*Asteraceae*) showed strong inhibition of *Trypanosoma brucei rhodesiense*. The active constituent was shown to be a guajanolide sesquiterpene lactone. Against *T. brucei rhodesiense* cynaropicrin had an IC_{50} of 0.3 µM. It was ten and fifteen times less active against *Plasmodium falciparum* (IC_{50}: 3.0 µM) and *T. cruzi* (IC_{50}: 4.4 µM), respectively. In a primary *in vivo* study with six mice infected with *T. b. rhodesiense* bloodstream forms were treated daily with 5 and 10 mg/kg bid cynaropicrin i.p. for three consecutive days. When treated with 10 mg/kg bid the mice showed 92% decreased parasitemia on day 7 postinfection. The test animals died of the infection on day 12, which is 9 days after the termination of the treatment, whereas the control animals died within six days. This is the first study of a natural product showing *in vivo* activity against *T. b. rhodesiense*. Preliminary structure activity studied will be shown.

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EFFICACY OF RADIO-FREQUENCY INDUCED HEAT THERAPY VESUS INTRALEISONAL SODIUM STIBOGLUCONATE TREATMENT IN LOCALIZED CUTANEOUS LEISHMANAISIS CAUSED BY *LEISHMANIA TROPICA* IN INDIA

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Localised cutaneous leishmaniasis (LCL) is a wide spread protozoal infectious disease caused by *Leishmania* parasite. LCL is endemic in the Bikaner, India and causative agent being L. tropica. In search of

a well tolerated, effective therapy with good compliance, we used radiofrequency heat therapy (RFH) and compared it with twice weekly intralesional sodium stibogluconate (SSG). One Hundred fresh established cases of CL were included in the present study. Alternate patient were categorized into two groups, Group A and B of 50 each. GroupA patients were treated with RFH (50°C for 30 seconds) once. Group B patients were given seven, twice weekly intralesional SSG in dosage of 50mg/cm2 of lesion. Lesions were evaluated at 6th, 8th, 10th 12th, 16th, 20th and 24th weeks. RFH and intralesional SSG injection were well-tolerated. Complete cure rate of lesions at 6th, 8th, 10th, 12th and 20th weeks were 24%, 42%, 50%, 82% and 98% in group A and 30%, 44%, 56%, 76% and 92% in group B respectively. In conclusion, both modalities are effective and well-tolerated. Intralesional injections of SSG are painful, cause localized edema, requires several visits, whereas RFH is a ruggedized, non invasive, painless, battery operated method, requires single session, cosmetically more acceptable. So, RFH is better alternative to intralesional injections of SSG in resource poor country like India.

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INTERFERON-Γ RELEASE ASSAY (MODIFIED QUANTIFERON) AS A POTENTIAL MARKER OF INFECTION FOR *LEISHMANIA DONOVANI*, A PROOF OF CONCEPT STUDY

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In areas endemic for visceral leishmaniasis (VL), a large number of infected individuals mount a protective cellular immune response and remain asymptomatic carriers. We propose an interferon-γ release assay (IFN-γRA) as a novel marker for latent L*eishmania donovani* infection. We modified a commercial kit (QuantiFERON) evaluating five different leishmania-specific antigens; H2B, H2B-PSA2, H2B-Lepp12, crude soluble antigen (CSA) and soluble leishmania antigen (SLA) from *L. donovani* with the aim to detect the cell-mediated immune response in VL. We evaluated the assay on venous blood samples of active VL patients (n=13), cured VL patients (n=15), non-endemic healthy controls (n=11) and healthy endemic controls (n=19). The assay based on SLA had a sensitivity of 80% (95% CI= 54.81-92.95) and specificity of 100% (95% CI= 74.12-100). Our findings suggest that a whole-blood SLA-based QuantiFERON assay can be used to measure the cell-mediated immune response in *L.donovani* infection. The positive IFN-γ response to stimulation with leishmania antigen in patients with active VL was contradictory to the conventional finding of a non-proliferative antigen-specific response of peripheral blood mononuclear cells and needs further research.

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LYMPH NODE DYSFUNCTION IN EXPERIMENTAL MODEL OF MALNUTRITION AND VISCERAL LEISHMANIASIS

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Undernutrition is a key factor for the development of visceral leishmaniasis (VL). However, the mechanistic relationship between malnutrition and the course of VL at the immunological level is poorly understood. In a murine model of polynutrient (protein, iron and zinc) deficiency that resembled moderate human malnutrition in children we found that malnutrition led to increased early visceralization following cutaneous *Leishmania donovani* infection. This increased early visceralization was related to altered lymph node barrier function so we sought to investigate the mechanism by which this occurs. Well-nourished (control) and malnourished mice were inoculated intradermally in the footpad with *L. donovani* promastigotes and the popliteal lymph nodes harvested 3 days post-infection. There was no gross difference in the lymph node histopathology or in the proportion or localization of endothelial cells, fibroblast reticular cells (FRC), B cells, or T cells between the infected malnourished and well-nourished groups as demonstrated by immunohistochemistry and flow cytometry. However, by flow cytometry the total number of DC, neutrophils and macrophages was significantly less in the lymph node of malnourished *L. donovani* infected mice compared with the well-nourished controls ($p<0.01$). With PCR array and real-time PCR techniques, we found that malnourished infected mice showed significant down-regulation of the expression of a group of genes that have been shown to play a role in dendritic cell chemoattraction and retention: chemokine (C-C motif) ligand 2 (CCL2), CCL7, CCL11, chemokine (C-C motif) receptor 2 (Ccr2), C-X-C motif chemokine 10 (CXCL10), interferon- γ, and secreted phosphoprotein 1 ($p<0.05$ for all). Notably, CCL2, CCL7, and CXCL10 are known to be produced by FRC in the lymph node. These results suggested that the impaired capacity of the lymph node to act as a barrier to dissemination of *L.donovani* infection is accompanied by dysregulation of the molecular signals involved in cellular trafficking and retention in the lymph node.

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CD8+CD28- T CELLS FROM CHRONIC CHAGASIC PATIENTS INVOLVED IN MYOCARDIC DESTRUCTION

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Human infection with *Trypanosoma cruzi* leads to Chagas disease, which presents as several different clinical phenotypes ranging from an asymptomatic form to a severe dilated cardiomyopathy. Several groups have demonstrated that T cells play a critical role in cardiac pathology as well as in immunoregulation during chronic disease. Given that CD28-negative T-cells are so frequent in chagasic patients and considering the precise phenotype, function and specificity of these cells remain elusive, this study was designed to better characterize CD28- cellular subsets in Chagas disease context. Patients from the polarized forms, indeterminate and severe cardiopathy, were carefully selected and screened for CD28 expression in peripheral blood by flow cytometry. Disease activity correlated with lack of CD28 expression since CD28+ cells were significantly less frequent in patients with severe cardiac damage. Cellular analysis with regards to their activation, migration and cytotoxic potential were performed by flow cytometry after *in vitro* stimulation with cardiac and parasite antigens. Our results show that CD4+CD28- T cells from chagasic patients display a phenotype related to effector functions (high expression of CD11a, HLA-DR and granzyme A). Previously, we have shown a correlation between CD4+CD28- cells from indeterminate and cardiac chagasic patients and the expression of IL-10 and TNF-α, correspondingly. These data suggest that CD4+CD28- T cells from indeterminate and cardiac patients, despite of their similar characteristics, exert their activities differently in asymptomatic or symptomatic patients, controlling or exacerbating the disease, respectively. Previous data showing a high frequency of CD8+ cells in chagasic myocardial tissue in association with our results showing CD8+CD28- T cells as activated, ended differentiated, able to migrate and having enhanced cytotoxic ability in a heart/parasite antigens environmental, might indicate that CD8+CD28- T cells are important effector cells in lesion site causing cardiac tissue damage observed in Chagas disease.

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IMMUNOLOGICAL PROFILE OF ASYMPTOMATIC LEISHMANIASIS INDIVIDUALS AFTER VISITING ENDEMIC AREAS IN PERU

Francesca Falconi[1], Angela Privat-Maldonado[1], Patricia Reyes-Uribe[1], Maria Cruz[2], Ivan Best[1], Jorge Arevalo[1]

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People who visit Leishmaniasis endemic areas could be exposed to the parasite but would not necessarily present any clinical manifestation. We decided to assess immune factors contribution to disease outcome. Because host immune response plays a central role in determining disease outcome, we investigated the cellular and humoral immune response in asymptomatic leishmaniasis patients. Sera and blood were collected from individuals with cutaneous (CL; 7), mucosal (ML; 12) leishmaniasis and those without any sign of the disease (28). The control healthy subjects (HS; 8), never visited endemic areas. Asymptomatic (ASY) were defined as individuals who showed T-cell proliferation with no disease. They represented 54% of those who visited endemic areas (SI median=4.70). ASY presented low levels of IFNγ, TNFα and IL10 (medians= 18.60, 6.83 and 31.50, respectively), but a remarkably low IFNγ/IL10 ratio (median=0.32) ($p<0.05$, when compared with HS). ELISA for IgG isotypes showed that IgG3 and IgG1 were detected in 40% and 6.7% of ASY respectively. These levels of cytokines and IgG isotypes were considerably lower than corresponding values from CL/ML patients ($p<0.01$). Exacerbated pro-inflammatory response were found in CL and ML (Medians: SI=77.60 and 22.20; IFNγ=3259.48 and 4,673.70; TNFα=66.30 and 155.90; IFNγ/IL10 ratio=61.27 and 64.30) respectively. IgG1 and IgG3 were detected in most of the CL/ML samples (100% and 83.3%). Immune response in ASY was characterized by both, moderate cell proliferative response and production of IFNγ and TNFα when compared with CL/ML, despite similar IL10 production in these groups. Furthermore, it is interesting to note the presence of IgG3 and absence of IgG1 in ASY, whereas both are present in CL/ML patients. This fact might suggest that other factors different than IL10 could be involved in the modulation of Th1 response in ASY.

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IMMUNOLOGICAL PROFILE OF ASYMPTOMATIC LEISHMANIASIS INDIVIDUALS AFTER VISITING ENDEMIC AREAS IN PERU

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People who visit Leishmaniasis endemic areas could be exposed to the parasite but would not necessarily present any clinical manifestation. We decided to assess immune factors contribution to disease outcome. Because host immune response plays a central role in determining disease outcome, we investigated the cellular and humoral immune response in asymptomatic leishmaniasis patients. Sera and blood were collected from individuals with cutaneous (CL; 7), mucosal (ML; 12) leishmaniasis and those without any sign of the disease (28). The control healthy subjects (HS; 8), never visited endemic areas. Asymptomatic (ASY) were defined as individuals who showed T-cell proliferation with no disease. They represented 54% of those who visited endemic areas (SI median=4.70). ASY presented low levels of IFNγ, TNFα and IL10 (medians= 18.60, 6.83 and 31.50, respectively), but a remarkably low IFNγ/IL10 ratio (median=0.32) ($p<0.05$, when compared with HS). ELISA for IgG isotypes showed that IgG3 and IgG1 were detected in 40% and 6.7% of ASY respectively. These levels of cytokines and IgG isotypes were considerably lower than corresponding values from CL/ML patients ($p<0.01$). Exacerbated pro-inflammatory response were found in CL and ML (Medians: SI=77.60 and 22.20; IFNγ=3259.48 and 4,673.70; TNFα=66.30 and 155.90; IFNγ/IL10 ratio=61.27 and 64.30) respectively. IgG1 and IgG3 were detected in most of the CL/ML samples (100% and 83.3%). Immune response in ASY was characterized by both, moderate cell proliferative response and production of IFNγ and TNFα when compared with CL/ML, despite similar IL10 production in these groups. Furthermore, it is interesting to note the presence of IgG3 and absence of IgG1 in ASY, whereas both are present in CL/ML patients. This fact might suggest that other factors different than IL10 could be involved in the modulation of Th1 response in ASY.

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FIRST REPORT OF NATURAL *LEISHMANIA* INFECTION OF *LUTZOMYIA AURAENSIS* IN MADRE DE DIOS, PERU, DETECTED BY A NOVEL REAL-TIME PCR ASSAY

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Leishmania species of the Viannia subgenus are responsible for most cases of New World leishmaniasis (NWL) in South America. Studying the prevalence and distribution of *Leishmania*-infected vectors is critical to understanding the dynamics of disease transmission and predicting the emergence of new endemic areas. In the year 2010 up to 6 761 new cases of leishmaniasis were reported in Perú. We used a novel real-time PCR assay to detect natural *Leishmania* infections in phlebotomines collected in ten households from a jungle community in Madre de Dios, Peru. Using miniature CDC light traps, we collected a total of 1299 female sand flies belonging to 33 species. *Leishmania* genus was detected by kDNA PCR and species were identified by real-time PCR of the genes 6PGD and MPI that allows the differentiation of up to 6 *Leishmania* species. Seven out of 164 pools (4.3%) were positive for *Leishmania*. We identified four positive pools of *Lutzomyia auraensis*, three with *Leishmania Viannia lainsoni* and one with *Leishmania Viannia braziliensis*. Our findings revealed a large predominance of *Lu. auraensis*, which comprised 63% of all collected sand flies in the study area and its minimal infection prevalence was conservatively calculated at 0.6% (5/821). Other sand fly species infected with *Leishmania Viannia braziliensis* were *Lutzomyia davisi, Lutzomyia punctigeniculata* and *Lutzomyia trinidadensis*. This novel real-time PCR assay allowed us to implicate for the first time *Lutzomyia auraensis*, a common sand fly in this region of the Amazon Basin, as a new carrier of pathogenic species of *Leishmania*. Further studies are needed to assess the importance of *Lutzomyia auraensis* and other sand fly species in the transmission of NWTL in hyperendemic areas of Peru.

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FUNCTIONAL CHARACTERIZATION AND GENETIC DISRUPTION OF IMPDH GENES IN *TRYPANOSOMA CRUZI*

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Trypanosoma cruzi is incapable of de novo purine biosynthesis and is dependent on the salvage of exogenous purines for growth. Inosine monophosphate dehydrogenase (IMPDH) is responsible for the rate-limiting conversion of inosine monophosphate to xanthine monophosphate in the synthesis of guanine nucleotides. The objective of this study was to evaluate the function and essentiality of *T. cruzi* IMPDH enzymes for parasite growth and viability. Two IMPDH enzymes, *TcIMPDH1* and *TcIMPDH2*, have been annotated in the *T. cruzi* genome, and transcriptome analysis of the four parasite life stages reveals high expression of both enzymes in amastigotes. Phylogenetic analysis demonstrated these TcIMPDHs group with other eukaryotic and

prokaryotic IMDPH proteins; interestingly, *TcIMPDH2* clustered with the *Cryptosporidium parvum* IMPDH, a gene apparently obtained in *C. parvum* by lateral transfer from an epsilon-proteobacterium. Bacterial complementation of IMPDH-deficient *E. coli* strains confirmed IMPDH activity of the *TcIMPDH1* gene product, however, no growth of *TcIMPDH2*-bearing colonies suggested an alternative function of *TcIMPDH2* gene products. The *Leishmania major* gene syntenic with *TcIMPDH2* was recently re-annotated as a guanosine monophosphate reductase (GMPR), so we asked if the *TcIMPDH2* might also be a GMPR. However, testing in a GMPR-deficient *E. coli* strain revealed no GMPR activity for either *TcIMPDH1* or *TcIMPDH2*. To further investigate the role of TcIMPDH2, we generated parasite lines where the gene was selectively disrupted by homologous recombination using a drug resistance gene. *TcIMPDH2* null parasites grew normally as epimastigotes and converted to metacyclic trypomastigotes, which were infective in C57BL/6 mice as evidenced by the induction of a strong *T. cruzi*-specific $CD8^+$ T cell response. Currently, we are developing *TcIMPDH1* KO parasites to compare the role of these two apparently functionally distinct genes. Our results confirm the function of *TcIMPDH1* while suggesting no IMPDH or GMPR function for the gene annotated as *TcIMPDH2*. Successful construction of null *TcIMPDH2* parasites suggests this gene is not essential for *T. cruzi*.

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TRYPANOSOMA CRUZI INTERACTS WITH HUMAN ADIPOCYTES AND ITS INFECTION IN HUMAN AND MICE RESULTS IN DIFFERENTIAL SERUM APOLIPOPROTEIN A1 PROFILE

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Chagas disease (CD) is caused by the protozoan parasite, *Trypanosoma cruzi*. Endemic in Central and South America where ~17 million persons are infected, latent infections can persist for decades, causing terminal, cardiomyopathy in ~30% of subjects. According to previous observations, CD patients, even those who die from cardiac complications have a lower incidence rate of atherosclerosis. However, levels of HDL and Apolipoprotein A1 (Apo A1) in CD patients are reported to be normal using ELISA. Recently, our laboratory discovered several novel biomarkers for CD using mass spectrometry (1). We have identified intact Apo A1 as a negative biomarker for CD and several truncated forms of Apo A1 as positive biomarkers for CD. Apo A1 is the principle protein found in high density lipoprotein (HDL). We intended to validate our human Apo A1 findings in the CD1 acute/chronically-infected mice model. However, we demonstrated that murine Apo A1, though sharing approximately 60% similarity with human Apo A1, did not share the same cleavage patterns with human Apo A1. In contrast, Apo A1 in infected mice serum formed several high molecular weight complexes. Adipocytes have recently come under the spotlight of CD research as the reservoir of *T. cruzi* during chronic infections. Because adipocytes and HDL are both major players in the host lipid metabolism and they closely interact with each other, we decided to investigate *T. cruzi* infections in the human adipocyte system. We utilized cultured primary human adipocyte as our *in vitro* model. We discovered that human adipocytes ameliorate *T. cruzi* infection based on the number of *T. cruzi* amastigotes housed by the adipocytes 3-day post infection and confirmed the result using real time PCR. As well, *T. cruzi* interacts closely with cellular lipid storage as observed by light microscopy. The phenomenon will be observed more closely by fluorescent microscopy. Our findings may shed light on the interaction of *T. cruzi* and human host system during the chronic infection stage. It may also uncover the impact of *T. cruzi* on host lipid homeostasis.

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EVALUATION OF THE AMASTIGOTE-SPECIFIC MITOCHONDRIAL MEMBRANE PROTEIN ENCODING GENE DELETED PARASITE AS A LIVE ATTENUATED VACCINE CANDIDATE AGAINST LEISHMANIASIS

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Leishmaniasis causes a significant morbidity and mortality worldwide. There is no vaccine available against leishmaniasis. Various approaches to develop a vaccine against leishmaniasis have had limited success so far. In this study, we are evaluating the use of live attenuated parasites as vaccines. *Leishmania donovani* parasites that are deleted for the amastigote specific protein p27 gene (Ldp27), which is a component of an active cytochrome c oxidase complex, were used as a vaccine in the mouse and hamster models. Ldp27 gene deleted parasites do not survive more than 13 weeks in BALB/c mice or hamsters and do not cause any pathogenesis as indicated by histological analysis of the liver. Immunization with p27 gene deleted parasites for 8 weeks and challenge with wild type virulent *L. donovani* for 12 weeks, showed a significantly lower spleen and liver parasite burden compared to non-immunized mice post challenge. Protection in immunized mice was correlated with the stimulation of multifunctional Th1 type CD4 cells after challenge with the wild type parasite. Mice immunized for 16 weeks, when no p27 gene deleted parasites are observed, and challenged with virulent parasite showed significant reduction in parasite burden post challenge suggesting the participation of memory T cell response in protection from infection. Currently we are investigating the mechanism of memory cell response to live *L. donovani* attenuated parasites in mice. We are also evaluating the vaccine potential of p27 gene deleted parasites in hamsters, a model for leishmaniasis disease.

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AN APPROACH TO DETERMINE THE TRANSCRIPTOME OF *TRYPANOSOMA BRUCEI RHODESIENSE* FROM SLEEPING SICKNESS PATIENTS IN UGANDA

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Trypanosoma brucei rhodesiense is a protozoan parasite that causes the acute form of Human African trypanosomiasis, commonly referred to as Sleeping Sickness. The disease is characterized by two stages, the early haemolymphatic stage and late meningoencephalitic stage, which leads to death if untreated. So far all expression profiling studies of African trypanosomes have been carried out on cultured parasites or high-density mouse infections. However, we have no idea to what extent these parasites are representative of a real human infection. My work is therefore aimed at analyzing the transcriptomes of clinical isolates of *T. b. rhodesiense* from patient peripheral blood and cerebral spinal fluid, using high throughput RNA sequencing technology. Furthermore the genomes from these infective parasites will be sequenced and analyzed for structural variations and possible drug resistance markers. But given the low parasitaemia during active infection, there is a need to amplify the trypanosome RNA above the human cellular RNA background. Hence, using the spliced leader sequence that is attached to the 5' end of all trypanosome mRNAs following *trans*-splicing, I am developing a technique to specifically amplify nanogram concentrations of trypanosome RNA against a background of microgram amounts of human RNA.

Subsequently, the method will be used on patient samples to analyze the transcriptomes of trypanosomes from blood and CSF for possible differential gene expression. Furthermore we would like to know whether the parasites from human infections differ from those cultured in the laboratory.

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MULTILOCUS GENOTYPING REVEALS A POLYPHYLETIC PATTERN AMONG NATURALLY ANTIMONY-RESISTANT *LEISHMANIA BRAZILIENSIS* ISOLATES FROM PERU

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Pentavalent antimonials (Sb^V) remain the mainstay treatment against leishmaniasis worldwide, but their efficacy is threatened by the emergence of drug-resistant parasites, as described in several endemic regions. In order to understand the epidemiological dynamics of Sb^V resistance in zoonotic tegumentary leishmaniasis and its link with treatment outcome, we analyzed the population structure of 24 Peruvian *Leishmania braziliensis* clinical isolates with known *in vitro* antimony susceptibility and clinical phenotype by multilocus microsatellite typing (14 microsatellite loci). The genetic variability as defined by the used loci in the Peruvian isolates was high and the multilocus genotypes were highly divergent from each other. No association was found between the genotypes and *in vitro* drug susceptibility or clinical treatment outcome. These findings, together with the polyphyletic pattern shown by the Sb^V-resistant *L. braziliensis* parasites in the Neighbour-Joining dendrogram might be explained by (i) independent events of drug resistance emergence, (ii) sexual recombination and/or (iii) other phenomena mimicking recombination signals. Interestingly, the polyphyletic pattern observed here is very similar to the one we observed in the anthroponotic *L. donovani,* as reported previously, hereby questioning the role of transmission and/or chemotherapeutic drug pressure in the observed population structure.

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IDENTIFICATION OF SYNTHETIC PEPTIDES FROM *GLOSSINA PALPALIS GAMBIENSE* SALIVA AS BIOMARKER CANDIDATE OF EXPOSURE

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Vertebrates have three efficient systems that make life potentially difficult for hematophagous animals: hemostasis, inflammation, and immunity. These three complex physiological responses interact with each other in order to counteract the host's barriers by using a complex mixture of pharmacologically active components, which are injected into the host skin during the probing and ingestion phases of feeding. In addition, some salivary proteins are immunogenic and can initiate a specific antibody (Ab) response that could be a potential marker of exposure to vector-borne diseases in individuals exposed to bites of arthropod vectors. The first objective of this work was to assess if the IgG response directed against *Glossina* saliva was representative of the human-tsetse contact. For this purpose, saliva from Gpg was collected by an experimental procedure, and reactivity of human plasma from active HAT foci (Guinée) and no infested foci (Burkina) was evaluated by indirect ELISA. Though the highest anti-saliva responses have been observed in the HAT foci of Guinea in contrary to Bobo and Loropeni (non infected foci), a low specificity of this marker have been noted. This is why, secondly we aimed to improve this tool by indentifying synthetic peptides from Gpg saliva to substitute whole saliva for its. To accomplish this purpose, we realized 2D gel electrophoresis follow up to blots with 2 pools of exposed and unexposed individuals' plasmas. Blots alignment by the Samespots software followed by mass spectrometry (MS, LCMS/MS) analysis allowed identifying of seven proteins, all in Gmm and in which three were specific: adenosine deaminase (41KDa), Tsetse Saliva Growth Factor (56-58KDa), and antigen 5 (29KDa). Bioinformatic analysis using epitopes prediction software and alignment Blast led us to target 3 sequences from these three proteins that can be use as good biomarker candidates. In the next stage, the potential biomarker of these peptides will be assessed by ELISA in order to retain the peptide that will give the best result.

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IFN-Γ AND IL-10 SEQUENCES AND EXPRESSION ANALYSIS IN *PEROMYSCUS YUCATANICUS*

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In southeast Mexico, *Peromyscus yucatanicus* is the primary reservoir of *Leishmania (Leishmania) mexicana,* main agent of localized cutaneous leishmaniasis (LCL). We have already demonstrated that this deer mouse reproduced both clinical and subclinical infections by *L. (L.) mexicana* similar to those of humans as well as production of nitric oxide in response to infection. We have also proposed to use *P. yucatanicus* as the experimental model to characterize the immune response to *L. (L.) mexicana* but immunological tools remain lacking. Thus, the aim of this study was to sequence INF-γ and IL-10 of *P. yucatanicus* in order to analyze their expression. These cytokines were amplified by RT-PCR using *P. maniculatus* primers. Partial cDNAs were cloned into p-GEMT Easy and sequenced. The INF-γ was 240 nucleotides long and shared 96% nucleotide identity with *P. maniculatus*. IL-10 sequence had 365 nucleotides and shared 84% nucleotide with *P. maniculatus*. RT-PCR analysis of splenocytes stimulated by Concanavalin A determined that maximal INF-γ expression was at 8 hour however, no peak happened during IL-10 expression. Results supported the use of *P. maniculatus* primers to study the immune response in *P. yucatanicus* infected by *L. (L.) mexicana.*

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TRANSPLACENTAL TRANSMISSION OF *LEISHMANIA INFANTUM* WITHOUT IMMUNOLOGIC TOLERANCE AS A MEANS FOR CONTINUED DISEASE INCIDENCE IN NORTH AMERICA

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Dogs are the predominant domestic reservoir for human *L. infantum* infection. Zoonotic visceral leishmaniasis (ZVL) is an emerging problem in some U.S. dog breeds, with an annual quantitative PCR prevalence of greater than 20% within an at-risk canine population. Classically *Leishmania* is transmitted by infected sand flies and phlebotomine sand flies exist in the United States, means of ongoing *L. infantum* transmission in U.S. dogs is currently unknown. Possibilities include vertical (transplacental/transmammary) and horizontal/venereal transmission. Several reports have indicated that endemic ZVL may be transmitted

vertically. Our aims for this present study were to establish whether vertical/transplacental transmission was occurring in this population of *Leishmania*-infected US dogs and determine the effect that this means of transmission has on immune recognition of *Leishmania*. A pregnant *L. infantum*-infected dam donated to Iowa State University gave birth in-house to 12 pups. Eight pups humanely euthanized at the time of birth and four pups and the dam humanely euthanized three months post-partum were studied via *L. infantum*-kinetoplast specific quantitative PCR (kqPCR), gross and histopathological assessment and CD4+ T cell proliferation assay. This novel report describes disseminated *L. infantum* parasites as identified by kqPCR in 8 one day old pups born to a naturally-infected, seropositive U.S. dog with no travel history. Despite presence of disseminated parasites, pups had a productive T cell proliferative response to parasite antigen at a day of age, also present at 12 weeks old, indicating absence of immunologic tolerance despite *in utero* infection. This is the first report of vertical transmission of *L. infantum* in naturally-infected dogs in North America, emphasizing that this novel means of transmission could possibly sustain infection within populations. Evidence that vertical transmission of ZVL may be a driving force for ongoing disease in an otherwise non-endemic region has significant implications on current control strategies for ZVL, as at present parasite elimination efforts in endemic areas are largely focused on vector-borne transmission between canines and people. Determining frequency of vertical transmission and incorporating canine sterilization with vector control may have a more significant impact on ZVL transmission to people in endemic areas than current control efforts.

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IMMUNOLOGICAL BIOMARKERS FOR THE DETECTION OF LOW-DENSITY INFESTATIONS OF *TRIATOMA INFESTANS* TO SUPPORT CHAGAS DISEASE CONTROL

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Early detection of low density or re-emerging triatomine populations is critical if the efficacy of Chagas disease control programmes is to be maintained. We developed an immunological monitoring approach that can be applied to measure low infestation and re-infestation of *Triatoma infestans* and other reduviid bugs. IgG and IgM antibody responses of guinea pigs and/or chickens to the saliva of *T. infestans* revealed significant differences between sera from animals exposed to low and high numbers of triatomines in the laboratory or field. We identified a highly immunogenic 14.6 kDa salivary protein of *T. infestans* and synthesised a recombinant form (rTiSP14.6) of the antigen. rTiSP14.6 was highly effective for detecting differences in exposure levels of *T. infestans* using IgG and IgM antibody responses of sera from laboratory-exposed and field-exposed chickens from households in Bolivia with low and high infestation levels of *T. infestans*. rTiSP14.6 was also confirmed as a sensitive exposure marker for at least four further triatomine species but it did not cross react with anti-saliva antibodies elicited by unrelated haematophagous arthropods. Differential analysis for IgG and IgM components of the anti-saliva response allowed the detection of very recent exposures due to the short persistence of the IgM response, and thus offers a method to detect re-infestation of triatomines even shortly after insecticide based control programmes have been completed.

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HOST CANDIDATE GENE POLYMORPHISMS AND CLEARANCE OF DRUG-RESISTANT *PLASMODIUM FALCIPARUM* PARASITES IN AFRICA

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The correlation of treatment outcome and molecular tests is not perfect, due in part to individuals who are able to clear drug-resistant parasites. This study aimed to refine and validate molecular markers in the human genome that correlate with the clearance of malaria parasites after specific treatment, despite the drug resistance profile of the protozoan as determined by molecular approach. 4541 samples from six African countries which were known to contain drug resistant parasites were analysed. These parasites were collected from patients who subsequently failed to clear or not their infection following drug treatment, as expected. 4418 samples were successfully analysed, using Sequenom's mass spectrometry iPLEX gold platform, for 67 human polymorphisms (SNPs) on 17 chromosomes, to identify regions of the human genome which contribute to enhanced clearance of drug resistant parasites. An analysis of all data from the six countries revealed significant associations between the phenotype of ability to clear drug-resistant *Plasmodium falciparum* infection and two human immune response loci common to all populations. Overall, two SNPs showed a significant association with clearance of drug-resistant parasites with Odds Ratios of 0.76 (95% CI 0.62-0.92, P= 0.005) and 0.67 (95% CI 0.45-0.99, P= 0.046). The first SNP (located at 5q31-33) has previously been reported to be involved in the control of malaria parasite density, and the locus contains genes encoding Th2 cytokines such as IL-4, IL-5 and Il-13.The second SNP (on chromosome 22) occurs within a Der1-like domain family gene, and linkage to this locus has not previously been reported in studies of malaria. The study showed significant association of three loci in the human genome with the ability of parasite to clear drug-resistant *P. falciparum* in samples taken from six countries distributed across sub-Saharan Africa. One locus has previously been linked to the control of malaria parasite density, and it is possible that patients able to clear drug-resistant infections have an enhanced ability to control parasite growth. Two loci are involved in the Th1/Th2 balance, and the association of SNPs within these genes suggests a key role for antibody in the clearance of drug-resistant parasites. The other locus encodes a protein involved in the degradation of misfolded proteins within the endoplasmic reticulum, and its role, if any, in the clearance phenotype is unclear.

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MALARIA INFECTED ERYTHROCYTES STIMULATE MONOCYTE-DERIVED MACROPHAGE INFLAMMATORY CYTOKINE PRODUCTION WHICH IS IMPAIRED BY HIV INFECTION

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Women in first pregnancy lack protective immunity to *Plasmodium falciparum* and are at increased risk of severe anaemia and low infant birth weight associated with monocyte infiltrates in the maternal circulation of the placenta. Antibody and complement opsonise infected erythrocytes (IE) sequestered in the placenta resulting in macrophage and monocyte phagocytic clearance. Production of pro-inflammatory cytokines and β chemokines by macrophages and monocytes cause an alteration in the placental cytokine balance. This immune response leads to both phagocytosis of IE which restricts infection and promotes antibody-mediated immunity and cytokine production which causes immunopathology and poor outcomes. We hypothesise that modulation of phagocytic function by antibody, complement and acquired cellular immunity is a key determinant of the balance between host protection and immunopathology in malaria infection. In addition, HIV infection increases the risk and severity of pregnancy-associated malaria by poorly defined mechanisms. The aim of this study was to measure human monocyte-derived macrophage (MDM) cell signaling events, inflammatory cytokine mRNA and secretion following exposure to IE opsonised with pooled patient sera with and without HIV-$1_{Ba\text{-}L}$ co-infection. IL-6 transcript and secreted protein were detected following stimulation by unopsonised CS2-IE in the absence of ingestion suggesting internalization is not required. Ongoing work involves determining the pattern recognition receptor responsible. In contrast, IL-1β and TNFα mRNA but not secreted protein was detected in response to IE. Antibody opsonisation resulted in ingestion, and in IL-6, IL-1β and TNFα transcription and secretion. Interestingly, the NF-κB pathway was activated in response to both opsonised and unopsonised CS2-IE. *In vitro* HIV infection reduced MDM phagocytosis and pro-inflammatory cytokine mRNA transcription and secretion. Thus inadequate phagocytosis and cytokine secretion in the context of HIV infection may reflect the poor immune response to parasitemia.

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ELEVATED PLASMA VON WILLEBRAND FACTOR AND PROPEPTIDE LEVELS IN MALAWIAN CHILDREN WITH MALARIA

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In spite of the significant mortality associated with *Plasmodium falciparum* infection, the mechanisms underlying severe disease remain poorly understood. We have previously shown evidence of endothelial activation in Ghanaian children with malaria, indicated by elevated plasma levels of both von Willebrand factor (VWF) and its propeptide. In the current prospective study of children in Malawi with mild and complicated malaria, we investigated the specificity of these markers for malarial disease, using the presence of retinopathy as an indicator that coma in an individual is likely to be due to *P. falciparum* infection. Children with cerebral malaria, mild malaria and controls without malaria were recruited into the study. All comatose patients were examined by direct and indirect ophthalmoscopy. Plasma VWF and propeptide levels were measured by ELISA. Mean VWF and propeptide levels were higher in patients with uncomplicated malaria than in children with non-malarial fevers of comparable severity, in whom mean levels were higher than in non-febrile controls. Mean concentrations of both markers were higher in cerebral malaria than in uncomplicated malaria, and were similar in patients with and without retinopathy. Levels of both VWF and propeptide fell significantly 48 hours after commencing therapy and were normal one month later. In conclusion, plasma VWF and propeptide levels are markedly elevated in both cerebral and mild paediatric malaria, with levels matching disease severity, and these normalize upon recovery. High levels of both markers also occur in retinopathy-negative cerebral malaria cases, many of whom are thought to be suffering from diseases other than malaria, indicating that further studies of these markers are required to confirm their specificity.

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POST-MORTEM ANALYSIS OF *VAR* GENE GROUP EXPRESSION IN MALAWIAN PEDIATRIC MALARIA PATIENTS

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Sequestration of parasitised erythrocytes (pRBC) in the microcirculation of tissues is thought to be important in the pathogenesis of severe *falciparum* malaria. A major variant surface antigen, *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1), expressed on the surface of the pRBC mediates parasite cytoadhesion to vascular endothelium. PfEMP1 is encoded by the *var* multigene family that is sub-divided into three main groups, A, B and C, according to sequence similarities in coding and non-coding sequences. Using real time PCR, we compared abundance of the three main *var* groups utilising the resources of a clinicopathological study of fatal Malawian paediatric malaria patients. 20 patients were recruited and divided into cerebral malaria and parasitaemic control groups. The cerebral malaria group was sub-divided into two groups; circulating and sequestered parasites (CM1); circulating and sequestered parasites as well as perivascular abnormalities (CM2). *var* transcripts A and C were more abundant in the CM2 and the parasitaemic control group. However, a significantly different expression pattern was observed in the CM1 group, with *var* gene group B being more abundant than in the other two groups. This data indicates that perivascular pathogenesis in naturally infected children is associated with differential *var* gene expression in the body.

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C5 ACTIVATION MEDIATES ADVERSE COGNITIVE OUTCOMES IN OFFSPRING FOLLOWING *IN UTERO* EXPOSURE TO EXPERIMENTAL PLACENTAL MALARIA

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Each year approximately 125 million pregnancies are at risk of complications due to malaria infection. Despite the enormity of the problem and the serious implications of placental malaria (PM) on maternal and child health, little is known about the impact of PM on neonatal and infant cognitive development. Previous work has reported increased C5a in women with PM and has suggested that excessive activation of the host immune response, in particular of the complement system, may mediate adverse outcomes of PM. We hypothesized that *in utero* exposure to experimental PM will result in adverse cognitive and

neurological outcomes in offspring and that blockade of complement signaling will improve offspring outcomes. We used a murine model of PM that replicates several aspects of the human PM pathology to examine the impact of PM on the cognitive and neurological development of offspring as well as the role of C5 activation (C5a) using both genetic and receptor blockade strategies. BALB/c wild-type and C5a receptor deficient ($C5aR^{-/-}$) dams were infected at gestational day 13 with the rodent malaria parasite, *Plasmodium berghei* ANKA. In C5a-C5aR blockade studies, BALB/c dams were given anti-C5a antibody. Control animals were offspring brought to term by uninfected dams. Offspring were tested in a battery of behavioural tests to assess learning and memory, hyperactivity and affect. Neurological changes will be examined using MRI and brain tissue analyzed for levels of biogenic amines. We show that offspring of malaria-infected dams display an abnormal behavioural phenotype, including impairments in learning and memory in the novel object recognition test ($p < 0.0005$), and the y-maze test ($p < 0.05$), hyperactivity in the open field test ($p < 0.05$), and increased immobility in the tail suspension test ($p < 0.001$). Genetic and pharmacological blockade of C5a signalling confers protection against the behavioural phenotype ($p > 0.05$ across all tests). There was no significant difference in weight between groups up to eight weeks of age. Our results indicate that malaria-induced activation of the complement cascade contributes to the abnormal behavioural phenotype observed in offspring from malaria-infected dams independent of low birth weight. Moreover, strategies to block C5a signalling in malaria-infected dams rescue the behavioural impairments observed in offspring.

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CAN NEGATIVE EPISTASIS IN THE MALARIA PROTECTIVE EFFECTS OF SICKLE CELL TRAIT (HBAS) AND A+ - THALASSAEMIA BE EXPLAINED BY CYTOADHERENCE?

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The important role of malaria on human evolution is seen through the selection of high frequency polymorphisms that confer protection against malaria. Examples include the sickle cell trait (HbAS) and α+ - thalassaemia. The distribution of these polymorphisms overlaps in some malaria endemic regions where they can therefore be inherited in combination. Although their effect on protection against malaria when inherited individually is well described, little is known about their effect when inherited in combination. We have shown previously that the occurrence of HbAS in combination with α+ - thalassaemia results in a negative epistatic effect with a loss of protection against malaria afforded by each polymorphism individually. The exact mechanism through which this is mediated still remains unclear. We hypothesised that it could be explained by some of the important host-parasite interaction phenotypes associated with the pathogenesis of severe malaria including cytoadherence. Our results show that individually, HbAS and α+ - thalassaemia are associated with a reduced binding ability of *Plasmodium falciparum* infected erythrocytes to important recombinant endothelial receptors CD36 and ICAM1. Interestingly, this reduced binding ability is lost in erythrocytes containing both polymorphisms in a pattern very similar to that seen with the loss of protection from malaria in our epidemiological study. Preliminary results indicate that mechanisms other than altered PfEMP1 expression may be responsible for the changes in binding ability and we are studying the effect of HbAS and α+ - thalassaemia interaction on other important host-parasite related phenotypes including rosetting.

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PLASMODIUM KNOWLESI NORMOCYTE BINDING PROTEIN XA INVASION GENE HAPLOTYPES FROM HUMAN INFECTIONS IN MALAYSIAN BORNEO

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Parasitaemia is associated with disease severity in *Plasmodium knowlesi*, a zoonotic malaria, found in Southeast Asia. *P. knowlesi* has a 24-hour erythrocytic cycle and invades host red blood cells using multiple invasion ligands. The relative efficiency of invasion may be critical to the rate of increase in parasitaemia. Therefore a study was designed to test the hypothesis that *P. knowlesi* invasion gene haplotypes are associated with parasitaemia at presentation. The *P. knowlesi* normocyte binding protein xa (*Pknbpxa),* an invasion gene which is closely related to reticulocyte homologs of *P. falciparum* (Rh2a and Rh2b) was chosen for this work. In the first instance, the full-length *Pknbpxa* (8500bp) gene from *P. knowlesi* isolates from five patients was cloned and sequenced at high stringency. The five patients were recruited at geographically distinct locations and at different time intervals to identify polymorphic sites on the *Pknbpxa* gene. Preliminary analysis of these isolates show that there are 296 polymorphic sites within the gene. Full-length *Pknbpxa* DNA sequences will be presented highlighting polymorphic regions of the gene. A haplotyping method for screening *P. knowlesi* isolates from clinically well-characterized patients is being developed. *Pknbpxa* haplotypes will be analyzed for

associations with parasitaemia and other markers of disease severity in patients with *knowlesi* malaria recruited between January 2008 to April 2011 and the results presented.

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TRANSFER OF 4-HYDROXYNONENAL FROM PARASITIZED TO NON-PARASITIZED ERYTHROCYTES IN ROSETTES: ROLE IN SEVERE MALARIA ANEMIA

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Alpha+ thalassaemia occurs at high gene frequencies in malaria endemic regions. It is associated with low expression of complement receptor 1; reduced formation of rosettes and protection is specific for malaria-anemia. During parasite development, natural hemozoin catalyzes peroxidation of membrane lipids resulting in formation 4-hydroxynonenal (4-HNE) that forms adducts with proteins of the red blood cell membrane resulting in modification of the cytoskeleton responsible for cell deformability which might cause the increased removal of non parasitized RBCs in the spleen as seen in malaria anemia. 4-HNE also has the ability to diffuse to surrounding cells. In this study, therefore, we hypothesized that in rosettes 4-HNE may diffuse from parasitized to non-parasitized RBCs, damage non-parasitized RBCs and induce their phagocytic removal, providing a mechanistic explanation for the association of rosetting with severe malaria anemia and protection afforded by alpha+ thalassemia. Cultures of varO variant of *Plasmodium falciparum* experiments showed transfer of 4-HNE to non-parasitized RBCs within rosettes. Ex vivo analysis of samples from malaria patients (N=40) showed an increase in the proportion of non-parasitized RBCs positive for 4-HNE adducts with increasing rosette frequency. Anemic children had a significantly higher proportion of non-parasitized RBCs positive for 4-HNE adducts than non-anemic children (P=0.0037). However, neither rosette frequency nor proportion of non-parasitized RBCs positive for 4-HNE-adducts differed by thalassemia genotype. 4-HNE adducts are present in infected and uninfected RBCs in culture. There is a dependency of 4-HNE positivity of uninfected RBC with ability to form rosettes. Increased 4-HNE modification of uninfected RBC may explain the correlation between rosetting and malaria anaemia. No correlation was found between thalassaemia genotype and rosetting or HNE modification.

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SEQUESTRATION ASSOCIATED LOSS OF PROTEIN C RECEPTORS LINKS COAGULATION, INFLAMMATION AND ENDOTHELIAL PERMEABILITY IN CEREBRAL MALARIA

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Studies in comatose Thai adults implicate coagulation abnormalities in cerebral malaria (CM). In African children, this relationship is supported by vascular hemorrhagic lesions found in the retina in life and in the brain post mortem. We hypothesised that these lesions indicate intravascular coagulation caused by an imbalance in the thrombin and protein C pathways. Immunohistochemistry (IHC) on post mortem brain tissue from 3 Malawian children with fatal CM showed that the characteristic vascular lesions contain fibrin thrombi, implying a state of thrombin dysregulation. Thrombin-antithrombin complexes in 71 Malawian children with CM were significantly raised when compared with healthy controls (n=19, p=<0.0001), mild febrile illness (n=30, p=<0.05) or uncomplicated malaria (n=30, p=<0.001). To explore the possibility that loss of the anticoagulant receptors thrombomodulin (TM) and endothelial protein c receptor (EPCR) explains this increased thrombin generation and fibrin deposition associated with CM, we performed IHC on post mortem CM brain, gut and subcutaneous tissue. This showed a unique pattern of TM and EPCR distribution with normal expression in healthy vessels but absence in vessels containing malaria parasite infected erythrocytes (IE). To confirm these findings pre-mortem, we developed a novel flow cytometry method to assess ICAM-1, TM and EPCR expression on the microvessels of subcutaneous fat, a tissue that sequesters IE. This revealed a marked decrease in endothelial TM (p=<0.005) and EPCR (p=<0.005) but an increase in ICAM-1 (p=<0.0001) compared with controls. This dysregulation in the thrombin and protein C pathways, with focal loss of the major anticoagulant and endothelial protective receptors at sites of IE sequestration, provides new insight into CM pathogenesis. It provides a link between key pathogenic features of the disease; coagulation, inflammation and vessel permeability. Furthermore, because TM and EPCR are constitutively expressed at lower levels in cerebral vessels, it may explain the vulnerability of the brain in this condition.

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A BIBLIOMETRICS ANALYSIS MALARIA RESEARCH

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Bibliometric analyses indicate trends and patterns within scientific disciplines, national and international strengths and biases in areas of research. In view of the importance of malaria research in the field of human health, it is essential to shed light on research activities carried out around the world. Since publications are one of the major outputs of any research activity which can be quantified, the objective is to map the international and Brazilian research production, skills and competence along with the key research topics/themes of malaria research in the world and in Brazil. We also identify the pattern of malaria research funding, which should provide subsidies to improve public policies. This project is part of the the National Institute of Science and Technology for Innovation in Neglected Diseases (INCT-IDN, http://www.cdts.fiocruz.br/AnnualActivityReport/), based at the Oswaldo Cruz Foundation in Brazil. References on the subject obtained from the Science Citation Index (SCI), PubMed Medline and Scopus databases for the period 1997-2010 are analyzed. A total of 78,742 articles were identified for each database respectively: SCI (41,022), Medline (35,539) and Scopus (2,181). The results show that the research takes place mainly in Europe and North America, not the peripheral countries who are directly affected by the disease. In accordance to other publications of bibliometric analysis of malaria and tropical medicine, the main bias with all these databases is that they are dominated by North American and European publications, and by default, authors. A further bias is their inaccessibility to authors from developing countries. Internationally, the most substantial funding for malaria research came from the National Institutes of Health, and World Health Organization. In Brazil, these were The National Council for Scientific and Technological Development and the State of São Paulo Research Foundation. These data are preliminary and we intend to extend the searches to other databases and also use network analysis to detect collaborative research groups or communities in this area.

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ANTI-GLYCOPHORIN A AND B ANTIBODIES INHIBIT ERYTHROCYTIC INVASION OF *BABESIA DIVERGENS*

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Babesiosis is an emerging tick borne zoonotic disease caused by intraerythrocytic parasites of the genus *Babesia*. *B. microti* and *B.*

divergens have been recognized as important infectious agents in humans and as a threat for transfusion medicine. Although the incidence of *B. divergens* is not as high as *B. microti*, *B. divergens* causes more severe disease. *B. divergens* is the only human babesia parasites that can be propagated in human red blood cells (RBC) *in vitro* with exceptionally high parasitemia (up to 80%). Because of the parallels in the invasion patterns of *Plasmodium* and *Babesia* into human erythrocytes, we are interested in building *B. divergens* into a parasite model to study malarial RBC invasion. In contrast to *Plasmodium*, little is known about the *Babesia*-host cell interaction process. *B. divergens* specifically invades RBCs. Previous work in our lab has identified the Glycophorins A (GPA) and B (GPB) as host receptors for parasite invasion. In this work, we evaluated the participation of GPA and GPB specific domains in RBC invasion by *B. divergens*. Inhibition-of-invasion assays were carried out in the presence of specific monoclonal antibodies directed against various GPA and GPB determinants. We have identified 2 MAbs and 2 Fab fragments that caused a significant decrease in invasion efficiency when compared to the control MAbs. These results represent an important advance toward the identification of binding domains on GPA and GPB involved in the receptor-ligand interactions that facilitate the entry of *B. divergens* merozoite into the human erythrocyte.

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CD36 RECRUITMENT AND ACTIN CYTOSKELETAL REARRANGEMENT VIA P130CAS ENHANCES AVIDITY OF *PLASMODIUM FALCIPARUM* ADHESION ON HUMAN MICROVASCULAR ENDOTHELIUM

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The adhesion of *Plasmodium falciparum*-infected erythrocytes (IRBC) to microvascular endothelium is critical in the pathogenesis of severe malaria. In this study, we used atomic force microscopy to analyze the adhesive force between IRBC and human dermal microvascular endothelial cells (HDMEC). A single live IRBC, attached to the end of the cantilever, served as a functionalized probe that monitored the IRBC-endothelial cell interaction in real time. Our results show that the initial IRBC-HDMEC interaction generated a mean adhesion force of 166.7 ± 4.2 pN pN from the formation of either single or multiple bonds. The adhesion force was reduced by an anti-CD36 but not an anti-ICAM-1 antibody. Interestingly, the adhesion force increased with time as the IRBC was left in contact with the endothelium, so that by 300 seconds the force of adhesion had increased to 559.3 ± 45.5 pN. The time-dependent increase in the strength of adhesion was mediated by CD36, Src family kinases, the adaptor protein p130CAS, and actin cytoskeletal rearrangement in a calcium-dependent manner. The end result was both an increase in the affinity of binding between IRBC and HDMEC that was alkaline phosphatase dependent, and an increase in the number of ligand-receptor pairs through cytoskeletal rearrangement. These findings were supported by fluorescence microscopy imaging that showed recruitment of CD36 and actin in response to ligation of CD36 by IRBC, anti-CD36 or parasite peptide coated beads. Functionally, the increase in adhesion force enabled IRBC to remain adherent in shear stresses of up to 15 dynes/cm2 in a flow chamber assay, an ability that was significantly reduced on HDMEC in which p130CAS expression was knocked down by siRNA. Collectively, the data suggest a novel mechanism by which IRBC adhesion to CD36 activates a signaling pathway that leads to changes in the membrane localization of CD36, actin recruitment and increased binding avidity between IRBC and HDMEC. These results provide new insight into the complex regulation of cytoadherence by *P. falciparum* that could be exploited in the development of novel therapeutic interventions.

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NOVEL MODEL OF SEVERE MALARIAL ANEMIA USING SEQUENTIAL *PLASMODIUM CHABAUDI* AND *P. BERGHEI* INFECTION

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Lack of an adequate animal model of *Plasmodium falciparum* severe malarial anemia (SMA) has hampered the understanding of this highly lethal condition. Therefore, we set out to develop a model in mice that reflected key characteristics of SMA in humans such as relatively low parasitemia and the requirement of pre-immunity. We found that C57BL/6 mice infected with *P. berghei* after recovery from *P. chabaudi* (Pch-Pb) developed an initial 9-10-day phase of relatively low parasitemia and severe anemia followed by a second phase of hyperparasitemia, more profound anemia, reticulocytosis, and death 14-21 days after infection. We studied the first phase of this infection as a model of SMA. Pch-Pb animals had more intense splenic hematopoiesis, higher IL-10/TNF-α and IL-12/IFN-γ ratios, and higher antibody levels against *P. berghei* and *P. chabaudi* antigens, than *P. berghei*-infected (Pb) or *P. chabaudi*-recovered (Pch-sham) animals. Early treatment with chloroquine or artesunate did

not prevent the anemia, suggesting that the bulk of red cell destruction was not due to the parasite. Red cells from Pch-Pb animals had increased surface IgG and C3 by flow cytometry. However, $C3^{-/-}$ mice still developed anemia. Cell tracking of *ex vivo* and *in vivo* labeled red cells and analysis of tissue sections by H&E and immunofluorescent microscopy demonstrated that red cells from Pch-Pb animals were removed at an accelerated rate in the liver by erythrophagocytosis. We conclude that this model is practical and reproducible. Its similarities with *P. falciparum* SMA in humans makes it an appealing system to study the pathogenesis of this condition and explore potential immunomodulatory interventions.

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A SYSTEMS IMMUNOLOGY APPROACH TO UNDERSTANDING THE ACQUISITION AND LOSS OF IMMUNITY TO MALARIA A BETTER

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A better understanding of how immunity to malaria is acquired and lost will be increasingly important as efforts to eliminate malaria proceed, not only to inform novel malaria vaccine strategies, but to understand how interventions such as mass drug administration might influence malaria susceptibility in populations at different stages of malaria control and elimination. In an area of Mali that experiences an intense six-month malaria season we conducted a longitudinal study in which flow cytometry, multiplex cytokine analysis, and genome-wide transcription profiling were used to analyze the impact of acute *Plasmodium falciparum* (Pf) infection on the human immune system Peripheral blood mononuclear cells (PBMCs) were collected from Pf-uninfected children at the end of the dry season, and again after the first malaria episode of the year. Genome-wide expression analysis identified 581 transcripts with a >2 fold change in expression from before to after the malaria episode. Both the innate and adaptive branches of the immune system were affected as evidenced by alterations in the expression of cytokines including IFNγ, TNFα, TGFβ, IL-1 and IL-4, as well as components of Toll-like receptor (TLR), B cell receptor (BCR) and T cell receptor (TCR) signalling pathways. Taken together, preliminary analyses are consistent with the hypothesis that the relatively rapid acquisition and loss of strain-transcendent immunity to severe malaria is due in part to down regulation of pro-inflammatory pathways, a hypothesis that we are testing further in a larger prospective cohort study in Mali and by several *in vitro* experimental approaches. This study highlights how advances in genome-based technology can be applied to longitudinal studies in which malaria exposure is clearly defined to gain insight into how malaria immunity is acquired and lost.

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REGULATION OF AKT/GSK-3 AND NEUROPROTECTION WITH CEREBRAL MALARIA

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Cerebral Malaria (CM) is the most severe neurological complication of *Plasmodium falciparum* infection, resulting in an encephalopathy. Previously, we demonstrated that infection of C57BL/6 mice with *P. berghei* ANKA (PbA) was associated with a vasculopathy which results in a reduction of cerebral blood flow, neuronal dysfunction and axonal impairment. These were associated with neuro-cognitive and motor deficits in CM mice both during acute infection and after successful anti-parasitic treatment. The mechanisms that underlie the lingering effects of the neuronal damage and cognitive deficits in CM remain largely unknown. Here we demonstrate that acute CM results in significantly abnormal phosphorylation of tau protein. This aberrant tau phosphorylation is associated with a significant reduction in the activation of Akt in the brains of mice with CM leading to a significant decrease in Akt inhibition of glycogen synthase kinase (GSK3-β). We demonstrate that regulation of GSK3-beta is neuroprotective in mice with CM. Treatment with lithium chloride, a compound that regulates GSK3- β activity, ameliorates the neuro-cognitive and motor deficits in PbA-infected mice after eradication of the parasite. This indicates that regulation of GSK3- β may reduce neuronal degeneration and have neuroprotective effects in CM. Our data present GSK-3 as a potential therapeutic target which might be used as adjunctive therapy directed at the reduction of neurological dysfunction in children with CM.

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SIMULTANEOUS QUANTIFICATION OF ASEXUAL AND SEXUAL STAGES DURING MALARIA INFECTION

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Determining the prevalence of *Plasmodium falciparum* gametocyte carriers in the population is important because peripheral gametocytemia is a major determinant of transmission in this devastating disease. Detection and quantification of gametocytes in the blood of malaria-infected patients is generally based on microscopy, which is tedious and lacks sensitivity. Newer transcript-based methods have succeeded in quantifying these stages, but there is not a standardized approach for quantifying asexual and sexual stages in the same sample with adequate sensitivity and detailed stage resolution. We have thus developed a model for predicting the sample proportions of two asexual (ring and schizont) and three sexual (early, intermediate and mature) stages based on transcript levels. Using published expression data from across the *P. falciparum* intraerythrocytic infection cycle, we identified five genes with peak expression occurring during one of the aforementioned stages and one gene with constitutive expression across all stages. This provided us with a set of six "sentinel" genes, each of which was informative about particular stage(s). We deliberately selected intron-containing genes so that primers could be designed across exon-exon junctions. This strategy increases the sensitivity of the process by preventing amplification of residual genomic DNA. We performed qRT-PCR for the sentinel genes at several time points during *in vitro* asexual and sexual development time course experiments. Using the qRT-PCR data in combination with stage-specific microscopy data for each time point, we developed a constrained linear regression model to predict the stage composition for an unknown sample. Testing and validation of model accuracy was done using *in vitro* samples with known stage composition. Finally, we applied our qRT-PCR assay and predictive model to a set of patient blood samples and obtained estimates for the percent of parasites in each life cycle stage category. We believe this system may be suitable for malaria control and elimination activities, where the identification and detailed resolution of gametocyte life cycle stages is required.

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EXPLORING PROVIDER AND COMMUNITY RESPONSES TO THE NEW MALARIA DIAGNOSTIC AND TREATMENT REGIME IN SOLOMON ISLANDS

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Improvements in availability and accessibility of artemisinin-based combination therapy (ACT) for malaria treatment and the emergence of multi-drug-resistant parasites have prompted many countries to adopt ACT as the first-line drug. In 2009, Solomon Islands (SI) likewise implemented new national treatment guidelines for malaria. The ACT, artemether-lumefantrine is now the primary pharmacotherapy in SI for *Plasmodium falciparum* malaria, *Plasmodium vivax* malaria and mixed infections. Targeted treatment is also recommended in the new treatment regime through maintenance of quality microscopy services and the introduction of Rapid Diagnostic Tests (RDTs). Ascertaining the factors that influence community and provider acceptance of and adherence to the new treatment regime will be vital to improving the effectiveness of this intervention and reducing the risk of development of drug resistance. To understand community and prescriber perceptions and acceptability of the new diagnostic and treatment regime, 12 focus group discussions and 12 key informant interviews were carried out in rural and urban villages of Malaita Province, Solomon Islands, four months subsequent to roll out of these interventions. Lack of access to microscopy or distrust in the accuracy of diagnostic tools were reported by some participants as reasons for the ongoing practice of presumptive treatment of malaria. Lack of confidence in RDT accuracy negatively impacted its acceptability. Artemether-lumefantrine had good acceptability among most participants; however, some rural participants questioned its effectiveness due to lack of side effects and the larger quantity of tablets required to be taken. Storing of left over medication for subsequent fever episodes was reported as common. To address these issues, further training and supportive supervision of healthcare workers will be essential, as will the engagement of influential community members in health promotion activities to improve acceptability of RDTs and adherence to the new treatment regime. Exploring the extent of these issues beyond the study population must be a priority for malaria programme managers. Practices such as presumptive treatment and the taking of sub-curative doses are of considerable concern for both the health of individuals and the increased risk it poses to the development of parasite resistance to this important first-line treatment against malaria.

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COSTING A LARGE-SCALE IMPLEMENTATION OF INTERMITTENT PREVENTIVE TREATMENT OF MALARIA IN CHILDREN DELIVERED THROUGH COMMUNITY HEALTH WORKERS IN SENEGAL

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Intermittent Preventive Treatment in children (IPTc) is a new strategy for malaria control in areas where transmission is strongly seasonal, shown to be highly effective in clinical trials. In Senegal, a pilot implementation of IPTc was conducted by four district health teams from 2008 to 2010 in order to evaluate the feasibility of delivering IPTc, its safety and effectiveness, when administered on a large scale to rural populations using community health workers (CHWs). In 2010, the intervention was delivered by 46 health-posts to a rural population of 175,000 children under 10 years of age in 1097 villages, and detailed information on costs was collected from each health facility in order to estimate the financial and economic costs of delivery. Delivery was coordinated by the head nurse in each health-post who assigned CHWs to a circuit of villages to visit over a 5-day period in September, October and November, to deliver IPTc house to house to all children 3-120 months of age. Tools were developed to collect data on costs and resource use at four levels: the project, the district, the health post, and the CHW. Data was collected from both "top-down", and "bottom-up" (using facility-based costs and extensive interviews on resource use). Data were collected from all 46 health-posts after each round of administration. The study takes a provider perspective with a focus on costs of implementation at the district level. Each health-post employed from 4-68 CHWs and delivery each month took from 2-5 days. High coverage was achieved with about 90% of eligible children treated each month. When the financial cost of delivery was estimated, it cost $233,714 to administer IPTc to a population of 175,000 children under 10 years of age at a cost of $0.50 per course. High coverage of IPTc can be achieved at moderate cost. Each year CHWs may visit households a number of times for distribution of Vitamin A, bednets, mass vaccination and other programmes. Opportunities therefore exist for economies of scope by combining IPTc with delivery of other interventions.

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ANTIMALARIAL DRUG UTILIZATION IN A CHILDREN HOSPITAL

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Although drugs have greatly transformed the practice of medicine in recent years, inappropriate drug use can lead to increased mortality and morbidity rates. National and international health organizations therefore employ policy changes in order to regulate and optimize the benefits of drug use. This study investigated drug utilization in a children's hospital in Ibadan, Southwest Nigeria. In particular it investigated the impact of a national antimalarial drug policy change on prescribing patterns. Patients' case note data on age, sex, diagnoses and drug therapies during a single hospital visit were reviewed and assessed using selected WHO drug use indicators. One out of ten prescriptions written in the first four months of 2004, 2005 and 2010 were studied retrospectively. Data analysis was done using SPSS 14 with confidence limits set at 95%. Percentage of prescriptions including artemisinin based combination therapies (ACTs) though negligible in 2004 increased to more than 60% in 2010. There was an attendant reduction in the proportion of prescriptions written in generic names as many ACTs were prescribed using brand names. In addition, compliance with the policy was not significant until nine months after the national adoption of the policy.This study provides information on trends of antimalarial drug use and responsiveness to antimalarial treatment policy. The study confirms that drug policy changes are not without difficulties and require sustained monitoring to succeed.

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IN VITRO ACTIVITY OF FERROQUINE, VERSUS CHLOROQUINE, AGAINST WESTERN KENYA *PLASMODIUM FALCIPARUM* FIELD ISOLATES AS DETERMINED BY A SYBR GREEN I ASSAY

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Ferroquine (FQ), a 4-aminoquinoline analogue of chloroquine (CQ), is being developed for treating CQ resistant and CQ sensitive *Plasmodium falciparum* malaria. Growing *in vitro* drug sensitivity data support these indications, and that FQ may be more potent than CQ. Continued *in vitro* testing, especially against CQ resistant *P. falciparum* field isolates, may be useful in understanding of FQ's potential. In 146 *P. falciparum* field isolates collected in western Kenya, most processed immediate *ex vivo* (IEV), we measured 50% inhibitory concentrations (IC_{50}; nM) of CQ and FQ by a SYBR Green I *in vitro* assay. Laboratory reference clones included D6 (CQ "resistant") and W2 (CQ "resistant"). Field isolates were assessed for PfCRT K76T mutation, *Pfmdr1* copy number and *Pfmdr1* single nucleotide polymorphisms (SNPs) at 4 codons by real time PCR. Geometric mean IC_{50}s for FQ were lower than CQ for *P. falciparum* field isolates ($p = 0.005$) and the CQ resistant clone W2 ($p < 0.001$). pfcrt K76 mutations, detected in > 80% of isolates, conferred higher IC_{50}s for CQ, and modestly lower IC_{50}s for FQ. For *Pfmdr1*, mean copy number was 1, with SNPs common at codon 86. In conclusion, *in vitro*, FQ is more potent than CQ against CQ-resistant *P. falciparum* field isolates, and the CQ-resistant clone W2, with seemingly little or no effect from pfcrt K76T mutations. This bodes well for the clinical use of ferroquine.

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FERROUS IRON-TARGETED DRUG DELIVERY IN ANTIMALARIAL CHEMOTHERAPY

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Artemisinin combination therapy is the current standard of care in treating uncomplicated malaria. While the precise mechanism of artemisinin action is still debated, the importance of an initial reductive activation by ferrous iron is broadly accepted in the field. We have been exploring novel means to deliver multiple drug activities to the malaria parasite in a ferrous iron-dependent fashion. This new approach has the potential improve drug efficacy and safety and to reduce the potential for resistance, particularly in the context of prophylaxis. Here we will describe prototypical ferrous-iron targeted prodrugs that deliver a pan protease inhibitor only after an initial 1,2,4-trioxolane (artemisinin-like) activity has been conferred. Activity-based probes were used to confirm ferrous iron-dependent drug delivery in cultured parasites. *Plasmodium berghei* infected mice were cured when treated for three days (40 mg/kg/day, ip) with the prodrug, treatment starting ten days post-inoculation. This delivery of a protease inhibitor in prodrug form was both more efficacious and much less toxic than direct administration of the protease inhibitor alone at a comparable dose.

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SIMULTANEOUS ANALYSIS OF PRIMAQUINE AND ITS METABOLITE CARBOXYPRIMAQUINE BY LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY

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Primaquine (PMQ) is the only tissue schizonticide that is widely used in the treatment of *Plasmodium vivax* malaria. However, a variety of dosage regimens are reported and the pharmacokinetics of PMQ and its principal active metabolite, carboxyprimaquine (CPMQ), have not been clearly established in some patient groups, including children. In most pharmacokinetic studies, PMQ and CPMQ have been extracted and analysed separately by HPLC methods, because PMQ is a base and CPMQ is an acid. There are few reported LC-MS methods that are reproducible and applicable in clinical trial assays. We have developed a simple, robust method to simultaneously extract and analyze both PMQ and CPMQ from plasma. Solid phase extraction was used for sample preparation (Waters Oasis HLB cartridges) with 8-aminoquinoline as the internal standard. Analyses were performed using a Shimadzu 2020 LC-MS with selected ion monitoring under electrospray ionisation mode (m/z for quantification was 260, 275 and 145 for PMQ, CPMQ and 8-aminoquinoline respectively). Separation was performed isocratically in water and methanol (20:80) containing 0.1% formic acid, using a Phenomenex Luna C18 column. Retention times for PMQ, CPMQ and 8-aminoquinoline were 3.4, 8.6 and 5.9 min respectively. Standard curves were linear across the concentration range 2-1000 µg/L. Analysis of samples containing three different PQ and CPMQ concentrations (5, 50 and 200 µg/L) spiked into five separate plasma samples were used to determine matrix effects (ion suppression/enhancement), absolute recovery, and process efficiency, all of which were within acceptable analytical ranges. The assay intra-day and inter-day relative standard deviations were <10% at 5, 50, 200, 500 and 1000 µg/L. LLOQ for PMQ and CPMQ were 1 µg/L and 2 µg/L respectively. Plasma concentration PMQ and CPMQ profiles for a representative patient were consistent with previously-reported data. A simple and reliable LC-MS method was developed, validated and successfully applied to determine PMQ and CPMQ plasma concentrations in patients treated with primaquine.

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POTENTIAL FOR CLINICAL IMPACT FROM THE NON-HEMOLYTIC 8-AMINOQUINOLINE CONSORTIUM

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Primaquine (PQ) is an 8-aminoquinoline (8AQ) critical for malaria elimination campaigns, although its widespread use is limited by hemolysis in G6PD-deficient (G6PDd) people and poor adherence. Tafenoquine (TQ), an analog with a two-week half life, is currently in Phase II/III clinical trials for *Plasmodium vivax* radical cure. In 2008, an expert committee reviewed the current state of knowledge and made key recommendations. All available safety and efficacy data from 1800+ 8AQ from the WRAIR chemical inventory system, the literature, and the 40 given to humans are available in a website database. To understand and overcome hemolytic risk of this drug class, one *in vitro*, two mouse and a Rhesus model of G6PDd have recently been qualified. These models are allowing us to implement a systematic plan to understand the mechanisms of hemolysis, the impact of drug combinations, and identification of non-hemolytic 8AQs. The 12 drugs documented to be hemolytic in G6PDd humans and 12 non-hemolytic analogs were used to make a model that correctly predicted an 8AQ efficacious in monkeys to be non-hemolytic. Both TQ and NPC1161B appear to have a clearly improved therapeutic index over PQ. In the human literature, chloroquine and quinine potentiate activity against hypnozoites, while quinine decreases methemoglobin, the mechanism of which is now being understood through key metabolism experiments. Initial studies in mice have suggested that a pan cytochrome p450 inhibitor blocks efficacy of 8AQ, but not hemolytic toxicity, providing initial evidence that efficacy can be separated from toxicity. Drugs in human use will be systematically evaluated in combination and with new approaches to determine if an improved therapeutic index is achievable in the newly qualified G6PDd models and efficacy models in the same animal strains/species. Combinations will target mechanisms to reduce hemolysis (e.g. antioxidants, metabolism inhibitors) or potentiate efficacy (e.g. chloroquine). Promising approaches will be pushed to human testing for definitive documentation of proof of concept.

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IN VITRO PLASMODIUM FALCIPARUM KILLING RATES CAN DIFFERENTIATE ANTIMALARIAL MODE OF ACTION

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Malaria is a major public health and economical issue affecting the world's most disadvantaged populations. Current treatments are compromised by the advance of resistance even to the newest antimalarial class, highlighting our constant need for new and efficacious drugs. Ideally new drugs should be fast-acting compounds in order to maximize their therapeutic efficacy and minimize their potential to induce resistance. Current assays to assess antimalarial potency of compounds are based on parasite metabolism measures, however these methods are not adequate to evaluate effects over parasite viability: metabolically inactive parasites can be viable because drug effect can be fully reversible or parasites committed to death might still appear metabolically active as is the case for most of antibacterials with antimalarial efficacy. We present an *in vitro* methodology to address these issues, that assess viability of drug-treated parasites over time. This method uses limiting-dilution technique to quantify the amount of viable parasites, and provides an *in vitro* pharmacodynamic profile for each antimalarial drug. Relevant PD parameters can be determined by this method such as - *in vitro* parasite reduction rate (PRR) representing the fractional reduction of initial parasites load per asexual life cycle or the lag phase that reflects the time needed to observe full killing effects of a drug. Furthermore, by testing increasing concentrations of the drug, conditions for the maximum killing rate can be determined. This can be useful to select the optimal doses for efficacy in clinical trials. Moreover, *in vitro* PRR profiles appear to be directly related to the antimalarial drug mode-of-action. Drugs acting on the same target or pathway display similar profiles. This observation suggests that PRR profiles can be used to distinguish different antimalarial mode-of-action and potentially identify the drugs with the fastest killing rate.

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"THEY JUST TOLD ME TO GO BUY DRUGS FROM THE SHOP." HOW THE 2010 ACT STOCK OUTS IN TANZANIA AFFECTED MALARIA CASE MANAGEMENT AND CARE-SEEKING BEHAVIORS IN MTWARA REGION

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We present qualitative data from two communities in Mtwara Region to examine how stock outs of Artemether-Lumefantrine (ALu) during February - June 2010 affected malaria case management and care-seeking behaviors. We conducted qualitative interviews to examine provider and community experiences with malaria diagnosis and treatment including 21 provider in-depth interviewees (IDI), 8 community focus groups (FGD), and 31 illness narrative interviewees (INI) who had experienced a recent malaria episode. Discussions were held with regional and district authorities to document malaria-related strategies and challenges. Data were collected twice to capture seasonal differences. Interview transcripts were entered into NVivo8 for content-analysis. Although all INI who sought malaria treatment reported using some type of antimalarial drug (AM), INI seeking care during the stock out period were half as likely to be treated with ALu compared to INI pre-stock out. Stock outs often resulted in patients having to travel back and forth between health facilities (HF) and drug shops (DS) in search of prescriptions and drugs. Although some health worker IDI said they provided patients with prescriptions for DS referrals, several INI reported being left on their own to figure out where to go and what AM to buy. Others noted they avoided particular HF altogether either because of their own or others' prior experiences of being told there were no drugs. Economic consequences of ALu stock outs also emerged. Both FGD and INI participants complained about having to pay HF registration fees only to be told there were no drugs; some INI resorted to buying AM half doses due to limited funds. District authorities adopted several measures to address the stock out including rationing of ALu to under-fives, moving ALu stocks between HFs, and treatment with available AM, although they did note their concern with the overuse of sulfadoxine-prymethamine and quinine. The region received 480 doses of Duocotexin from the Medical Stores Department to use as an alternative ACT treatment but it eventually

expired. HF stock outs of ALu in 2010 had negative effects on timely access to effective antimalarial treatments. Attention to improving timely acquisition and distribution of ACTs is of utmost importance.

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IN VITRO METABOLISM OF PIPERAQUINE IS PRIMARILY MEDIATED BY CYP3A4

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Piperaquine (PQ) is a quinoline antimalarial that has been recently added as a first-line treatment option for uncomplicated *Plasmodium falciparum* malaria by the World Health Organization. The primary objective of this investigation was to determine the major metabolic pathway(s) of PQ *in vitro*. A reliable, validated tandem mass spectrometry method was developed to quantify PQ. Concentrations of PQ were measured over time after incubation with both human liver microsomes (HLMs) and expressed cytochrome P450 enzymes (P450s). In pooled HLMs, incubations with an initial PQ concentration of 0.3 μM resulted in a 34.8 + 4.9% loss of substrate over 60 min, corresponding to a turnover rate of 0.009 min-1 (r2 = 0.9223). Miconazole, at non-specific P450 inhibitory concentrations, resulted in almost complete inhibition of PQ metabolism in HLMs. The greatest inhibition was demonstrated with selective CYP3A4 (100%) and CYP2C8 (66%) inhibitors. Using a mixture of recombinant P450 enzymes, turnover for PQ metabolism was estimated as 0.0099min-1; recombinant CYP3A4 had a higher metabolic rate (0.017 min-1) than recombinant CYP2C8 ($p < 0.0001$). Inhibition of CYP3A4-mediated PQ loss was greatest using the selective inhibitor ketoconazole (9.1 + 3.5% loss with ketoconazole vs 60.7 + 5.9% with no inhibitor, $p < 0.0001$). The extent of inhibition of *in vitro* metabolism with ketoconazole (83%) denotes that PQ appears to be primarily catalyzed by CYP3A4.

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DETERMINANTS OF PROMPT ANTIMALARIAL TREATMENT OF FEVER IN CHILDREN UNDER FIVE: EVIDENCE FOR TARGETED COMMUNICATIONS IN FIVE AFRICAN COUNTRIES

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In malaria endemic settings, decisions made by children's caregivers at the onset of fever are critical. While increasing access to artemisinin combination therapy (ACT) is important for improving effective fever treatment, a number of demand-side factors likely influence treatment outcomes. Identifying determinants of treatment-seeking behavior can inform demand creation activities aimed at achieving optimal uptake of ACTs. This study uses a behavior change framework to guide examination of opportunity, ability and motivation factors theorized to influence prompt antimalarial treatment for fever in children under five. Formative in-depth interviews and focus group discussions informed development of quantitative scaled constructs. Nationally-representative surveys were conducted in DRC, Madagascar, Nigeria, Uganda and Zambia during 2008-2010 as part of the ACTwatch research program. Logistic regression was used to build country-specific models predicting prompt antimalarial treatment of fever. Results show that each country context is unique with respect to determinants of behavior and the gap between current and ideal levels of those determinants among caregivers. Implications for program design are discussed. Cross-country trends include significance of opportunity (perceived antimalarial availability) and motivation factors (beliefs favorable to modern medicine and positive outcome expectations for use of antimalarials). Ability factors that were tested (malaria knowledge and forms of social support) are not typically significant behavioral determinants. Relative household wealth is not associated with prompt antimalarial treatment, with the exception of a significant disparity in Nigeria. As effective antimalarials become more affordable and available, locally-relevant evidence-based communications will play an important role in ensuring that caregivers promptly seek and acquire these treatments. Practical methods for investigating behavior can provide necessary evidence to create targeted communications.

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TEN YEARS EXPERIENCE WITH COARTEM: A PATIENT-CENTRIC APPROACH TO FIGHTING MALARIA

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We review the experience made with Coartem (Artemether-Lumefantrine), the gold standard artemisinin-based combination therapy (ACT) for malaria that has been deployed to endemic countries for the last 10 years, delivering over 400 million treatments. Over the years, our focus shifted from providing a quality medicine in public/private partnership with WHO to a holistic, 'patient-centric' approach, focusing on educating caregivers and patients to ensure, timely treatment and adherence to full course of medication, involving multiple partnerships. To meet the specific needs of children, a dispersible formulation was developed jointly with Medicines for Malaria Venture (MMV). Coartem Dispersible tablets meet the specific needs of children as they can be given dispersed in a small amount of liquid and are sweetened to mask the bitter taste, which is typical of most antimalarials. More recently, we are evaluating novel approaches that may be of use in malaria elimination strategies. A study assessing the utility of Coartem in mass screening and targeted treatment for malaria in entire village populations, including carriers of the malaria parasite that are asymptomatic has been undertaken in an effort to reduce parasite transmission. New strategies to expand access to ACTs have been implemented: the Affordable Medicines Facility - malaria (AMFm) initiative, where funds from donors will be used as subsidies to lower the price of ACTs at retail outlets, and the SMS for Life initiative, part of the Roll Back Malaria (RBM) program, a tool for supply chain management based on electronic mapping technology and short text messages sent via mobile phones. These initiatives that maintain and further evolve a patient-centric approach, and go beyond a mere deployment of drugs are essential for achieving a sustained health benefit in developing countries. Sharing these learnings with relevant stakeholders may allow developing strategies that achieve similar results also for other diseases, worldwide.

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CYCLOPROPYL CARBOXAMIDES A NEW ANTIMALARIAL CHEMICAL CLASS WITH *IN VIVO* EFFICACY

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Discovery of new classes of antimalarial drugs has become an urgent task to counteract the increasingly problem of drug resistance. Screening directly for compounds able to inhibit parasite growth *in vitro* is one of the main approaches the malaria research community is now pursuing for the identification of novel antimalarial drug leads. Very recently, thousands of compounds with potent activity against the parasite *Plasmodium falciparum* have been identified and information about their molecular descriptors, antiplasmodial potency and cytotoxicity is publicly available. Now the challenge is how to identify the most promising chemotypes for further development and how best to progress these compounds through a lead optimization program to generate antimalarial drug candidates. We report here the first chemical series to be characterized from one of those screens, a completely novel chemical class designated with the generic name of cyclopropylcarboxamides and never before described to have antimalarial or other pharmacological activities. Cyclopropylcarboxamides are potent inhibitors of drug sensitive and resistant strains of *P. falciparum in vitro*, and show *in vivo* oral efficacy in malaria mouse models. In this work we describe the biological characterization of this chemical family,

showing that inhibition of their still unknown target has very favorable pharmacological consequences but the compounds themselves seem to select for resistance at high frequency.

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NEUROLOGICAL SEQUELAE OF CEREBRAL MALARIA IN CHILDREN

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Out of 604 Rwandan children admitted with *falciparum* malaria to Ndera hospital hospital between August and December, 2009, 308 had cerebral malaria and 203 were severely anaemic (haemoglobin less than 60 g/l). 14% of those with cerebral malaria died, as did 7.8% of those with severe anaemia. 32 (12%) of children surviving cerebral malaria had residual neurological deficit. 69 other children were admitted with clinical features strongly suggestive of cerebral malaria but with negative blood films; 16 of these died and 3 had residual neurological deficits. The commonest sequelae of cerebral malaria were hemiplegia (23 cases), cortical blindness (11), aphasia (9), and ataxia (6). Factors predisposing to sequelae included prolonged coma, protracted convulsions, severe anaemia, and a biphasic clinical course characterised by recovery of consciousness followed by recurrent convulsions and coma. At follow up 1-6 months later over half these children had made a full recovery, but a quarter was left with a major residual neurological deficit. Cerebral malaria in childhood may be an important cause of neurological handicap in the tropics.

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METEOROLOGICAL, VECTORIAL AND SOCIAL-ECONOMIC FACTORS RELATED TO MALARIA TRANSMISSION IN TIBET

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Malaria has been endemic in Linzhi Prefecture in the Tibet Autonomous Region (TAR) over the past 20 years, especially in Motou County with a highest incidence in the country in recent years. Considering spatial aggregation of malaria cases and specific vectors, the meteorological, vectorial and social-economic factors were analysed to determine the key factors related to malaria transmission in this particular area. Meteorological factors were incorporated in the spatio-temporal models. Seven models were established by Bayesian hierarchical models and Markov Chain Monte Carlo methods in comparison based on Deviance Information Criterion (DIC). In Tibet, malaria patients are scattered along the Brahmaputra River with spatial cluster, where inhabited by members of the Zang, Menba and Luoba nationalities. Relative humidity was the greatest influence factors, which affected the mosquito survival directly. The relationship between malaria incidence and rainfall was complex and it was not directly and linearly. The lags of temperature and relative humidity were similar and smaller than that of rainfall. Entomological investigation, which included adult anopheles collections, morphological and molecular identification was to identify the species of *Anopheles* including *An. maculatus* group, *An. peditaeniatus*, *An. barbumbrosus*, and *An. kochidonitz*. *An. pseudowillmori* was considered the sole malaria vector and the larval habitats only were paddy field. *An. pseudowillmori* accounted for 98.1% of the *Anopheles* composition. Human blood index, sporozoites natural infection rate, vector capacity and entomology inoculate rate were 29%, 0.56‰, 2.795 and 0.004389, respectively. Social-economic or household factors have been identified as increasing human-vector contact within a given ecological environment, which were collected by household investigation including the average income,domestic animals, the usage of bed-nets and malaria awareness. In Tibet, the risk of infection with malaria was high among residents of "poor" family without usage of bed-nets and few domestic animals. In conclusion, considering the unique and special characteristic of meteorological factors in Tibet, it may be speculated that the meteorological factors play major role in malaria transmission. Of course, vector species and abundance and social-economic status, are also known to have significant influence on the transmission of malaria.

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IDENTIFICATION OF MALARIA TRANSMISSION AND EPIDEMIC HOTSPOTS IN THE WESTERN KENYA HIGHLANDS: ITS APPLICATION TO MALARIA EPIDEMIC PREDICTION

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Malaria in the Western Kenya highlands is characterized by unstable and high transmission variability that causes epidemics during hyper transmission seasons. This study examined how terrain in the highlands affects the exposure and sensitivity of a site to malaria. The study was conducted in western Kenya highlands; two U-shaped valleys (Iguhu, Emutete), two V-shaped valleys (Marani, Fort-Ternan) and one plateau (Shikondi) for 16 months among 6-15 years old children. Malaria Exposure was tested using circum-sporozoite protein and merozoite surface protein immunochromatographic antibody test; malaria infection was tested by microscopic examination of blood smears, children's homes were georeferenced using global positioning system. Paired t-test was used to compare the mean prevalence rates of the sites. The mean antibody prevalence was 22.6% in Iguhu, 24% in Emutete, 11.5% in Shikondi, 8.3% in Fort-Ternan and 9.3% in Marani. The mean malaria infection prevalence was 23.3% in Iguhu, 21.9% in Emutete, 4.7% in Shikondi, 2.9% in Fort-Ternan and 2.4% in Marani. The difference in antibodies and malaria infection prevalence among the two valley systems and the plateau was significant ($P<0.05$). The difference in antibodies and malaria infection prevalence within the U-shaped valleys and within the V-shaped valleys was not significant ($P> 0.05$). There was clustering of malaria antibodies and infections around flat areas in the U-shaped valleys and random distribution of the infections in the V-shaped valleys and less clustered at the plateau. This study showed that the V-shaped and plateau ecosystems have low malaria parasites prevalence and few individuals with immune response to malaria parasites, they can be considered as epidemic hotspots. The U-shaped ecosystems are transmission hotspots.

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ANALYZING THE IMPACTS OF MALARIA INTERVENTION TECHNIQUES WITH A GIS-EQUIPPED, SPATIAL AGENT-BASED MODEL OF MALARIA

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My research is about spatial agent-based model (ABM) of malaria. We developed several ABMs of the vector dynamics lifecycle of malaria, and performed verification and validation of these. The ABMs were non-spatial: none of the agents possessed any spatial attributes. However, spatial heterogeneity is one of the most important factors for an effective representation of the mosquito environment. The dynamics of malaria can be affected by substantial local variations (e.g. locations of aquatic habitats and bloodmeal events) resulting from various spatial differences. Also, some events in the mosquito lifecycle (e.g. host-seeking, oviposition) are by nature spatial. A spatial ABM thus may provide opportunities for more realistic modeling of these events, and to obtain new insights from analyzing the spatial heterogeneity. We recently presented a spatial extension of the malaria ABM, and designed a landscape simulator to simulate landscapes used by the mosquito vectors. The landscapes, to be

used as inputs to the spatial malaria ABM, can be simulated with varying spatial heterogeneity of resources that are required by female mosquito agents to complete their gonotrophic cycles. The next step would be to augment the spatial ABM with georeferenced data. Several successful research efforts have shown the increasing use of GIS (Geographic Information System) and RS (Remote Sensing) for the study of spatial and temporal patterns of vector-borne diseases (including malaria). This would allow us to apply the ABM for a specific geographic location (e.g. some cluster of villages in Kenya). In association with the Center for Research Computing (CRC) at Notre Dame, we have collected data on various types of vector breeding sites, human habitats, and weather (primarily rainfall and temperature). Once the spatial ABM is run with these data, we can investigate the impacts of various intervention strategies for malaria. Out of many possible interventions, our primary emphasis is on habitat reduction, insecticide-treated bed nets (ITNs), and indoor residual spraying (IRS). Other than analyzing impacts of interventions, the ABM, equipped with georeferenced data, may also serve as a decision-making tool. For a particular region of interest, it can identify human populations at risk and the geographical spread of malaria. It can also help in stratifying malaria risk factors, and planning resource allocation more effectively.

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ASSESSMENT OF TREATMENT PRACTICES FOR MALARIA IN CHILDREN UNDER FIVE YEARS, NIGERIA, 2008

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Nigerian Demographic and Health Survey (NDHS) is a 5-year survey carried out to assess the impact of public health measures. Malaria is the commonest cause of febrile illness in Nigeria and *Plasmodium falciparum* accounts for >95% of these episodes. The 2002 drug efficacy study showed monotherapies, e.g. Chloroquine (CQ) and Sulfazodine-Pyrimethamine (SP) were no longer adequate for first line treatment. The reviewed national antimalarial treatment policy(2005) recommends the use of Artemisinin based-Combination Therapy (ACT) as the first line treatment for the management of malaria. The data of the 2008 NDHS was reviewed to assess the treatment practices for fever in children. We analysed NDHS data of 24,975 mothers whose children less than 5 years (U5) had fever 2 weeks preceding the survey and may have had antimalarial treatment.Descriptive analysis and comparison of treatment practices including promptness, type of drugs administered was conducted by chi-square test. Prompt treatment was defined as administration of antimalarial drugs within the first 24hours of the onset of fever.Altogether 3,968 (15.9%) children of 24,975 mothers interviewed had fever. Of the 3,968 children, 1,317(33.2%) received any type of antimalarial. Of the 3,968 children, 95 (2.4%) received ACT, 762 (19.2%) CQ and 234 (5.9%) received SP. Of the specific antimalarials administered, 41 (43.2%) children had ACT (n=95), 229(30%) had CQ (n= 762) and 80 (34.2%) had SP (n=234) readily available to their mothers at home ($p<0.01$). Of the 3,968 children, overall 595(15%) had prompt treatment with any antimalarials, 71(1.8%) received prompt treatment with ACT, 349(8.8%) had CQ and 99(2.5%) had SP. Children of mothers with at least secondary education were more likely to receive prompt treatment ($p<0.01$) and more likely to receive CQ and SP rather than ACT ($p<0.01$). Prompt treatment of fever and the use of the recommended antimalarial (ACT) by mothers of U5 is low. There is a need to sensitise mothers on prompt and appropriate treatment of febrile episodes in children with ACT.

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IMPACT OF HEALTH RESEARCH CAPACITY STRENGTHENING IN LOW AND MIDDLE INCOME COUNTRIES: THE CASE OF WHO/TDR PROGRAMS

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Measuring the impact of capacity strengthening support is a priority for the international development community. Several frameworks exist for monitoring and evaluating funding results and modalities. We report on the impact of individual and institutional capacity strengthening programmes conducted by the UNICEF/UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR) and on factors that influenced the outcome of its Research Capacity Strengthening (RCS) activities. Quantitative and qualitative methods (questionnaires and in-depth interviews) were applied to a group of 128 individual and 20 institutional capacity development recipients that completed their projects between 2000 and 2008. A semi-structured interview was conducted on site with scientists from four institutions. Most grantees, both individual and institutional, reported beneficial results from their grants. However, glaring inequities stemming from gender imbalances and a language bias towards English were identified. The study showed that skills improvement through training contributed to better research proposal formulation, but not necessarily to improved project implementation or results communication. Appreciation of the institutional grants' impact varied among recipient countries. The least developed countries saw the grants as essential for supporting basic infrastructure and activities. Advanced developing countries perceived research grants as complementary to available resources, and particularly suitable for junior researchers who were not yet able to compete for major international grants. There is need for a more equitable process to improve the effectiveness of health research capacity strengthening activities. Support should be tailored to the existing research capacity in disease endemic countries and should focus on strengthening national health research systems, particularly in the least developing countries. Stakeholders' engagement at country level would facilitate design of more specific and comprehensive strategies based on local needs.

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THE GENETIC RELATEDNESS OF *PLASMODIUM FALCIPARUM* PARASITES *VIS A VIS* THEIR SPATIAL DISTRIBUTION

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Understanding the genetic relatedness of *Plasmodium falciparum* parasites is important in providing insight on how these parasites are being transmitted in various localities. In this study: 1. Since *P. falciparum* mixed clone infections are common in nature, PyrosequencingTM was validated as a technique enabling the identification of each genetically distinct clone represented in an infection by assigning proportions to the SNPs representing each genetically distinct clone and enabling the identification of parasite genotypes in every isolate analysed; and, 2. The genetic relatedness between the identified clonal genotypes was determined. These results comprise a total of 58 samples; 8 samples collected from Cameroon, 15 from Kenya and 35 from Mali. The data consists of 6 SNPs analysed by PyrosequencingTM. 83 clonal genotypes were identified by PyrosequencingTM from the analysed isolates. Genetic relatedness (GRs) was determined and Pairwise comparisons conducted between clones occurring (i) within an isolate i.e. a major and minor clone (ii) between isolates within the countries and, (iii) between isolates from the different countries. The results indicated that parasites within one isolate in a polyclonal infection were found to have higher GR compared to parasites from another isolate within a region and beyond it. This offers the possibility that parasites occurring within households and in close neighbourhoods would be more closely related than those separated by large geographic distance. On this basis, it is recommended that a larger

study should be conducted to determine the level of genetic relatedness in parasites collected within households and in close neighbourhoods to clearly establish the level of genetic relatedness of these parasites in natural populations. This information would enable the detection of foci of malaria transmission which is important for effective deployment of malaria interventions.

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FEVER AND PARASITE DENSITY IN PASSIVE CASE DETECTION: IMPLICATIONS FOR ENDPOINTS USED IN MALARIA VACCINE TRIALS

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The measurement of clinical malaria is complicated in high transmission areas where parasitemia during a febrile presentation may be incidental. Fever at the time of presentation and parasite density cut-offs derived from attributable fractions of fever due to malaria among the general community have been used to refine case definitions. However, whether these criteria are valid in passively detected cases, who have histories of fever, is not known. We measured the proportion of ill patients presenting with current fever (≥37.5°C) and estimated the effect of parasite density on fever during home and clinic-based care from 2006-2009 in a vaccine trial site population of 2,204 people residing in eight forest villages of Orissa, India. We modeled the odds of fever as a function of continuous parasite density by age category and adjusted for year, village, month, sex, and correlated observations using unconditional logistic regression and general estimating equations. 4,889 screenings for malaria among 1,493 ill persons were conducted. The prevalence of current fever was 45% and 52% among those with parasitemia. Parasite prevalence and the incidence of malaria, 39% and 215/1000years respectively, varied with age. Mean parasite density decreased with age and increasing densities were associated with higher odds of current fever (p for trend<0.0001). The unadjusted odds ratio (OR) of fever for a one log increase in parasite density was 1.06 (95%CI: 1.04, 1.07); including interaction between parasite density and age and adjusting for village, month and intra-subject correlation the OR of fever was 1.14 (95%CI: 1.08, 1.21). Overall, 28% (95%CI: 26, 29) of current fevers were attributable to malaria. A parasite density cut-off of 500 provided 87% sensitivity and 84% specificity in classifying malaria-attributed current fever. Increasing parasite density increased the odds of current fever in forest areas of India. In the context of persons who sought screening because they were ill, the fraction of current fevers attributable to malaria was low in spite of high malaria transmission. The results of this study, which was akin to a vaccine trial using passive case detection such as Phase 3 RTS,S, suggest that in order to preserve power and reduce bias the primary endpoint should not use fever at the time of presentation or parasite density cut-offs as case definition criteria.

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SCALING UP THE IMPLEMENTATION OF HMM WITH THE USE OF RDT IN SARAYA HEALTH DISTRICT: FEASIBILITY, PHARMACOVIGILANCE, HEALTH SEEKING BEHAVIOR AND IMPLICATIONS FOR SURVEILLANCE SYSTEM

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From 2005 and 2008 malaria morbidity and mortality have respectively dropped from 32, 5% and 20,6% to 5,6% and 7,1% in Senegal health services ; data did not include the underserved communities in a context of 70% of the population living at least at 15 kilometers from the nearest health unit. To fill this gap Home Management of Malaria has been scaled up in this district through Community Health Workers and malaria volunteers. The objective of the study is to assess HMM and enhance its surveillance system. Following a community census and a survey on CHW and volunteers' profiles, a baseline household cluster survey including KAP and Health Seeking Behavior has been completed. CHW and volunteers have been trained on the use of RDT, Artemisinin-based Combined Therapy administration and malaria pharmacovigilance. Supervisions were held to follow the process in 47 community health units. 57% of community agents are CHW, 43% are malaria volunteers. The mean age is 33 years, 14% are illiterate, 74,5% have previously involved in malaria treatment, 40% have traditional healers in their area. The KAP survey has concerned 981 households; the respondents' mean age is 56 years, 86% are male, 69% are farmers, and 22% are literate. Among them 84% are aware of malaria transmission, 72% recognize malaria symptoms, 94% know RDT as a confirmation tool and 68% LLINs as protective. Treatment by ACT and quinine is known by 41%, 39% are aware on adverse events. 91 households (9.3%) have responded to the health seeking behavior survey 14% are close to traditional healers among these latter 16% are "treating" malaria. From June 2010, in a 10050 population, 3699 consultations have been completed at the community level; 59% of clinical malaria confirmed by RDT, 23.6% negative and 0.03 invalid; 865 referrals completed, 12 adverse events and 29 deaths notified. Large scale implementation of HMM is feasible and useful for malaria surveillance; it is fundamental to follow up RDT and data quality, outcomes of executed referrals, and the potential role of traditional healers.

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ESTIMATING THE CLINICAL BURDEN OF *PLASMODIUM FALCIPARUM* MALARIA IN INDONESIA IN 2010

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The contemporary map of population at risk of *Plasmodium falciparum* in Indonesia estimated that 132.8 million (57.1%) of the population lived at risk of *P. falciparum* transmission in 2010. Of these, 70.3% inhabited areas of unstable transmission and 29.7% in stable transmission zones. However, the burden of *P. falciparum* in this archipelago has not been estimated. The new cartographic technique for deriving the clinical burden estimates of *P. falciparum* developed by Malaria Atlas Project (MAP, http://www.map.ox.ac.uk) was used in this study. The mapped of unstable and

stable *P. falciparum* malaria transmission was first determined. Estimates of the plausible incidence range of clinical cases were then calculated within the spatial limits of unstable transmission. A modelled relationship between clinical incidence and prevalence was used to estimate incidence areas of stable transmission. Geostatistical joint simulation was used to quantify uncertainty in these estimates at provincial scales. These results are summarized across main islands of Indonesia and the implications for evaluation of malaria elimination elaborated.

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AGE AND SEX DISTRIBUTION OF GAMETOCYTE-POSITIVE INDIVIDUALS IN SOUTHERN ZAMBIA

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The incidence of malaria has decreased in Zambia as a result of malaria control interventions, including increased coverage with insecticide treated nets (ITNs), indoor residual spraying (IRS) and artemisinin-combination therapy (ACT). Gametocytes are the sexual stage of the malaria parasite critical to transmission. Malaria is commonly diagnosed based on the presence of asexual parasites and most anti-malarial drugs act on these stages. However, of importance to malaria control and elimination is the prevention of gametocytemia. The present study compares the age and sex distribution of gametocyte-positive individuals in 2007, 2008 and 2009 malaria transmission seasons in rural southern Zambia. A cross-sectional survey of individuals residing in randomly selected households was conducted in Choma District, Southern Province, Zambia. A total of 174, 317 and 675 blood samples were collected in 2007, 2008 and 2009, respectively. The samples from 2007 were examined by microscopy after making thick and thin films. Samples collected in 2008 and 2009 were assayed using RT-PCR to detect the pfs25 mRNA expressed in *P. falciparum* gametocytes. The proportion of individuals with detectable gametocytes was 2.8% in 2007 by microscopy, and 4.5% and 1.5% by RT-PCR in 2008 and 2009, respectively. There were no significant differences in gametocyte positivity between males and females in any year. Gametocytemia was more frequent in the 5-20 year age-group among the 2008 and 2009 cohorts, with 79% and 90% of gametocyte-positive individuals within this age group, respectively. In contrast, 60% of gametocyte-positive individuals were children younger than five years of age in 2007. The prevalence of gametocytemia decreased over the three years, with the lowest prevalence of gametocytemia recorded in 2009, concurrent with the scale-up of malaria control interventions. The prevalence of gametocytemia was highest in school-age children and young adults as malaria transmission decreased, facilitating targeted treatment in schools.

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ANTIBODIES TO *PLASMODIUM FALCIPARUM* IN A REGION OF DECLINING MALARIA TRANSMISSION IN SOUTHERN ZAMBIA

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The prevalence of malaria has decreased dramatically in southern Zambia due to high coverage with insecticide-treated nets and artemesinin-combination therapy. We assessed the prevalence of antibodies to *Plasmodium falciparum* in this region of declining malaria transmission to investigate whether the prevalence of seropositivity would decrease concurrently. Participants were residents of randomly-selected households in the catchment area of Macha Hospital in Choma District, Southern Province, Zambia. Residents of some households participated once (cross-sectional households) and others were assessed at repeated visits (longitudinal households). Samples were tested for the presence of *P. falciparum* antigen using a rapid diagnostic test. An enzyme immunoassay was used to measure IgG antibodies to whole *P. falciparum* asexual stage parasites. Seropositivity was defined based on a threshold value established using plasma from persons never exposed to malaria. A total of 433, 742 and 822 blood samples were collected from participants in 2008, 2009 and 2010, respectively, for the cross-sectional survey. A total of 118, 150 and 185 participants were recruited into the longitudinal cohort in 2008, 2009 and 2010, respectively, of whom 39, 36 and 47 had complete follow-up. The parasite prevalence decreased from 8.1% in 2008 to 0.24% in 2010 within the cross-sectional cohort and from 3.4% in 2008 to 0.54% in 2010 within the longitudinal cohort. The prevalence of seropositivity, however, did not decrease and was 47.8%, 69.3% and 68.4% in 2008, 2009 and 2010, respectively. Mean OD values in the cross-sectional cohorts similarly did not decrease (0.634, 0.958 and 0.904 respectively). In general, seropositivity increased with age and was higher in females than males. Although the parasite prevalence declined over the study period from 2008 to 2010, seroprevalence to whole *P. falciparum* antigens remained high. Serology is a useful marker of exposure to *P. falciparum*, but antibodies to whole parasite antigens are not a sensitive indicator of recent decline in parasite prevalence.

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KNOWLEDGE, ATTITUDE, AND PRACTICES (KAP) REGARDING MALARIA IN MUMBAI, INDIA

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According to the National Vector Borne Disease Control Program, malaria incidence has been increasing in India and we hypothesize that the most important reason for this rise is the lack of appropriate human behavior in preventing malaria. This study was thus focused on studying the relationship between various behavioral risk factors and malaria infection in and around Mumbai, India. Four cluster groups in and around Mumbai were identified and a total of 30 households were selected from each cluster. Cluster groups were divided into urban middle class (subjects living in city apartment complexes), urban lower class (subjects living in slums), immigrants (construction workers living at construction sites) and rural lower class (subjects living in a village near Mumbai). Relative poverty between regions is city<slums<Construction workers<village. A structured questionnaire focusing on socio-demographic factors, environmental factors, malaria knowledge, malaria preventive practices and any previous malaria infection was administered to the eligible participants by a trained interviewer. Malaria varies by regions (City: 77% of respondents (or a member of their family) have had malaria, Construction workers: 67%, Village: 17%, Slums: 60%, $p<0.001$). Reported knowledge also varies by region (City: average of 80% of knowledge questions correct, Slums: 73%, Construction workers: 67%, Village: 62%, $p<0.001$). Malaria status appears to be related to knowledge, with those reporting having had malaria scoring higher on the knowledge test (average 55% correct) compared to those reporting never having had malaria (average 45% correct, $p=0.029$). Mosquito nets usage in Construction workers showed 83 % usage while city did not use it (Construction workers: 83%, Village: 24%, Slums: 13%, City: 0%). The usage of insecticide treated nets were mostly seen in Slums (13%) and Village (3%) while other regions did not report any usage. Low rates of reported malaria in the village may reflect a lower chance of surviving malaria. Lack of malaria knowledge may adversely affect this group's ability to recognize and report malaria symptoms and take preventive measures. Thus, our study highlights an urgent need to incorporate malaria education as well as use of insecticide treated nets as an integral measure for all malaria control programs from national to local levels.

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KNOWLEDGE, ATTITUDE, AND PRACTICES (KAP) REGARDING MALARIA IN MUMBAI, INDIA

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REINTRODUCTION OF *FALCIPARUM* MALARIA IN THE NORTH COAST OF PERU, 2010 - 2011

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In Peru, the number of cases of *falciparum* malaria has decreased in the last years and the last case in the North Coast was reported in 2006. After the identification of two cases of *falciparum* malaria in Tumbes in October 2010, an investigation was conducted to confront this reintroduction in the North Coast of Peru.The study was led by the Peruvian Ministry of Health. After identifying new cases, cross-sectional case-finding studies were conducted in the surrounding areas. Cases were diagnosed by smear microscopy and confirmed by nested-PCR when whole blood was available. Genotyping was conducted using microsatellite molecular markers. An epidemiological questionnaire was applied to all cases. Five 12-hour live bait (human landing) mosquito collections took place in two locations where initial cases presented. Twenty-three cases were identified between October 2010 and April 2011, and nine cases were confirmed by nested-PCR (39%). Almost 1200 people were evaluated in areas surrounding the case houses searching for other febrile cases; two additional cases were found. All cases were symptomatic and the most common symptoms were fever and headache (95%), followed by muscle pain (90%), chills (90%), and sweating (80%). No cases had a severe presentation. On average cases were 28 yo, 55% were male and only two reported a previous malaria episode (11%). More than half did not use bed nets (53%) and, 74% lived with people with malaria-like symptoms. The mean time before seeking treatment was 11 days (0-48 days). Twenty-two cases were treated with artesunate/mefloquine (96%); one pregnant woman was treated with quinine. All the subjects reached clinical cure and none reported side effects. No cases have been reported in neighboring regions of Piura, Peru and Ecuador to date. We could not differentiate strains between all six isolates genotyped and we found high similarity to a strain circulating previously in Peruvian Amazon Basin. We collected 194 mosquitoes but only 34 adults of *Anopheles albimanus* were found in the locations assessed. In conclusion, this outbreak demonstrates the latent, permanent risk of reintroduction of *falciparum* malaria in the North Coast of Peru and suggests that transmission continues to date. Better clinical, epidemiological and control data will facilitate control measures executed by Peruvian authorities.

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IDENTIFICATION OF RISK FACTORS FOR MALARIA INFECTION IN MACHA, ZAMBIA DURING THE DRY SEASON FOR EPIDEMIOLOGICAL MODELING AND OUTBREAK PREDICTION

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Current control methods for malaria in Southern Africa include indoor residual spraying and insecticide-treated bed nets which have been responsible for the reduction of malaria transmission in some endemic areas by up to 80 percent. However, these tactics do little to remove asymptomatic cases from the environment. Particularly in areas of unstable transmission, asymptomatic cases act as reservoirs that sustain malaria through the dry season, facilitating transmission the next year. Asymptomatic infections tend to cluster; by detecting and determining risk factors for these foci, local outbreaks of malaria can be predicted, enhancing elimination of asymptomatic cases. Detection can be accomplished by implementing both active and passive surveillance plans, including the use of Rapid Diagnostic Tests and routine collection of malaria case data by a central location. Current work in Macha, Zambia has demonstrated that malaria incidence data can be collected in real time through the use of RDT and Short Message Service data sent via cell phone. Symptom-free household members of malaria-positive cases are tested for infection to detect asymptomatic carriers, and a Global Positioning System coordinate is taken at the homestead. Currently lacking is a means to standardize the data collection and mobilize epidemiological methods, such as Geographical Information Systems (GIS), to develop surveillance frameworks that can be incorporated into a national strategy for malaria detection. We report on a procedure to determine appropriate data stratifications to form site-specific malaria surveillance systems for risk-factor analysis in the Macha area of Zambia serviced by 12 rural health clinics. Homesteads of positive malaria cases were visited and the ecologic, demographic, and socioeconomic status of each was recorded. These data are used in conjunction with GIS and malaria outbreak analysis to determine risk factors for malaria and to develop the framework necessary to implement an effective and efficient malaria surveillance system.

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DECLINING BURDEN OF MALARIA IN A RURAL COMMUNITY IN MUHEZA DISTRICT NORTHEASTERN TANZANIA OVER A PERIOD OF 18 YEARS (1992-2010) AND ITS IMPACT ON ANTIMALARIAL PRESCRIPTION

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The declining burden of malaria in some endemic countries indicate that most of febrile cases may not necessarily be due to malaria; thus, over-diagnosis and -prescription of anti-malarials may pose an increasing challenge to the health system. This study was conducted to assess the changing burden of malaria and examine the effect of decreasing malaria prevalence on dispensing of anti-malarials. Blood smears were prepared from finger prick/venous blood and examined for malaria parasites by microscopy during cross-section surveys conducted between September 1992 and June 2010 in two villages (Magoda and Mpapayu) in Muheza district, Tanzania. Prevalence of *Plasmodium falciparum* infections was compared across the years and study villages. Data from patients treated for malaria were obtained from health records at Magoda dispensary (without diagnostic laboratory facilities) and Mkuzi Health Centre (with capacity for malaria diagnosis by microscopy which serves as a referral centre for the study villages) and used to assess the level of antimalarials dispensing at the two health facilities. The prevalence of *P. falciparum* infections in Magoda village decreased significantly from 83.5% in 1992 to 15.0% in 2010 and in Mpapayu, the prevalence dropped from 83.3% in 1998 to 11.7% in 2010. Spleen rate (from >40% to <1%), anaemia prevalence (69% to <25%) and gametocyte rates (23% to <1%) also declined over the same period. From January 2008 to December 2010, a total of 5755 patients were attended and treated presumptively for malaria with Artemether/Lumefantrine at Magoda dispensary, and the number of patients increased from 114 in January 2008 to 289 patients in October 2010. At Mkuzi, over 50% of patients (n=564) with negative test results by microscopy were prescribed with anti-malarial drugs. Although a remarkable decline in the burden of malaria occurred between 1992 and 2010, the number of patients treated with antimalarial drugs in the same area did not decline, leading to over-prescription of anti-malarials. Accurate diagnosis and treatment of patients with positive malaria results is urgently needed to target anti-malarials to patients with malaria, reduce wastage of expensive drugs and improve management of other causes of febrile infections.

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PYRETHROID RESISTANT *CULEX QUINQUEFASCIATUS:* AN OBSTACLE TO THE USE OF INSECTICIDE TREATED NET?

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In many West African countries, human activities often create mosquito breeding sites, such as improper drainage systems or choked gutters that hold water that *Culex quinquefasciatus* especially like to breed in. This problem has not been adequately addressed because *Cx. quinquefasciatus* are currently not of much importance as disease vectors in most of these countries. However, some studies have suggested their possible negative effect on the use of insecticide-treated nets, especially when resistant to pyrethroid insecticides. As a result, efficacy of alphacypermethrin treated net were evaluated in tunnel test bioassay. A 6-week trial was further conducted in experimental huts to assess its entomological impact on wild *Cx. quinquefasciatus* in Southern Benin. In tunnel test, 40mg/m^2 of alphacypermethrin treated netting induced <15% mortality, however it was effective in preventing blood feeding (50-70%). Similar trend were observed in the experimental hut, low mortality rate (23.8%) but a marked inhibition in blood feeding rate (81.2%). Relative to the number of mosquitoes exiting naturally into the verandah of control hut, alphacypermethrin treated net induced exophily of the pyrethroid-resistant *Cx. quinquefasciatus* into the verandas of the huts. Pyrethroid insecticide treated nets may still offer protection to users against pyrethroid resistant *Cx. quinquefasciatus*. Nevertheless, combination of insecticide treated nets and other mosquito strategies may be require to manage their populations.

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FIELD EVALUATION OF AN ATTRACTANT (OVIPOSITION PHEROMONE IN COMBINATION WITH INSECT GROWTH REGULATOR) FOR SURVEILLANCE AND CONTROL OF DENGUE AND CHIKUNGUNYA IN KERALA, INDIA

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Dengue has been known to be endemic in India as a benign and self-limited disease, the principal vector of which is *Aedes aegypti*. In recent years, the disease has changed its course manifesting in the severe form as DHF, with increasing frequency of outbreaks in many urban and rural parts of India. Similarly, chikungunya is another illness caused by chikungunya virus (CHIKV), transmitted by *Ae. aegypti*. In India, chikungunya re-emerged after a lapse of three decades in a virulent epidemic form in late 2005. In 2006, a total of 1.39 million suspected cases from 213 districts in 15 states and about 565.42 million people were at the risk of infection. Kerala state reported 70,731 suspected cases mainly from three coastal districts viz., Alappuzha with a maximum of 58,308 (82.44%), Thiruvananthapuram with 8,311 (11.75%) and Eranakulam with 1,840 (2.60%) mostly confined to urban areas including small townships. A longitudinal evaluation was undertaken during 2009 - 2010 to assess the field efficacy of attracticide in Kadakkarapally and Vettackal of Alappuzha district, Kerala, India. 216 houses (951 population) were selected and 748 ovitraps (control and experimental) were placed both inside and outside the house. Monitoring of ovitraps was done on weekly basis and the ovitrap positivity in experimental and control bowls in Vettackal ranged from 14.5 to 55% and 9.9 to 46.7% respectively. The corresponding results in Kadakkaraplly were 19.8 to 40.8% and 15.4 to 36.5%. Oviposition active index (OAI) of the pooled data ranged from 0.2 to 0.5 indicating effective attractant property of the compound. The study clearly indicated that the compound lured Aedes mosquitoes to oviposit in ovitraps placed inside and outside the house. Besides, the increase in number of eggs in experimental bowls revealed pheromonal action of the compound and its feasibility as an effective tool in surveillance and control of Aedes mosquitoes in Kerala, India.

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MODELLING OF THE EFFICIENCY OF THE INSECTICIDE FENITROTHION ON THE *ANOPHELES GAMBIAE* TO MALANVILLE

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The control methods used against mosquitoes nowadays are the effective ways of protection against malaria in Africa. The control of the vector is however subject to increasing problems of resistance of the wild anopheles to conventional insecticides. This has led the IRD and WHO to test the quality of new formulations of the insecticide in indoor residual spraying in Malanville (North Benin). Fenitrothion has presented five forms according to the dose, the liquid or the powdered nature; these have been evaluated in six experimental huts belonging to the Anopheles Biology and

Control (ABC) network. A longitudinal study was carried out to evaluate the product according to four entomological criteria, among these; two have the characteristics that are essentially deterrent and lethal. So we have focused our research work on these two aspects. As far as this study is concerned, 2735 anopheles were caught in six trap-huts for six months. Our objective was to determine in what form (liquid or powdered) the insecticide was more effective at limiting the number of mosquitoes entering the huts and kill them. To do this, we used two tests including the comparison, the nonparametric Kruskall-Wallis' test (to determine if there is a significant difference in terms of the median among the six trap-huts of the study) which one is added to two by two comparison's test and the equality of several proportions with Holm correction (to establish a significant difference in terms of the death proportion in the six trap-huts). Then two generalized linear models including mixed effect (with random variable, day) for deterrent criteria were established to compare which of the five trap-huts has repelled or has effectively killed mosquitoes. An observation of significant differences between the numbers of mosquitoes captured and their survival in the control hut in relation to the treated huts, we can thus conclude that the mixed Poisson model explains better the phenomenon of deterrence while the joint logistics best explains the phenomenon of survival of mosquitoes in the treated cells.

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EVALUATION OF HUMAN EXPOSURE TO *AEDES ALBOPICTUS*, TOWARD A BIOMARKER OF VECTOR CONTROL EFFICACY

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The global expansion of *Aedes albopictus* stresses the need to improve current entomological methods. Recent findings suggest that human antibody (Ab) response to arthropod salivary proteins can provide new tool for monitoring vector populations. In La Reunion urban area, *Ae. albopictus* is the only anthropophilic *Aedes* specie. We aim to evaluate human IgG response to *Ae. albopictus* salivary proteins in order to get an insight of the adult population exposed to *Ae. albopictus* bites as well as the efficacy of vector control program. We measured the IgG response to *Ae. albopictus* saliva before (T0), two (T+2), four (T+4) and six (T+6) weeks after deltamethrine pulverisation. The use of individual protection devices was investigated and the abundance of *Ae. albopictus* adult female population was monitored. At T0, the immunoassays indicate high prevalence (83%) of IgG anti *Ae. albopictus* saliva which is consistent with the high density of adult female population. This Ab response is maintained at T+2 and T+4 while significant decrease is observed at T+6 (61%) coinciding with the decline of *Ae. albopictus* population. Additionally, the level of IgG anti saliva is lower in individuals using an individual protection compared to those unprotected (P=0.034). We assessed the Ab cross reactivity between *Ae. albopictus* and *Ae. aegypti* saliva. Low level of IgG cross reactivity is noticed between these two closely related species, underlining the specificity of the Ab response. These results suggest that IgG to *Ae. albopictus* saliva could be a potential biomarker of exposure to *Ae. albopictus* bites and a direct tool for the evaluation of control strategies; therefore, allowing to assess accurately the risk of *Ae. albopictus*-borne diseases transmission. To improve the reproducibility, the identification of specific *Ae. albopictus* salivary protein is under investigation.

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DEVELOPMENT OF ALLELE-SPECIFIC LOOP-MEDIATED ISOTHERMAL AMPLIFICATION METHOD (AS-LAMP) FOR DETECTION OF THE L1104F KDR MUTATION IN *ANOPHELES GAMBIAE* S.L

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The knock down mutation (kdr) in Western Africa is due to a substitution of the Leucine (L) by the Phenylalanine (F) in the position 1014 of the sodium channel gene sequence. This mutation induces resistance to pyrethroids and impacts negatively vector control against *Anopheles gambiae* s.l. The management of resistance to insecticides requires simple and effective tools for its early detection and for early decision-making. Our study aimed to develop simple and cost/effective method to detect the West African-type *kdr* mutation in field collected mosquitoes. Specific primers to detect the mutation have been designed with the mutation on the 5' end of the BIP primer to allow distinguishing between the resistant type (L1014F) and the sensitive type (L1014L) of the kdr mutation. Genomic DNA of three mosquitoes homozygous (L1014F/L1014F), heterozygous (L1014F/L1014L) and homozygous (L1014L/L1014L) confirmed by DNA sequencing has been used as template to set

the reaction conditions. The reaction has been processed in a real time turbidimeter at 63°C for 75 min. The reaction has been detected by the turbidity values as well as by naked eye. The sensitivity, the specificity of this method has been compared to the DNA sequencing method using 120 field-collected mosquitoes. The detection time for the L1014F/L1014L and L1014L/L1014L genotypes were around 62 min and 75 min respectively using L1014L type primers. Using these primers, there is no amplification for the resistant type untill 75 mins after incubation. For the resistant genotype detection, the amplification starts around 60 min and 65 min after incubation and there is no amplification for the sensitive type until 75 min when using the resistant type primers. The specificity and the sensitivity of the AS-LAMP compared to the DNA sequencing were respectively 0.92 (CL: 0.74 - 0.98) and 0.99 (CL: 0.94 - 1) This AS-LAMP method can be performed using minimum equipment like a water bath at 63°C. The reaction result is detectable by naked eye due to the deposit of magnesium pyrophosphate a by-product of the reaction. AS-LAMP can be use for *kdr* mutation detection for earliest decision-making in the context of less equiped laboratory.

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FIELD USE OF GENETICALLY ENGINEERED (GE) MOSQUITOES

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Recent advances in insect genetic engineering have opened new possibilities for the control of mosquitoes and hence of mosquito-borne diseases. Oxitec has developed engineered strains of *Aedes aegypti* and *Ae. albopictus* which are homozygous for one or more dominant lethal genes and are "genetically sterile" unless provided with the repressor molecule tetracycline in the diet. Use of such strains for mosquito control, a method known as RIDL, is based on the Sterile Insect Technique (SIT) which has been used successfully since the 1950s for the area-wide suppression or elimination of several major agricultural pest insects. Sterile males are released continually over a wide area to mate with the target pest population; no progeny result from these matings and the target population declines. Engineered strains with the necessary genetic properties ('RIDL strains') have been constructed. Field trials of the lead strain of *Ae. aegypti*, OX513A, have been initiated in several countries. This followed extensive testing in contained conditions and mathematical modeling to predict the outcome both of such trials and of potential programmatic use. Such models indicate that this would be both effective and cost-effective as a dengue control strategy, potentially as a stand-alone method but even more so if integrated with other existing methods such as larviciding and source reduction. This presentation will summarise the results of experiments to date and discuss the options for further testing and programmatic use of such technology.

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INCREASE IN SPOROZOITE RATES AND KDR ALLELE FREQUENCIES AFTER INITIATION OF IRS AND ITN INTERVENTIONS IN CONTINENTAL EQUATORIAL GUINEA

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Anti-vector approaches reliant on insecticides may be compromised as a result of selection for target site mutations conferring resistance to pyrethoid and carbamate-class insecticides. In 2007, The Equatorial Guinea Malaria Control Initiative (EGMCI) was initiated in the continental region of Equatorial Guinea with funding from the Global Fund. The EGMCI comprises a comprehensive anti-malaria program with a strong emphasis on anti-vector interventions consisting of multiple rounds of indoor residual application of pyrethroids, a single round of a Bendiocarb as well as distribution of deltamethrin-treated bed nets. Entomological monitoring provides important data on vector abundance, sporozoite infection levels and insecticide resistance alleles. We analyzed 1,462 *Anopheles gambiae* mosquitoes from nine sentinel sites prior to the start of the intervention activities in 2007 and 3,261 mosquitoes from eleven sites in 2009-10 after the start of intervention activities. Conventional and quantitative PCR assays were employed to determine species and molecular form identification, sporozoite rates, *kdr* and *ace*-1 (carbamate target site mutation) allele detection in individual mosquitoes. Significant increases in *kdr* allele frequencies were detected in seven of the nine sites for which pre- and on-going intervention data were analyzed. Sporozoite incidence in sentinel sites increase between 2007 and 2010 and this change was highly significant. To date, no *ace*-1 alleles have been observed in any sentinel site. In conclusion, prior to the initiation of intervention activities, both *kdr* alleles were present in all sentinel sites with the L1014F allele occurring at significantly higher frequencies than the L1014S and wildtype alleles. The dramatic increase in *kdr* allele frequencies since the start of anti-vector interventions is likely due to the selection pressure imposed by the intensive use on pyrethroids as part of the IRS and LLIN distribution programs. Given the absence of *ace*-1 alleles, carbamate insecticides would be a suitable choice for continued IRS applications. Significant increases in sporozoite rates were observed in five of the six sites for which paired data was available. Although no comparative data on mosquito abundance before and after control is available, these results suggest that the risk of infection to humans remains high despite ongoing anti-vector activities.

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PUBLIC POLICY FAILURES AND THE DEVELOPMENT OF NEW INSECTICIDES

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Public health insecticides (PHIs), on netting or sprayed on walls, are vital to control vector-borne diseases and are the primary means of preventing such diseases. The current arsenal of PHIs for malaria control is limited to just 12 old chemicals, most belonging to one class (pyrethroids). This is the direct result of environmentalist opposition and misinformation campaigns, stifling regulatory hurdles, weak public health advocacy, limited commercial markets, and activism by some United Nations agencies; conditions which are worsening. While the World Health Organization (WHO) Global Malaria Program and Roll Back Malaria Partnership recognize the need for new PHIs and are beginning to take steps to help encourage investment, other UN agencies are conducting a global campaign to eliminate the use of PHIs. This old campaign was reinvigorated in 1997 when the World Health Assembly passed resolution 50.13 calling on member countries to reduce their reliance on PHIs. As a continuation of this campaign, the UN Environment Program (UNEP), the Global Environment Facility, the Stockholm Convention Secretariat (SCS), and environmental sectors of WHO and the Pan American Health Organization falsely claimed success of 'environmentally sound malaria control interventions' in Mexico and Central America without PHIs, specifically DDT. Additionally, even though the Stockholm Convention provides an exemption for use of DDT until a safe, effective and affordable alternative is available, the SCS has proposed plans to eliminate use and production of DDT by 2020. In response and to ensure continued availability of DDT to malaria programs where it is needed, the Southern African Development Community has officially announced intent to begin local production of DDT. The fact that this region has committed to producing a PHI in the 21st century that was first created in the late 1800's, highlights severe regulatory and policy flaws governing insecticide

development and disease vector control. Also it prominently displays the environmental movement's war against control programs in disease endemic countries.

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NOVEL TECHNIQUES FOR EVALUATING SPATIAL REPELLENTS

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Spatial repellency which refers to the ability of an insecticide which in the vapour state, prevents mosquitoes from gaining access to the host and inhibit blood feeding, is gaining considerable attention in the recent past. There is a slow paradigm shift from dependence upon the toxicity of insecticides as the only mode of action towards mosquitoes that transmit malaria. Evidence in the past shows that the main method by which DDT reduced malaria prevalence in most parts of the world was by its spatial repellency characteristic. The guidelines put in place by the World Health Organization for evaluating spatial repellents are not sufficient and do not encompass most aspects of spatial repellents. In this study we have developed novel throughput semi-field systems and full field experimental hut assays for quantifying the main outcome indicators of spatial repellents which include; deterrence, irritancy/excito-repellency, feeding inhibition, effective dose and distance of spatial repellents and mortality. We have compared transfluthrin and metofluthrin coils to DDT which is the gold standard spatial repellent. The techniques used for testing in this study are safe, capture all outcome indicators for testing spatial repellents and are a reasonable representation of what goes on in the real world where spatial repellents are used. Our results indicate that the pyrethroids tested are more effective in terms of deterrence compared to DDT. Transfluthrin has the highest deterrence when compared to metofluthrin and DDT. It is probably worth noting that the greatest effects of DDT in comparison to those of transfluthrin and metofluthrin are irritancy and toxicity rather than deterrence as indicated in other studies. Further studies are being done to determine the most effective and acceptable format of delivering spatial repellents.

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BEHAVIORAL RESPONSES OF *AEDES AEGYPTI* TO DUET™ AND ITS TWO PYRETHROID COMPONENTS UNDER LABORATORY CONDITIONS

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DUET™ is an insecticide composed of two pyrethroids (1% prallethrin and 5% sumithrin) that is applied as an ultra low volume (ULV) spray to kill adult mosquitoes. It has previously been shown to activate *Culex quinquefasciatus* females in the laboratory resulting in greater mortality. Formulations of DUET™ and its two active pyrethroids were studied to evaluate behavioral responses in 4-8 day-old bloodfed and non-bloodfed ("unfed") *Aedes aegypti* females. Sub-lethal formulations of the pyrethroids and DUET™, and inert ingredients (control) were delivered in a spray cloud of ULV droplets in a wind tunnel and the responses of individual mosquitoes were videotaped. Videotapes were prepared and analyzed during pre-spray, spray and post-spray periods for 80 females using three behavioral analysis programs (*e.g.*, Motus, Ethovision, and Observer) and various behavioral responses (*e.g.*, time to flight, speed of flight, distance flown, duration of flight, and overall flight speed) were measured to determine the impact of the exposure to the different treatments. We found that all three insecticide treatments produced significantly more movement in all groups of females than in the control group. Unfed females moved greater distances when exposed to DUET™ and sumithrin than when they were exposed to prallethrin alone while bloodfed females traveled about the same distance regardless of insecticide treatment. A similar behavioral pattern was observed for overall flight velocity. No distinct pattern of percent time moving was observed regardless of bloodfed status and insecticide exposure. Comparative results of similar studies with *Ae. albopictus* will also be presented and implications of these laboratory results for field applications will be discussed.

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RESISTANCE OF *AEDES AEGYPTI* TO INSECTICIDES IN MARTINIQUE (FRENCH WEST INDIES) AND IMPLICATIONS FOR DENGUE VECTOR CONTROL

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Dengue virus, transmitted by *Aedes aegypti*, is reemerging dramatically in Martinique Island (Caribbean). One of the principal recourses to reduce the transmission remains the fight against the vector by the use of insecticides. Unfortunately, insecticide resistance (metabolic and target site mutation mechanisms) to conventional insecticides (pyrethroid and organophosphate) is strong and widespread among local mosquito populations. The present study was designed to measure and understand the phenotypic impact of resistance on the efficacy of adulticide and larvicide treatments at an operational scale. To assess the impact of pyrethroid resistance on the efficacy of treatments, 3 rounds of applications of deltamethrin and natural pyrethrins were performed with vehicle-mounted thermal foggers in 9 localities of Martinique. Efficacy was assessed by monitoring mortality rates of naturally resistant and laboratory susceptible female mosquitoes placed in sentinel cages. Results showed high mortality rates of susceptible sentinel mosquitoes treated with deltamethrin while resistant mosquitoes exhibited very low mortality. There was no reduction of either larval or adult *Ae. aegypti* population densities after treatments. This suggested a limited efficacy of pyrethroid treatments for reducing the virus transmission during epidemics. Conversely, we showed the potential of using alternative larvicides (spinosad, pyriproxyfen and diflubenzuron) for the control of organophosphate resistant *Ae. aegypti* larvae. Spinosad (naturalyte) and pyriproxyfen (growth regulator) were also used in mixture to measure the residual efficacy of the combination of their different modes of action. Under field conditions, pyriproxyfen and Bti failed to curtail *Ae. aegypti* populations after 4 weeks. Conversely, diflubenzuron and spinosad showed a residual efficacy of 16 weeks suggesting that these chemicals may be promising alternatives to Bti and temephos for controlling insecticide-resistant mosquitoes in Martinique. The mixture remained effective for 18 weeks, showing that the combination of the 2 larvicides acted to increase the residual activity of the treatment. The mixture could preserve the utility of both insecticides in public health programs. This study emphasizes the urgency in the need for further research to provide new tools and innovative strategies to manage insecticide resistance in dengue vectors.

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SUSCEPTIBILITY TEST OF FEMALE *ANOPHELES* MOSQUITOES TO TEN INSECTICIDES FOR INDOOR RESIDUAL SPRAYING (IRS) BASELINE DATA COLLECTION IN NORTHEASTERN NIGERIA

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Malaria is a major public health problem in Nigeria, accounting for about 60% of all outpatient attendances and 30% of all hospital admissions. Several insecticides have been proposed for Indoor Residual Spraying (IRS). However, insecticides vary considerably in their effectiveness against different species and strains of *Anopheles* mosquito.WHO standard insecticide- impregnated papers were used to conduct bioassays in the study area against local populations of *Anopheles* species with a view of selecting the suitable insecticides for IRS. These include: Cyfluthrin (0.15%), DDT (4%), Deltamethrin (0.05%), Lambdacyhalothrin (0.05%), Malathion (5%), Permethrin (0.75%), and Propoxur (0.1%), untreated blank papers were used as control. Two to three days old, female *Anopheles* species, glucose fed, none blood fed, were exclusively used in the bioassay. The result of the knockdown time periods of female *Anopheles* mosquitoes exposed to insecticides-impregnated filter papers after one hour indicated that Alphacypermethrin had the lowest KD_{50} (time taken to knockdown fifty percent of the exposed mosquitoes) values of 4.8 minutes. Relatively moderate KD_{50} values (minutes) were obtained with Propoxur (11.34), Deltamethrin (13.20), Malathion (15.82), Bendiocarb (17.29), Permethrin (18.43), Cyfluthrin (20.28) and Lambdacyhalothrin (23.11). Relatively higher KD_{50} values were obtained with Bifenthrin (27.29) and DDT (32.12) impregnated papers. The results of the 24 h post-exposure mortality indicate that *Anopheles* mosquitoes were susceptible to Alphacypermethrin and Malathion with 100.00% mortality achieved. Suspected cases of resistant were noted with Permethrin and Bendiocarb having mortality values of 96.67% each. On the other hand, cases of resistant were noted with Lambdacyhalothrin (93.33%), Deltamethrin (83.33%), DDT (78.33%) and Cyfluthrin (55.00%). The *Anopheles* species identified during the study were *A. gambae, A. funestus and A. nili.* The public Health significance of these findings is discussed.

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ENTOMOLOGIC BASELINE SURVEILLANCE IN PRELUDE OF INDOORS RESIDUAL SPRAYING IN BAROUELI, MALI

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Baseline data are important for assessing interventions used for disease control. In prelude of implementing IRS in the Baroueli district through PMI/USAID's support, an entomologic baseline study was conducted. Mosquito collections involving pyrethrum spray catches and human landing catches were conducted monthly in three villages from June to October. Bioassays were conducted to assess insecticide resistance in vectors using WHO test kits and five insecticides. Results showed 51% of all mosquitoes collected were *Anopheles* spp, 46% *Culex* spp and 3% *Aedes* spp (n=3399). *Anopheles gambiae s.l.* represented 39% while *An. rufipes* represented 12%. Within *An. gambiae s.l., An. gambiae s.s.* predominated over *An. arabiensis* (89.2% vs 10.8%, n=1403) and the M molecular form predominated over the S molecular form (93.3% vs 6.7% n=1251). Entomological inoculation rates were 0, 2.3, 7.8, 5.8 and 1.4 infective bites per human per month from June to October, respectively. The indoor *vs.* outdoor biting behavior assessment showed a slightly higher mean number of vectors indoors (98.7, SD=109.9) than biting outdoors (85.8, SD=106.2) but the difference was not statistically significant (independent T-test: t=0.187, df=8, p=0.856). Bioassays showed resistance to deltamethrin, permethrin, lambda-cyhalothrin and DDT while bendiocarb, a carbamate, showed a very high efficiency (100% mortality). These data provide valuable updated information on malaria vectors in the district. Of particular interest is the biting/landing behavior. More investigations are needed to better understand the biting behavior as this could be crucial for vector control of malaria.

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SEMI-FIELD EVALUATION OF DURABLE RESIDUAL WALL LINING AS AN ALTERNATIVE TO INDOOR RESIDUAL SPRAYS

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Insecticide-treated nets (ITNs) and indoor residual spraying (IRS) have been widely promoted as primary methods for controlling malaria vectors. However, achieving the desired ITN coverage and usage is challenging and IRS requires regular, repeated insecticide applications. In this study, we are assessing the efficacy of insecticide-treated ZeroVector™ (Vestergaard Frandsen) Durable Lining (DL) as an alternative to IRS for indoor control of *Anopheles* mosquitoes and prevention of malaria. ZeroVector™, a thin, blue sheet of woven shade cloth impregnated with deltamethrin, is being evaluated against 3 species of mosquitoes (*Anopheles quadrimaculatus*, *Culex quinquefasciatus*, and *Aedes aegypti*) and a stable fly (*Stomoxys calcitrans*) under semi-field conditions. Studies are being conducted at the USDA Center for Medical, Agricultural and Veterinary Entomology in Gainesville, FL. The study utilizes 5 wooden huts, with interior measurements of 9'5" x 7'5" x 7'11". Five panels of the DL have been cut, 8'4" L x 7'6" W, and attached vertically to the interior walls of each hut (with a staple gun), with an approximately 2" fold at the ceiling and floor. Untreated panels have been placed in 2 "control" huts and deltamethrin-treated panels have been placed in 3 "treatment" huts. Two types of evaluations are being conducted to determine durable wall liner efficacy. The WHO cone bioassay touch test is being used to evaluate knockdown and mortality caused by the DL against the 3 mosquito species; this test forces the exposed insects to come into contact with the wall lining. Three replicates (consisting of 10 female mosquitoes/cone) of each species were conducted per hut monthly. In the second test, 100 free-flying female mosquitoes of each species and 100 stable flies were released into the huts and able to fly freely and land wherever they chose. One hour after release, a Biogents Sentinel (BGS) trap (baited only with BG-Lure), which had previously been placed in the center of each hut, was activated and allowed to run overnight (ca. 15 hrs). The following day, the traps were collected and all dead mosquitoes were vacuumed from the floor, identified to species, and counted. All remaining flying insects were collected separately in an aspirator and held for 24 hrs and monitored for additional mortality. In the first 6 months of the study, 100% mortality was observed with the cone tests and few mosquitoes or flies were captured in the BGS trap in the treated huts.

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TRANSCRIPTOMICS OF PYRETHROID RESISTANCE IN WILD *ANOPHELES GAMBIAE* MOSQUITOES

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Pyrethroid resistance is one of the most important challenges in malaria vector control. As a consequence, determination of resistance mechanisms and development of cost-effective and reliable resistance monitoring

tools are of primary importance. The understanding of the molecular mechanisms of insecticide resistance in mosquitoes has progressed with technological advancement. Gene amplification-based techniques allowed the identification of two alternative point mutations in the para-type sodium channel gene that leads to knock-down resistance (kdr). Microarray techniques took the analysis of insecticide resistance mechanisms to genome-wide expression profiling and allowed the identification of resistance mechanisms related to transcript expression levels. However, microarrays are limited to the genes spotted on the array and provide relative expression levels, with no sequence variation information. Moreover, additional mechanisms of resistance (i.e. cuticular proteins and/or mitochondrial genes) have been hypothesized but poorly investigated due to the absence of appropriate methodological tools. The recent RNA-seq technology has emerged as an improved method for transcriptome analysis allowing both the absolute transcript quantification and the detection of coding sequence variation. On this basis, we classified field-derived *Anopheles gambiae* mosquitoes into deltamethrin resistant or susceptible on the basis of the standard WHO bioassay test and generated RNA-seq data from both pools. We are analyzing both the difference in transcript expression level and genetic variation in mRNA between RNA-seq libraries from both pools because either gene expression changes or genetic variation may contribute to insecticide resistance. Preliminary analysis detected 433,885 and 364,046 unique SNPs in the susceptible and resistant sample, respectively. Among these SNPs, 140,883 were common in both samples.

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REPORTED ADVERSE AND SERIOUS ADVERSE EVENTS AFTER THE ADMINISTRATION OF INFLUENZA A (H1N1) VACCINE IN CENTRAL GHANA

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The emergence of influenza A (H1N1) virus prompted the development of influenza A (H1N1) monovalent vaccines (2009-H1N1). The use of the vaccine was recommended by the Centers for Disease Control and Prevention (CDC) Advisory Committee on Immunization Practices (ACIP). Adverse events after vaccinations occur but are generally rare. The study was carried out to identify reported adverse and serious adverse events associated with an influenza A (H1N1) vaccine. This cross sectional study was carried out between mid of July 2010 to the 31st of August 2010 in Kintampo North Municipality and Offinso South Municipality in Ghana. The study was carried out as part of the INESS pharmacovigilance study of KHRC. Data were collected from consented participants using questionnaire. Of the 420 forms that were given out to consented participants in the two regions, 379 (90.2%) were returned with completed information related to the Influenza A (H1N1) vaccine. Participants who took the vaccine reported of adverse events such as fever, headache, chills, stomach ache, diarrhoea, pain in the heart and fast heartbeat. 4.4% (16/366) of those who received the vaccine were hospitalized for the adverse event they reported to have experienced after vaccination. Of the 4.4% of the vaccinated participants that were hospitalized, 43.8% (7/16) were males and 56.3% (9/16) were females. There was no difference between the proportions ($p=0.97$) of males and females that were hospitalized after vaccination. In conclusion, Ghana started use of Pandemrix- Influenza A (H1N1) vaccine in June 2010. Symptoms reported ranged from expected to reported death case. This survey of 379 people recorded 16 hospitalizations due to symptoms reported after vaccination. The study was not controlled and therefore could not make claims of whether the serious adverse events were associated with the vaccine. We would want to recommend post-marketing monitoring of adverse events after vaccinations.

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EPIDEMIOLOGICAL TRENDS OF RABIES IN DOMESTIC ANIMALS IN SOUTHERN THAILAND 1994-2008

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Rabies is an acute viral encephalomyelitis that affects wild and domestic mammals. Worldwide, human death due to rabies is approximately 55,000 cases annually. In Thailand, dogs are the main reservoirs and play an important role in rabies transmission. Although recent work indicated that clinical signs and demographics can be used as epidemiological tools for rabies diagnosis, few studies have been published on rabies risk factors in veterinary research. In Thailand, it is thought that rabies prevalence is highest during the hot dry season. People are less aware of rabies outside of this season making it difficult to control rabies year round. This study addresses the relationships between rabies infection and season, time and regions. We identified the risk factors most strongly associated with rabies and the degree to which each factor increased or decreased the odds of rabies infection. We also evaluated spillover assumption of rabies from dogs. Rabies and associated risk factors in dogs, cats and cattle (3,454 animals from 14 southern Thailand provinces submitted between 1994 and 2008) were evaluated using a mixed-effect logistic regression model. The overall prevalence of rabies infection was 48%, with 73%, 51% and 16% of tested cattle, dogs and cats positive for rabies, respectively. There was no seasonal variation in this region so rabies can occur year round in southern Thailand. Among unvaccinated dogs, the odds of rabies were 1.7 times higher than in vaccinated dogs. The odds of rabies were twice as high in dogs with a bite history compared to dogs with no bite history. Dogs less than one year of age had the highest likelihood of rabies. Owned and stray dogs had the same risk of rabies. Aggression was strongly associated with rabies in dogs; thus, most of dog rabies cases in the southern region were the furious form. In cattle, aggression, pharyngeal paralysis, hyperactivity and depression were associated with rabies. The annual fluctuation of the species-specific rabies prevalence suggested a positive correlation between canine and either feline ($r = 0.60$; $P = 0.05$) or bovine rabies ($r = 0.78$; $P = 0.004$). Increased vaccine coverage and public education are needed to reduce the risk of rabies in this population. In highly endemic areas, vaccination in cattle was recommended.

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CLIMATE, LAND USE AND TRAVEL TIMES PREDICT THE SPATIAL ADVANCE OF CASES OF CHIKUNGUNYA DURING AN OUTBREAK IN SOUTHERN THAILAND

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In 2008, Chikungunya re-emerged in Thailand after decades of absence. Cases appeared first in the extreme south of the country and advanced ~300 km over the next 18 months to the middle of the country. The spatial advance of cases appears to have two rates, first advancing slowly from October 2008 to April 2009 then rapidly after April 2009. We hypothesize that climatic variation affected the efficiency of spread in the country, slowing the spatial advance during one period of the year. To try to determine the effect of climate on Chikungunya transmission, we created 4 classes of transmission models, hypothesizing that climate affects; a) the transmission rate from mosquitoes to humans, b) the extrinsic incubation period, c) the fertility rate of mosquitoes and d) the mortality rate of mosquito larvae. We find that models that assume that

temperature and rainfall affects the transmission probability provide the best fit to data from 109 districts in southern Thailand. We find evidence that transmission intensities were high in all parts of southern Thailand at the time of emergence, but the spatial advance was too slow to spread cases throughout the south before changes in climate conditions led to low transmission of Chikungunya. The spatial advance resumed the next season when rainfall totals and mean daily temperature increased. Using a model that explicitly models the spatial transmission process from district to district, we find that a) forestry coverage, b) travel flows of individuals estimated using a gravity model, c) rainfall density and d) temperature and e) driving distance estimated using Google Maps were associated with the speed at which cases advanced.

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MOLECULAR CHARACTERIZATION OF HEMORRHAGIC FEVER VIRUSES CIRCULATING IN NORTHERN GHANA

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Haemorrhagic Fever (HF) viruses are prevalent in West Africa and have led to outbreaks with considerable morbidity and mortality. However, information on prevalence and geographic distribution of these viruses in Ghana is largely lacking. Molecular and serological tools to diagnose typical viral haemorrhagic fevers (VHFs) and research programmes identifying and characterising VHF agents, as well as estimating their public health relevance rarely exist in Ghana. This study seeks to establish prevalence of the causative agents of VHFs and help inform public health policies in Ghana. 8 selected health facilities in Northern Ghana have since July 2008, served as sentinel sites. Patients who meet the case definition are recruited as study subjects. Following informed consent, 5 ml of whole blood is collected by venipuncture and processed onsite into serum. Virus detection and characterization by serological and molecular techniques is then done at the NMIMR, Accra and BNITM, Hamburg, Germany for viral agents associated with VHF. Laboratory analyses have been conducted on 263 serum samples as at January, 2011. Investigations with RT-PCR assays for all the clinical specimens have been negative for HF virus types, Lassa, Crimean Congo, Yellow fever, Dengue, Ebola, Marburg, and Rift Valley. Anti-Lassa fever IgG antibody titers have been recorded for 1 case; one case with both (titers ≥ 1:20); anti-Dengue type-2 IgG (titer ≥ 1:80) and anti-Yellow Fever. Two cases exhibiting specific IgG (titers 1:1280 and 1:1280) and IgM (titers 1:20 and 1:20) against Chikungunya virus respectively were found. Viral RNA were however detected upon differential diagnoses of clinically similar pathogens from the total serum samples for agents including one case of Leptospira *interrogans,* 16 (6.1%) for Hepatitis C, 15 (5.7%) for Hepatitis A, 92 (35.8%) for Hepatitis E and 59 (22.4%) for Hepatitis B viruses. In conclusion, results so far obtained do not indicate a significant presence of VHF viral agents in the Northern regions of Ghana. However, the data generated suggest that viral hepatitis infections, which often share clinical symptoms with viral haemorrhagic fevers, are quite prevalent illustrating the need for differential diagnosis to be implemented.

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CASE-CONTROL STUDY OF RISK FACTORS ASSOCIATED WITH HEPATITIS C

This study was undertaken to identify risk factors for hepatitis C virus (HCV) infection among pregnant women seeking antenatal care in tertiary care hospitals of Karachi, Pakistan. We enrolled 119 cases and 238 controls. Cases were enzyme-linked immunosorbent assay (ELISA III) positive pregnant women for antibodies to HCV; controls were anti-HCV ELISA negative pregnant women. The mean age of study subjects was 26 years (SD 5) ranging from 15 to 50 years. The mean number of pregnancies for cases was 4 (SD 3) and for controls was 3 (SD 2). Among cases an average number of injections in any month was 40%, history of hospitalization was 61% and household contact with jaundice or hepatitis was 35%. In the final multivariable logistic regression model, five or more gestations (aOR = 1.99; 95% CI = 1.08-3.33), ‡1 injection (aOR = 2.33; 95% CI = 1.38-3.91) per month, hospitalization (aOR = 1.78; 95% CI = 1.01-2.99) and household contact with jaundice hepatitis (aOR = 3.32; 95% CI = 1.89-5.83) were independently associated with HCV. Iatrogenic exposure (health care injections, hospitalizations and gestations) is the major risk factor for transmission of HCV among pregnant women. To identify risk factors for hepatitis C virus (HCV) infection among pregnant women seeking antenatal care in tertiary care hospitals of Karachi, Pakistan. We enrolled 119 cases and 238 controls. Cases were enzyme-linked immunosorbent assay (ELISA III) positive pregnant women for antibodies to HCV; controls were anti-HCV ELISA negative pregnant women. results The mean age of study subjects was 26 years (SD 5) ranging from 15 to 50 years. The mean number of pregnancies for cases was 4 (SD 3) and for controls was 3 (SD 2). Among cases an average number of injections in any month was 40%, history of hospitalization was 61% and household contact with jaundice or hepatitis was 35%. In the final multivariable logistic regression model, five or more gestations (aOR = 1.99; 95% CI = 1.08-3.33), 1 injection (aOR = 2.33; 95% CI = 1.38-3.91) per month, hospitalization (aOR = 1.78; 95% CI = 1.01-2.99) and household contact with jaundice hepatitis (aOR = 3.32; 95% CI = 1.89-5.83) were independently associated with HCV. Iatrogenic exposure (health care injections, hospitalizations and gestations) is the major risk factor for transmission of HCV among pregnant women.Iatrogenic exposure (health care injections, hospitalizations and gestations) is the major risk factor for transmission of HCV among pregnant women. Iatrogenic exposure (health care injections, hospitalizations and gestations) is the major risk factor for transmission of HCV among pregnant women.

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THE DETECTION AND MOLECULAR CHARACTERIZATION OF HUMAN ROTAVIRUS G12 GENOTYPES IN THE EASTERN PART OF KENYA

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Globally, rotaviruses (RVs) are the most common cause of severe infantile viral diarrheal disease in infants and children < 5 years of age with ~ 527,000 deaths occurring annually in developing countries. In Africa alone approximately 300,000 young children < 5 years die each year due to RVs. The objective of this surveillance was to determine the epidemiology and the disease burden caused by hospitalization due to rotavirus in children <5 years of age in the Eastern region and to ascertain whether the distribution of rotavirus serotypes in circulation differs from the available rotavirus vaccines strains. Hospital surveillance data for rotavirus infections among children aged < 5years of age was started September 2009 in the Eastern region of Kenya. Cases of acute watery diarrhoea lasting 7 days or less, who are below 5 years of age and had been admitted to the Hospital were enrolled for surveillance. Diarrhoea faecal samples collected from children under 5 years of age with acute gastroenteritis were analyzed by enzyme immunoassays (EIA) and the positive samples genotyped by reverse transcriptase/polymerase chain reaction (RT-PCR) with RV specific primer pairs used for amplification of the VP7 and VP4 gene. From this study G12 was detected for the first time with a G/P combination as G12P [6]. The other genotypes detected were G9 P[4] and G9 P[8]. The common strain detected was G2 P[4]. It was interesting to see that most of the common strains G1 P[8] and G3 P[8] that are already included in the licensed rotavirus vaccines were not identified in this study. In conclusion, rotavirus is an important cause of acute watery diarrhoea in the Eastern region of Kenya among the under five children. The detection of G12 strains from different parts of the world in recent years suggests the possibility of its emergence as an important global genotype. Thus, Monitoring of cocirculating rotavirus strains and detection of emerging strains is important in the context of the availability of rotavirus vaccines. This study has extended our knowledge on the circulating G-genotype circulating in the Eastern region of Kenya.

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PREVALENCE AND CLINICAL OUTCOMES OF CONGENITAL CYTOMEGALOVIRUS INFECTION IN URBAN AND RURAL KENYAN MATERNAL/INFANT COHORTS

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Congenitally acquired cytomegalovirus (CMV) is one of the most prevalent viral congenital illness and amongst the most severe with outcomes including fetal demise, mental retardation, hearing and vision loss. 10% of infants with congenital CMV infection develop mild or severe disease. Estimates of congenital CMV prevalence in industrialized countries range from 0.4-2% while the few studies reporting CMV prevalence in developing countries found >5% prevalence. This study examined congenital CMV prevalence in two maternal/infant Kenyan cohorts, one in an urban setting and one in a rural setting. Congenital CMV is typically diagnosed by culturing infant urine for CMV virus which was not possible in this study. An alternative method of congenital CMV detection is the presence of CMV IgM antibodies in infant cord blood. This study used a high through-put microsphere-based multiplex method to quantify CMV IgM and IgG antibodies in infant cord blood. 521 infant cord blood samples were tested. 77 were found to be positive for CMV IgM antibodies giving an overall prevalence of 12.7%. Significant differences between rural (6.9%) and urban (17.2%) cohort CMV prevalence were found. We will correlate these findings with infant clinical outcomes including hearing and neurocognitive development as well as maternal HIV and malaria co-infections.

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INVESTIGATION OF POTENTIAL CIRCULATION OF HANTAVIRUS AMONG KENYAN WILD RODENTS AND THE IMPLICATIONS FOR PUBLIC HEALTH AND ZOONOSES MONITORING

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Hantaviruses (Family *Bunyaviridae*) have been associated with the human disease Hantavirus pulmonary syndrome (HPS). This virus has been documented to be transmitted to humans through rodent (Family *Murinae*) feces and urine. Evidence of Hantavirus across Africa remains quite scanty, although serological evidence has been published in several countries, including Kenya. To date, two Hantaviruses have been isolated from wild a rodent and a shrew in Guinea, *Sangassou* virus (*Hylomiscua stella*) and *Tanganya* virus *(Crocidura therasae)* respectively. Presence and epidemiology of these viruses across East Africa remain largely unknown. From June 2008 through June 2010, rodents and mammals were trapped across a broad geographic range within Kenya, selected based on varying ecological and climatic conditions, thereby increasing rodent species diversity in the sample population. Total nucleic acid was extracted from lungs of necropsized rodents and reverse transcription Polymerase Chain Reaction used to amplify S and L segment of the Hantavirus genus genome. A total of 392 rodents and small animals consisting of 18 different species of rodents and shrews were trapped. Six samples generated a 494bp fragment with primers designed to amplify the S segment coding for nucleocapsid protein, depictive of Hantavirus presence. Two of these samples were isolated from shrews *(Crocidura species)* and four from *Mastomys species* trapped in semi arid areas of Marigat (Rift valley) and Garissa (North Eastern) Kenya respectively. High throughput pyrosequencing and bioinformatic analysis is underway to characterize the nature, genetic similarity and identity of the PCR positive samples.

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DETECTION OF ALPHA VIRUSES IN MOSQUITOES FROM SEMI ARID AREAS OF KENYA

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Alphaviruses are diverse group principally mosquito-borne RNA viruses that cause diseases in humans worldwide. They include Chikungunya, O'nyong-nyong and Sindbis viruses. They cause febrile illnesses with encephalitis or arthritis. They are of significant public health concern with Venezuelan equine encephalitis virus having potential to be weaponised. To determine the presence and circulation of alphaviruses and the associated vector species responsible for their maintenance and transmission, a surveillance study was undertaken in two semi arid regions of Kenya.Mosquitoes were trapped using CO_2-baited CDC light traps from December 2009 to June 2010 in 6 selected sites in Ijara and Marigat districts during the wet seasons. Mosquitoes were morphologically identified to species, pooled to 25 mosquitoes per pool and homogenized in minimum essential medium using copper beads.The homogenates were clarified by centrifugation at 10,000 rpm and the supernatants inoculated in monolayers of VERO cells in 24 well plates. The cultures were incubated at 37˚C and observed daily for cytopathogenic effects (CPE). Cultures showing CPE were harvested and viruses identified by RT-PCR and sequencing.Over 92,000 mosquitoes were collected, identified into 37 species and pooled into 4,382 pools. Eleven NDUV isolates were obtained from pools of *Aedes mcintoshi* (7), *Ae. ochraceus* (1) and *Ae. tricholabis* (2) all collected from Ijara and *An. pharoensis* (1) from Marigat. SFV was isolated from *Ae. ochraceus* (3) and *Ae. tricholabis* (2) from Ijara and one isolate of SINV from *Culex antenattus* from Marigat.This study shows that SFV, SINV and NDUV are circulating among mosquito species in the two semi arid regions of Kenya and could account for some of the febrile illnesses of unknown etiology observed in these areas. NDUV was found in both sites. SFV was detected in Ijara while SINV was found in Marigat. Human surveys are being conducted to establish the actual involvement of human population in the circulation of the viruses. Control strategies to prevent alphavirus transmission should target the three mosquito genera.

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DIFFICULTIES IN ACCESSING SPECIALIZED MEDICAL CARE BY ENCEPHALITIS CASES DURING A NIPAH VIRUS OUTBREAK IN BANGLADESH

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Nipah virus (NiV) causes fatal encephalitis in humans. Previous outbreak investigations in Bangladesh have identified drinking raw date palm sap contaminated by Pteropus bats, the reservoir host of NiV, and person-to-person transmission as the major risk factors for NiV transmission. Reluctance of hospital health workers to provide hands-on care to NiV cases has been reported. During December 2010 - February 2011, we investigated an outbreak of NiV infection in Bangladesh to understand the risk factors for acquiring the disease and to explore the medical care received by NiV cases. We collected clinical and exposure history of the cases. We conducted a case control study to identify risk factors; 4 neighborhood controls were selected for each case. We explored

the medical care received by NiV cases at hospitals through in-depth interviews, informal and group discussions with family members and health care providers. We visited isolation wards in two hospitals to observe the care received by admitted cases. We identified 31 Nipah cases, of which 18 were Nipah IgM antibody positive. All cases died. In bivariate analysis, drinking raw date palm sap was the only risk factor for NiV infection (OR 17; 95% CI 4-70). Among the 31 cases, 30 (97%) were hospitalized. Twenty four cases were directly admitted or referred to tertiary hospitals for specialized care. Seven were transferred twice or more from tertiary hospitals. Family members of cases and a health care provider reported that some hospitals refused admission or transferred patients to a different facility if they were from a Nipah affected area or had a history of consuming raw date palm sap. Family members also reported unwillingness of providers to attend admitted NiV cases. One case, who had been weaned from mechanical ventilation, was forced to leave the hospital after laboratory confirmation of NiV infection. Subsequently, she was refused admission to another tertiary hospital. In isolation wards, patients were admitted without proper evaluation, NiV and non NiV cases were kept close to each other and had to share oxygen masks. Bangladeshi people should avoid drinking raw date palm sap to prevent transmission of NiV infection. Hospitals should develop strategies to triage and treat patients with diseases that transmit person-to-person, organize training for hospital staff on providing appropriate care to these patients and ensure implementation of infection control practices.

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COMPLEX SEASONAL FLUCTUATION IN ARBOVIRAL ACTIVITY IN TROPICAL AND TEMPERATE AUSTRALIA

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Mosquito-borne diseases typically exhibit strong seasonal patterns with a series of outbreaks followed by low endemic levels outside the typical season. Occasionally, the period of low incidence contains secondary peaks of smaller magnitude that are difficult to detect using traditional methods and aggregated monthly data across years. More precise methods that capture the variability in incidence across a series of annual cycles may enhance the design of early warning systems for such diseases. To illustrate, we applied a Poisson harmonic regression model with polynomial components to capture non-linear trends in the incidence of three arboviruses - Barmah Forest virus (BFV), Ross River virus (RRV), and dengue virus (DENV) - as reported to Australia's National Notifiable Diseases Surveillance System from 1991 to 2010. For each infection, we estimated major seasonality characteristics - peak timing and amplitude - and their confidence intervals using recently introduced delta-methods. Strong annual periodic fluctuations were observed for BFV and RRV, with increased seasonal activity (defined as incidence >1SD above the annual mean) occurring within a narrow time interval (generally February through April). Nevertheless, the onset of increased seasonal activity varied by as much as 2 months between years. Further, clearly defined outbreaks (incidence >2SD above the annual mean) were only noted in certain years. Secondary peaks, occurring in October through December, were an emerging phenomenon, appearing in the latter half of the time series for both BFV and RRV. In contrast, the seasonal pattern for DENV comprised floating primary and secondary peaks that varied considerably in terms of timing and amplitude between years. We also detected very specific oscillations in DENV every 5-7 years. These findings suggest that triggering environmental or other factors may vary from year to year, and may be changing over time. The findings illustrate how application of advanced analytical tools can enrich our understanding of the complex seasonal fluctuations of mosquito-borne diseases. Future work will explore the extent to which these predictive models can be enhanced by incorporating local environmental conditions, both temporal (e.g. precipitation, temperature) and spatial (e.g. proximity to estuarine habitat), using remote sensing and meteorological data.

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DEVELOPMENT OF A REVERSE GENETIC SYSTEM TO STUDY THE IMPACT OF THE P GENE PRODUCTS ON THE ENDOTHELIAL CELL INNATE ANTIVIRAL RESPONSE AGAINST NIPAH VIRUS

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The henipaviruses, Nipah virus (NiV) and Hendra virus (HeV), are highly pathogenic zoonotic paraymyxoviruses that cause fatal encephalitis in up to 75% of infected humans. Like other paramyxoviruses, henipaviruses employ a process of co-transcriptional mRNA editing during transcription of the phosphoprotein (P) gene to generate additional mRNAs encoding the V and W proteins. The C protein is translated from the P mRNA, but in an alternate reading frame. Sequence analysis of multiple, cloned mRNAs showed that the mRNA editing frequencies of the P genes of the henipaviruses are higher than those reported for other paramyxoviruses. Mouse antisera against synthetic peptides from the P, V, W, and C proteins of NiV were generated to study their expression in infected cells. All proteins were detected in both infected cells and in purified virions. In infected Vero cells, the W protein was detected in the nucleus while P, V, and C were found in the cytoplasm. Since endothelial cells and neurons are important targets for NiV pathogenesis in humans, we measured viral replication and innate immune responses in NiV infected primary endothelial cell types and one neuronal cell line. NiV infected endothelial cells generated a functional IFN-β response, which correlated with the unexpected localization of the NiV W protein to the cytoplasm. There was no antiviral response detected in infected neuronal cells. NiV infection of endothelial cells induced a significant increase of inflammatory chemokines secreted into the cellular supernatant, and these supernatants induced a corresponding increase in monocyte and T-lymphocyte chemotaxis. Our results suggest that the induction of pro-inflammatory chemokines in NiV infected primary endothelial cells *in vitro* is consistent with the prominent vasculitis observed in infections, and provide initial molecular insights into the pathogenesis of NiV in physiologically relevant cell types. We have now developed a reverse genetic system to study the individual roles of the NiV P gene products on the endothelial cell innate immune response.

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CROSS PROTECTIVE IMMUNITY AGAINST O`NYONG-NYONG VIRUS AFFORDED BY A NOVEL RECOMBINANT CHIKUNGUNYA VACCINE

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Emerging mosquito-borne alphavirus infections caused by chikungunya virus (CHIKV) or O`nyong-nyong virus (ONNV) are responsible for sporadic and sometimes large explosive epidemics. In particular, ONNV that is transmitted by anopheles mosquitoes has been the cause of a major epidemic in Africa which involved at least 2 million patients between 1959 to 1962. For decades, CHIKV has been an important etiologic agent of

human disease in Africa and Asia. The virus recently reemerged in the Indian Ocean islands, India and Southeast Asia causing several million cases of severe and often chronic arthralgia. Recently, we developed a candidate CHIKV vaccine by employing a genetic attenuation mechanism. The internal ribosome entry site (IRES) from encephalomyocarditis virus was used to replace the sub-genomic promoter in a cDNA CHIKV clone, thus altering the level and host-specificity of structural protein gene expression. The testing of vaccine in both normal outbred mice and interferon response-defective (A129) mice demonstrated that it is highly attenuated, immunogenic and efficacious after a single dose. Furthermore, the genetically attenuated vaccine virus was incapable of replicating in mosquito cells or infecting mosquitoes *in vivo*. In this study we sought to investigate the capacity of the CHIK/IRES vaccine to induce cross protective immunity against the closely related ONNV. Our studies demonstrated that the CHIKV/IRES candidate vaccine elicited strong cross neutralizing antibodies against ONNV and conferred protection against challenge with this virus after a single administration. Moreover, the role of antibodies in protection was established by demonstrating their efficacy in two models; i) CHIKV/IRES immune A129 dams transfer antibodies to their offspring that protect against ONNV challenge, and ii) anti-CHIKV/IRES antibodies confer protection in AG129 mice against ONNV independently of a functional IFN response.

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TICK-BORNE ENCEPHALITIS VIRUS ANTIGEN IN TICKS AND MILK SAMPLES IN SOUTHERN AREAS OF THE REPUBLIC OF KAZAKHSTAN

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Tick-borne encephalitis is a serious human disease, whose incidence and geographic location is expanding in several areas of the world including regions in Central Asia. Tick-borne encephalitis virus (TBEV), a flavivirus, is harbored by ticks in Kazakhstan and evidence also shows high levels of seroconversion in human sera. Using an antigen-capture ELISA, we analyzed ticks and milk from cows and sheep for TBEV in one endemic and three non-endemic territories of Kazakhstan. In the Almaty oblast, where TBEV is endemic, we tested samples from 1,295 ticks that were collected and pooled into 28 groups based on size and maturity. TBEV antigen was found in 3 of 19 groups of 1056 *Dermacentor marginatus* ticks, 0 of 5 groups of 235 *Haemaphysalis punctate* ticks, and 0 of 4 individual *I. persulcatus* ticks. In the same area, we examined 45 milk samples (15 sheep and 30 cows) collected from local individuals. TBEV antigen was detected in 3 sheep milk samples and 0 cow milk samples. We also conducted testing in three oblasts considered non-endemic: Kyzylorda, Zhambyl, and South Kazakhstan. In Kyzylorda, TBEV antigen was found in 6 of 142 pooled groups of 3,500 *D. marginatus* ticks. In Zhambyl, TBEV antigen was also found in 6 of 10 pooled groups of 250 *D. niveus* ticks. In South Kazakhstan, TBEV antigen was found in 17 of 40 pooled groups of 1,338 *H. asiaticum* ticks. Our data clearly show that ticks infected with TBEV are present not only in areas previously considered to be endemic (e.g., Almaty oblast), but also in the additional oblasts of Kyzylorda, Zhambyl and South Kazakhstan where TBE disease is not registered by public health authorities. Also, we show that in the endemic Almaty oblast, risk of infection with TBEV may take place not just through an infected tick, but also by consumption of infected milk or milk products.

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FETAL EFFECTS OF INFLUENZA IMMUNIZATION IN PREGNANCY

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We report the association of maternal antenatal influenza immunization with fetal and neonatal outcomes. The Mother's Gift project was a blinded, randomized trial of 340 pregnant urban Bangladeshi women who were randomized to receive either inactivated influenza vaccine or pneumococcal vaccine (control group). Gestational age, proportions of small for gestational age (SGA) infants, and mean birth weights were compared in 327 neonates.There was a reduction in % SGA infants from 38% in influenza vaccinees to 28% in controls ($p = 0.05$) and a trend of increased birth weights in flu vaccine recipients ($p = 0.09$), with no differences in mean gestational ages. Influenza virus did not circulate from August 2004 through January 2005, and the study groups were similar in the incidence of respiratory illness with fever (RIF) ($p=0.99$); during this interval, % SGA infants and mean birth weights were similar between the study groups. In contrast, during the interval of influenza virus circulation from February to June 2005 there was a 49% reduction of RIF episodes in the influenza vaccine group ($p=0.0003$). During the interval of influenza circulation, the % SGA infants was substantially decreased in the influenza vaccine group to 29% versus 44% in controls ($p=0.03$). Similarly, the mean birth weight of infants of influenza vaccinees was 3,178gm vs. 2,978gm in controls ($p=0.03$). In conclusion, influenza immunization of pregnant women substantially reduces the proportion of SGA infants and increases mean birth weights in this South Asian setting. These data suggest that influenza infections in pregnancy adversely effect fetal development, and further studies are needed to assess this unique observation.

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THE BURDEN OF PEDIATRIC DIARRHEA: PERCEPTIONS OF COST AMONG BOLIVIAN CAREGIVERS

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Bolivia has high rates of diarrhea related child morbidity and mortality. While child diarrhea is known to be costly to the Bolivian state, Bolivian caregivers benefit from universal insurance for their children and are believed to have minimal expenses associated with diarrhea. The study goal was to characterize caregiver costs and cost perceptions associated with seeking treatment for pediatric gastroenteritis in Bolivia. From 2007 to 2009, researchers interviewed 1101 caregivers of pediatric patients (<5 years of age) seeking treatment for diarrheal illness in five healthcare settings, in three geographic regions, and participating in a diarrheal surveillance program throughout Bolivia. Caregivers were surveyed on child demographics, clinical symptoms, direct (e.g., medication, consult fees) and indirect (e.g., lost wages) costs, and perceived economic burden of the child's diarrheal illness. Patient populations were similar across hospitals in terms of gender, age, appointment type, and duration of illness, while familial income varied when stratified on appointment type. Direct and indirect costs to families were significantly higher for inpatients as compared to outpatients ($p < 0.01$). Overall, 74% of caregivers reported that the cost of treatment affected their family economy, and this proportion differed significantly among hospitals ($p < 0.0001$) and by cost burden (cost of treatment as a percentage of family income; $p < 0.0001$). Logistic regression indicated significant positive associations of cost perception with cost burden (OR 20.83 95% CI [4.39 - 98.98]) and appointment type (outpatient vs. inpatient, OR 2.06, 95% CI [1.22 -3.49]). Diarrhea related costs were a large burden on Bolivian families, and those with a high cost burden were most likely to perceive these

costs as posing economic hardship. While overall costs were higher for hospitalized patients as compared to outpatients, perceptions of cost were higher among caregivers of outpatients. Families who perceive outpatient treatment as costly may delay care, possibly resulting in the need for more expensive inpatient care and potentially poorer health outcomes.

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PARTICIPATORY MAPPING AS A COMPONENT OF OPERATIONAL MALARIA VECTOR CONTROL IN TANZANIA

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Global efforts to tackle malaria have gained unprecedented momentum. However, in order to move towards the ambitious goal of eliminating and eventually eradicating malaria, existing tools must be improved and new tools developed. The City of Dar es Salaam, Tanzania, is home to the first operational community-based larviciding programme targeting malaria vectors in modern Africa. In an attempt to optimize the accuracy of the application of larvicides, a participatory mapping and monitoring approach has been introduced in 2005 that includes (1) community-based development of sketch maps of the target areas, and (2) verification of the sketch maps using laminated aerial photographs in the field which are later digitized and analyzed using Geographical Information Systems (GIS). The participatory mapping approach developed enables gap-free coverage of targeted areas with mosquito larval habitat control, and more equal distribution of the workload of field staff. The procedure has been tested, validated and successfully applied for five years within the operational larviciding programme. During the same period, the mapping coverage has been scaled up from 56 km^2 to an area of about eight times that size, thus covering the urban area of Dar es Salaam. The Government of Tanzania is currently scaling up the larviciding programme to the whole city region, using the map data and mapping procedure as a basis. The procedure is simple, straightforward, replicable and at relatively low cost. It requires only minimal technical skills and equipment. In the case of Dar es Salaam, the resulting database provides a spatial resolution of administrative boundaries that is almost 50 times higher than that of previously available data. This level of detail can be very useful for a wide range of other purposes rather than merely malaria control, for example implementation of council programmes in a variety of sectors and spatially-explicit analyses for research and evaluation purposes.

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DISTRICT-BASED HOUSEHOLD SURVEY DATA AND ASSOCIATED BIOMARKERS IN INDOOR RESIDUAL SPRAYING (IRS) AND NON-IRS DISTRICTS IN NORTHERN UGANDA

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Indoor residual spraying (IRS) with insecticides is a primary intervention to reduce malaria transmission. In highly malaria-endemic Northern Uganda, selected districts have been sprayed since 2007 with DDT and pyrethroids, before localized political opposition to DDT and documented resistance to pyrethroids prompted a shift to carbamates in 2010. Data from a household survey and associated biomarker collection in late 2010 in three contiguous districts of Northern Uganda were used to compare one non-sprayed district (Lira) with two IRS districts (Apac, sprayed once with carbamates in 2010 after one round each of DDT (2008) and pyrethroids (early 2010) and Pader, which received two rounds of carbamate spraying in 2010, following four rounds of pyrethroids (2007-2009)). District-level anemia and parasitemia prevalence estimates from a total of 1,773 children less than five years of age were calculated from the two-stage, cluster sample survey, using sampling weights and accounting for clustering. Parasitemia levels were significantly lower in both IRS districts compared to the non-sprayed district. In Apac, 37.2% of children had positive malaria blood smears, compared to 50.1% of children in non-sprayed Lira district, $p<0.01$. Parasitemia prevalence was lowest in Pader (16.9%, $p<0.001$ compared to both Apac and Lira), which had been sprayed twice with carbamates in 2010. Anemia (hemoglobin<11g/dL) was less common in Apac (38.4%) and Pader (36.9%), compared to Lira (53.0%), $p<0.001$. Bednet use by children was significantly higher in the IRS districts (69.6% in Apac and 64.6% in Pader) than in Lira (49.5%), but there were no significant differences between the districts in terms of food security or distance to the nearest health facility. These results indicate lower malaria burdens, according to biomarkers, in IRS districts compared to non-sprayed districts in Northern Uganda. Additional research is needed to better define causal relationships between IRS schedules and formulations and reductions in malaria indicators in areas of high transmission intensity.

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SPATIAL DISTRIBUTION OF BEDNET COVERAGE UNDER ROUTINE DISTRIBUTION THROUGH THE PUBLIC HEALTH SECTOR IN A RURAL DISTRICT IN KENYA

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Insecticide-treated nets (ITNs) are one of the most important and cost-effective tools for malaria control. Maximizing individual and community benefit from ITNs requires high population-based coverage. Several mechanisms are used to distribute ITNs, including health facility-based, targeted distribution to high-risk groups; community-based mass distribution; social marketing with or without private sector subsidies; and integrating ITN delivery with other public health interventions. Here we use data from a population-based census of more than 44,000 households to examine the extent of coverage with bednets in a district in western Kenya where the primary mechanism for distribution is to pregnant women and infants who attend antenatal and immunization clinics. We use both multivariable logistic regression and spatial techniques to explore the relationship between household bednet ownership and sociodemographic and geographic variables. We show that only 21% of households own any bednets, far lower than the national average of 60%. Ownership in households that include a member of a targeted group, either a pregnant mother or child under-5, was slightly higher; 24% and 25%, respectively, compared to 17% in households with neither. Pregnant women attending antenatal clinic were not more likely to own a bednet than pregnant women not attending antenatal clinic. We also show that coverage is spatially heterogeneous with less than 2% of the population residing in zones with adequate coverage to experience indirect effects of ITN protection. Wealth indicators such as land ownership and animal ownership had a larger effect on bednet ownership in urban than rural areas. The type of nearest facility (hospital, health centre or dispensary) was more important than the absolute distance to the facility in predicting bednet ownership, although both were significant in the multivariable regression model.

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FROM INTERVENTION TO IMPACT: MODELLING THE POTENTIAL MORTALITY IMPACT ACHIEVABLE BY DIFFERENT LONG-LASTING INSECTICIDE-TREATED NETS (LLIN) DELIVERY STRATEGIES

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The current target of universal access to long-lasting insecticide-treated nets (LLINs) is 80% coverage, with a goal of reducing malaria deaths by 75% or more by 2015. So far, mass distribution campaigns have been the main channel for large-scale delivery of LLINs, and more recently WHO has recommended that equal priority should be given to delivery via routine antenatal care (ANC) and immunisation systems (EPI) to target pregnant women and children from birth. These various channels of LLIN delivery are targeted to children of different ages. Since risk of mortality varies with child age, and LLIN effectiveness declines with net age, we hypothesise that the age at which a child receives a new LLIN, and therefore the delivery channel, is important in optimising the health impact of that net. We developed a dynamic mathematical model of delivery and impact of LLINs among children under five years of age and their household members, incorporating data on age-specific malaria death rates at different endemicities, net efficacy over time and net use by household structure. LLINs are assumed to be discarded at a constant rate after delivery. Our analysis found that a universal campaign giving 2 LLINs per household every 3 years with 80% coverage at delivery in a high transmission setting would achieve an annual average 23% reduction in under-five malaria mortality. If supplemented by an ANC distribution system giving one LLIN per birth with 80% of eligible women receiving a net, the mortality reduction achieved is 1.4 times higher, with very little additional redundancy in impact per LLIN, reflecting that children born in the years between distribution campaigns would otherwise have access to old nets or no nets at an age of high risk. This advantage holds if campaign delivery is targeted to under-fives giving one LLIN per child or if malaria endemicity is medium-to-low. Our results indicate that LLIN delivery policies must take into account the age of greatest malaria risk. Strong emphasis should be placed on supporting routine delivery of LLINs to young children as well as campaigns.

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SURVEILLANCE OF VECTOR POPULATIONS AND MALARIA TRANSMISSION DURING AN EL NIÑO IN THE WESTERN KENYA HIGHLANDS: OPPORTUNITIES FOR EARLY DETECTION OF MALARIA HYPER-TRANSMISSION

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Vector control in the highlands of western Kenya has resulted in significant reduction of malaria transmission and a change in the vectorial system. Climate variability as a result of events such as the El Nino increases the suitability of malaria transmission in the highlands. Surveillance and monitoring of transmission is an important component of early risk identification and management. However below certain disease transmission thresholds the traditional tools for surveillance such as the entomological inoculations rates may become insensitive. We carried out a study to determine the usefulness of a rapid diagnostic kit based on the prevalence of *Plasmodium falciparum* circumsporozoite surface protein and merozoite surface protein antibodies in humans for early detection of transmission surges in the western Kenya highlands. Indoor resting adult malaria vectors were collected in Western Kenya highlands in four selected villages categorized into two valley systems, the U shaped (Iguhu and Emutete) and the V shaped valleys (Marani and Fort Ternan) for eight months. Members of the *Anopheles gambiae* complex were identified by PCR. Blood samples were collected from children 6-15 years old and exposure to malaria was tested using Circum-sporozoite protein and Merozoite surface protein immunchromatographic rapid diagnostic test kit. Sporozoite ELISA was conducted for detection of circum-sporozoite protein. Among the four villages studied an upsurge in antibody levels was first observed in October 2009. *P. falciparum* sporozoites were then first observed in December 2009 at Iguhu village and February 2010 at Emutete. Despite an upsurge in antibody levels in Marani and Fort Ternan no sporozoites were detected throughout the eight month study period. The antibody based assay had much earlier transmission detection ability than the sporozoite based assay. Prior to 2002, no *An. arabiensis* had been reported in the western Kenya Highlands. In this study the proportion of *An. arabiensis* among *An. gambiae* s.l. ranged from 2.9-66.7% indicating a rearrangement of the species complex. This is an adaptation to insecticide interventions and climate change. The changing malaria transmission rates in the western Kenya highlands will lead to more unstable transmission, decreased immunity and a high vulnerability to epidemics unless surveillance tools are improved and effective vector control is sustained.

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IMPACT OF ARTEMETHER-LUMEFANTRINE (AL) AS FIRST LINE TREATMENT POLICY ON MALARIA TRANSMISSION AND UNDER FIVE MORTALITY IN A RURAL AREA WITH HIGH INSECTICIDE-TREATED NET (ITN) COVERAGE IN TANZANIA

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Wide use of artemisinin-based combination therapy (ACT) in addition to vector control measures is recommended in the fight against malaria. The ALIVE [Artemether-Lumefantrine In Vulnerable patients: Exploring health impact] project, assessed the impact of AL as first line treatment for uncomplicated malaria on transmission and <5yrs (U5) child mortality in Tanzania. Parasite prevalence was obtained by repeated cross-sectional surveys in two rural districts during two separate periods of first line anti-malarial therapy (2004-2006: sulfadoxine-pyrimethamine [SP], and 2008-2010: AL). Mortality rates were obtained using a demographic surveillance system. Changes in community malaria parasitaemia and U5 mortality between both periods were compared taking into account the contribution of malaria interventions and contextual factors such as rainfall and rice yields using linear and Poisson regression models. Overall, asymptomatic parasite prevalence (%) progressively declined from 25.0 in 2004 to 3.9 in 2010. A 10% increase in community net ownership was associated with a 4.6% reduction in parasitaemia (95% CI= -8.0% to -1.2%). Mean U5 mortality rate decreased by 33% over the entire period, from 27.0 per 1,000 person years in 2005 to 17 in 2009. The introduction of AL was associated with an 11% decrease in U5 mortality when adjusted for other key malaria interventions and contextual factors (IRR= 0.89; 95% CI= 0.79-1.0). One unit (ton of rice/ha) annual increase in rice yields, was associated with a 36% reduction in annual U5 mortality (IRR= 0.64; 95% CI= 0.54-0.75). On the contrary, ITN coverage was not responsible for significant reduction in U5 mortality. ACT implementation with AL in Tanzania together with other major malaria control programmes was associated with a considerable decline in malaria and U5 mortality. Food security with other key malaria interventions is crucial to support malaria control hence elimination.

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ANOPHELES SALIVARY GSG6-P1 PEPTIDE, AN IMMUNO-EPIDEMIOLOGICAL BIOMARKER FOR PERTINENT EVALUATION OF EXPOSURE HETEROGENEITY TO *ANOPHELES* BITES AND EFFICIENCY OF MALARIA VECTOR CONTROL STRATEGIES IN URBAN SETTINGS OF AFRICA

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Urban malaria is becoming a serious public health problem in Africa. Classical entomological and parasitological methods to assess malaria risk and vector control strategies (MVCS) present considerable limitations in urban context. A simple and highly sensitive tool is needed for a precise evaluation. Human antibody (Ab) responses to the specific *Anopheles* salivary gSG6-P1 peptide was describe to be a pertinent biomarker evaluating human exposure to *Anopheles* bites and the efficacy of MVCS. The aim of this work was to validate the gSG6-P1 as an epidemiological indicator evaluating malaria risk heterogeneity and MVCS efficiency used by urban populations of Dakar (Senegal), one of the biggest cities in West Africa. One cross-sectional study (October-December 2008) concerning 3,000 randomly selected children and adults (1,435 households) living in 45 districts of Dakar and its suburbs was performed from October to December 2008. Results show considerable variations in individual anti-gSG6-P1 IgG levels between and within districts. In spite of this inter-individual heterogeneity, the level of specific IgG and the percentage of immune responders differed significantly between districts. According to anti-gSG6-P1 IgG results, three groups of exposure's intensity to *Anopheles* vectors (low, medium and high) were constituted. More significant differences between exposure groups were obtained using the anti-gSG6-P1 IgG tool ($P < 0.0001$) compared to results of exposure to *An. gambiae* bites evaluated by classical entomological method. In addition, multivariate analysis shows that specific IgG responses was age-dependant and significantly lower for individuals who especially used particular MVCS such as bed-nets and spray bombs. Specific IgG responses to gSG6-P1 peptide could represent a new alternative tool to evaluate the heterogeneity of exposure level to bites, malaria transmission risks and used MVCS efficiency in urban settings, at the population and individual levels.

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CONFIGURATION OF THE BG-SENTINELTM (BGS) MOSQUITO TRAP FOR AN *AEDES AEGYPTI* (*DIPTERA:CULICIDAE*) "PUSH-PULL" CONTROL STRATEGY AT THE HOUSEHOLD LEVEL

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Our previous studies have quantified recapture rates of *Aedes aegypti* using varying BioGents Sentinel™ (BGS) trap densities and mosquito release numbers under screen house (140m^3) conditions. Further studies determined the potential effects of exposure to spatial repellent chemicals on host-seeking behaviors of female *Ae. aegypti* mosquitoes and subsequent BGS trapping success. Optimization of the physical parameters - location and distance of traps from huts- that may affect BGS efficacy were also performed. We report here on validating the findings from these experimental conditions (i.e., screen house and experimental huts) in a local village environment in Thailand to determine correlates between the two scenarios. This current work is also aimed at describing the optimum BGS conditions for a planned pilot evaluation of a push-pull system (combining both a spatial repellent and the BGS trap) in a selected community in Chiangmai, Thailand. Results show that the use of BGS traps under the predetermined experimentally optimized conditions, to include distance, number and location of the BGS trap at a single household, functioned to capture *Ae. aegypti* adults despite the presence of competing resting sites and hosts under real-home settings. Findings will elucidate the full potential of the BGS trap as the pull component, as well as the challenges in implementing the complete system, under a typical endemic environment in support of our larger proof-of-concept research program.

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SPATIAL REPELLENCY RESPONSES OBSERVED IN *AEDES AEGYPTI* TO REDUCED DOSES AND SURFACE AREA COVERAGE OF CHEMICAL COMPOUNDS IN WESTERN THAILAND

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Current control strategies for adult *Aedes aegypti*, a vector of dengue, are focused on toxic actions of chemical compounds but increasing case numbers and distribution of dengue fever world-wide highlight that other approaches are warranted. Our larger research program is focused on evaluating sub-lethal chemical approaches in a Push-Pull system to reduce *Ae. aegypti* densities inside homes using minimal chemical dose and treatment coverage of spatial repellents. Here we report on behavioral responses of female *Ae. aegypti* in response to transfluthrin, one of the promising candidate spatial repellent compounds. Insecticide treated material strips of different surface area coverage and doses were placed on the interior walls of experimental huts at our Thailand field site. Entry movement patterns, knock down and toxicity rates of mosquito test populations were quantified and compared to a matched control. Our finding revealed that transfluthrin produced a strong insecticidal action at field application rate using high surface area coverage while rates below the field application rate applied at minimal coverage also significantly reduced the densities of *Ae. aegypti* entering into the huts but without toxic effects. This suggests that spatial repellency can reduce human-vector contact inside homes while minimizing insecticide resistance selection pressure, a key factor in future insecticide management. Data from this study will be used in modeling efforts to determine potential disease impact on dengue transmission using spatial repellency and to guide selection of the repellent compound treatment scheme for a Push-Pull pilot study.

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POOLED SEQUENCING AND ARRAY HYBRIDIZATION TO IDENTIFY INSECTICIDE RESISTANCE GENES IN A MALARIA MOSQUITO VECTOR

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The *Anopheles gambiae* mosquito is the principal vector of malaria in sub Saharan Africa, where malaria-induced mortality is most severe. The use of insecticides to control disease transmission has been demonstrated to be highly effective. Resistance to insecticides is increasingly common in *A. gambiae*, however, and threatens to undermine the efficacy of malaria control programs. To learn more about the genetic basis of insecticide resistance in this vector, we undertook both pooled Illumina sequencing and pooled SNP array hybridization of mosquitoes typed as resistant or sensitive to lambda-cyhalothrin, a pyrethroid insecticide commonly used to treat bed nets. A total of 40 pools were generated using an average of 15 sibling females with consistent phenotypes, which were reared from eggs laid by wild-caught Ugandan mosquitoes. This pooling strategy was devised to hedge against the extremely short linkage disequilibrium in the *A. gambiae* genome and improve our power to observe phenotype-associated SNPs in the array analysis. Comparison of the pools resulted in a surprisingly polygenic profile of the insecticide resistance phenotype in Ugandan *A. gambiae* mosquitoes. Analysis yielded several dozen genomic regions significantly associated with permethrin resistance, in both the array data and sequencing results. Candidate resistance loci include probable insecticide targets (voltage gated ion transporters) as well as genes most likely involved in metabolic resistance mechanisms. Despite the high density of markers on the SNP array (400K total SNPs/1 per 600 bp), most hits on the array were based on single markers due to the extremely short LD in *A. gambiae* mosquito populations. This makes sequence data very useful in detecting markers not associated with recent selective sweeps. Given the prevalence and volatility of insecticide resistance in *A. gambiae* populations, discovery and monitoring of resistance markers through sequencing or pooled array hybridization could be of great importance in the strategic implementation of future malaria control programs.

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ANOPHELES GAMBIAE-SELECTIVE AND RESISTANCE-BREAKING ACETYLCHOLINESTERASE INHIBITORS FOR MALARIA CONTROL

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Recent advances in malaria control in sub-Saharan Africa are threatened by growing resistance to pyrethroids, the class of insecticides used on current generation insecticide-treated nets (ITNs). To address this problem, we seek to develop acetylcholinesterase (AChE) inhibitors that are 1) safe to humans and 2) possess low cross-resistance to *Anopheles gambiae* carrying the G119S AChE resistance mutation. Agricultural carbamate insecticides show very low selectivity for inhibition of *An. gambiae* AChE over human AChE, but we found that appropriate structural modification of these compounds can confer up to 500-fold selectivity. Such levels of selectivity could significantly reduce human toxicity of carbamates. Reasoning that the G119S resistance mutation reduces the volume of the AChE active site, we explored carbamate inhibitors in which the typical 6-membered aromatic ring was replaced with a smaller core. We will disclose a series of pyrazole-based carbamates that show good contact toxicity to AKRON strain *An. gambiae*, which carries both the G119S AChE mutation and the L1014F kdr mutation of the voltage-gated sodium ion channel. Kinetic studies of the inhibition of WT and G119S *An. gambiae* AChE demonstrate that greater potency against the G119S enzyme accompanies their observed higher toxicity relative to standard carbamate insecticides.

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DYNAMICS OF INSECTICIDE RESISTANCE IN *ANOPHELES GAMBIAE* S.L. ACCORDING TO COTTON CULTIVATION SCHEMES IN BURKINA FASO, WEST AFRICA

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Resistance to insecticides in the mosquito, *Anopheles gambiae* is a major threat to sustainable malaria vector control in Africa. Here, we present new data from Burkina Faso, where longitudinal and cross-sectional surveys were to conducted to i) explore the level of resistance to the four classes of insecticides available for public health and ii) monitor the frequency of the L1014F and L1014S kdr mutations in field *An. gambiae* populations throughout the country. Our sampling sites were chosen to belong to one of three ecological settings including: areas of extensive industrial cotton cultivation with high levels of insecticide usage for crop treatment, areas of limited experimental parcels of biological cotton cultivation with no insecticide usage, and areas of transgenic cotton cultivation with low insecticide usage. Mosquitoes were collected as larvae during the spray and non-spray periods in 2008 and 2009. They were brought back to the laboratory and reared to adults. Adult susceptibility tests were carried out using standard WHO protocols: susceptibility to DDT, permethrin, deltamethrin, Chlorpyriphos Methyl (CM) and bendiocarb was assessed. Test specimens were further identified to species and molecular form and their genotype at the kdr locus was determined using RFLP-PCR and HOLA protocols. A pronounced increase in resistance levels to all insecticides except CM had occurred across the test period, and it is readily apparent that resistance increased during spray periods. Concomitantly, we detected an increase in the frequency of the L1014F kdr mutation, especially in the M form in areas of industrial and biological cotton cultivation. We further report for the first time the occurrence of the L1014S kdr mutation we found floating at a low frequency in both the M and S forms of *An. gambiae* as well as in *An. arabiensis*. Analyses showed that the frequency of the L1014F kdr mutation is not statistically different in mosquitoes that died or survived to insecticide exposure, suggesting that the kdr mechanism might act together with other resistance mechanism(s) yet to be identified. Areas of extensive industrial and biological cotton cultivation are sustaining selection pressure for insecticide resistance in mosquito vector populations, prompting for collaboration between pest management in areas of cotton growing and vector control programmes to better face the challenge of increasing insecticide resistance in malaria mosquitoes.

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DYNAMICS OF INSECTICIDE RESISTANCE IN MALARIA VECTORS IN BENIN: FIRST EVIDENCE OF THE L1014S KDR MUTATION IN *ANOPHELES GAMBIAE* FROM WEST AFRICA

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Insecticide resistance monitoring is essential to help national programs to implement more effective and sustainable malaria control strategies in endemic countries. This study reported the spatial and seasonal variations of insecticide resistance in malaria vectors in Benin, West Africa. *Anopheles gambiae* s.l populations were collected from October 2008 to June 2010 in four sites selected on the basis of different use of insecticides and environment. WHO susceptibility tests were carried out to detect resistance to DDT, fenitrothion, bendiocarb, permethrin and deltamethrin. The synergist piperonyl butoxide was used to assess the role of non-target site mechanisms in pyrethroid resistance. *Anopheles gambiae* mosquitoes were identified to species and to molecular M and S forms using PCR techniques. Molecular and biochemical assays were carried out to determine kdr and Ace.1R allelic frequencies and activity of the detoxification enzymes.Throughout the surveys very high levels of mortality to bendiocarb and fenitrothion were observed in, *An. gambiae* s.l populations. However, high frequencies of resistance to DDT and pyrethroids were seen in both M and S form of *An. gambiae* s.s and *An. arabiensis*. PBO increased the toxicity of permethrin and restored almost full susceptibility to deltamethrin. *An. gambiae* s.l. mosquitoes from Cotonou and Malanville showed higher oxidase activity compared to the Kisumu susceptible strain in 2009 whereas the esterase activity was higher in the mosquitoes from Bohicon in both 2008 and 2009. A high frequency of L1014F kdr allele was initially showed in *An. gambiae* from Cotonou and Tori-Bossito whereas it increased in mosquitoes from Bohicon and Malanville during the second year. For the first time the L1014S kdr mutation was found in *An. gambiae* M form and in *An. arabiensis*. The ace.1R mutation was almost absent in *An. gambiae* s.l. Pyrethroid and DDT resistance is widespread in malaria vector in Benin and both metabolic and target site resistance are implicated. Resistance was not correlated with a change of malaria species and/or molecular forms. The L1014S kdr allele was first identified in wild population of *An. gambiae* s.s and *An. arabiensis* hence confirming the expansion of pyrethroid resistance alleles in Africa.

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INSECTICIDE RESISTANCE MANAGEMENT: THE KEY TO CONTINUED SUCCESS OF MALARIA VECTOR CONTROL IN ZAMBIA

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In the absence of a vaccine, insecticide-based vector control has been harnessed for prevention of malaria transmission in endemic countries. In Zambia indoor residual spraying (IRS) with DDT (2g/m^2) and pyrethroids (25mg/m^2) and insecticide treated nets (ITNs) are implemented as frontline interventions. However, their continued efficacy for successful and sustainable malaria vector control is threatened by emergence of resistance in *Anopheles* species in Africa. Studies to evaluate the spatiotemporal resistance profiles in malaria vectors were conducted in spatially segregated localities. A total of 4,581 F1 generation *An. gambiae* s,l (2,745) and *An. funestus* (1,836) were assayed for susceptibility using WHO standard discriminating dosages. Both Leu-Phe (west) and Leu-Ser (east) knock down resistance (kdr) mutations assays were investigated. By 2004, no resistance had been detected in either *An. gambiae* s.l. or *An. funestus* in Zambia. Between 2009 and 2011 significant levels of resistance to pyrethroids (0.05% deltamethrin, 0.05% lambda-cyhalothrin and 0.75% permethrin) and DDT (4%) were detected in both species ($p < 0.001$). High levels of Leu-Phe (west) kdr mutation and monooxygenases (P450) have been detected in *An. gambiae* s.s and *An. funestus* respectively. Marked levels of resistance were detected in IRS than in ITNs areas. No resistance was detected to the carbamate (0.01% bendiocarb) or the organophosphate (5% malathion) in either species. This implies that resistance selection is due to scaled up IRS and ITNs and could potentially undermine malaria control. This has resulted in a change of IRS policy from pyrethroids and DDT to carbamates. To preserve the limited arsenal of insecticides, good stewardship through a rational insecticide resistance management strategy is critical. Thus a strong partnership has been set up and data on potential underlying mechanisms of insecticide resistance, factors contributing to its emergence and distribution is being collated. This will ensure evidence-based choice of insecticides and their prolonged efficacy in Zambia.

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CHIKUNGUNYA INFECTION AMONG HOSPITALIZED FEBRILE PATIENTS IN NORTHERN TANZANIA

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Little is known about Chikungunya virus (CHIKV) as a cause of undifferentiated febrile illness in non-epidemic settings in sub-Saharan Africa. To investigate the prevalence of CHIKV infection, acute serum was collected from consecutive febrile inpatients at two hospitals in northern Tanzania from September 2007 to August 2008. Confirmed acute CHIKV infection was defined as a positive PCR result for CHIKV RNA. Among 870 participants, PCR testing was performed on 700 (80.5%). Of these, 55 (7.9%) had confirmed acute CHIKV infection. CHIKV infection was more common during dry months (OR 3.2, p=0.001) and cold months (OR 3.9, p<0.001), and was more common among infants and children than adults and adolescents (OR 1.9, p=0.026). Clinical signs and symptoms, hematologic results, and radiographic features were largely unhelpful in distinguishing CHIKV-infected patients from other febrile inpatients, with the exception of hepatomegaly (OR 2.3, p=0.043) and an absence of vomiting (OR 0.49, p=0.043). We report the first case series of patients with HIV and CHIKV co-infection. Among HIV infected patients, CHIKV infection was strongly associated with lymphopenia (OR 5.6, p=0.017) and severe immunosuppression (OR 10.5, p=0.007). The most common clinical diagnosis among CHIKV-infected participants was malaria in 23 (41.8%); no participant received a clinical diagnosis of CHIKV infection. Five CHIKV-infected patients died, one of whom likely had CHIKV meningoencephalitis. In conclusion, CHIKV infection is an important but unrecognized cause of febrile illness in northern Tanzania, even in the absence of a recognized outbreak. The preponderance of cases among pediatric participants observed is in marked contrast to the age distribution observed in outbreaks in non-immune populations outside of Africa. CHIKV infection was commonly misdiagnosed as malaria, however

CHIKV infection was more than twice as common as malaria in this study. Further research is needed to fully understand the epidemiology of this and other arboviruses in sub-Saharan Africa in non-epidemic settings.

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LABORATORY CONFIRMED PERINATAL TRANSMISSION OF DENGUE VIRUS IN PUERTO RICO

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Dengue is a mosquito-borne, acute febrile illness (AFI) with a 7-10 day period of viremia. During this time dengue virus (DENV) transmission can be blood-borne by receipt of blood products or donor organs or tissue and transmission from mother to fetus *in utero* or to infants at parturition (perinatal transmission). The determinants of perinatal transmission and the rate at which transmission occurs are unknown. Perinatal transmission may be under recognized in dengue endemic areas, including Puerto Rico. We present a laboratory confirmed, perinatal case of dengue from Puerto Rico. In 2010, a pregnant woman presented to a hospital with one day history of headache and fever. She went into labor while being evaluated and gave birth to a healthy male infant at 38 4/7 weeks gestation by vaginal delivery. Because the woman had been Group B Streptococcus (GBS) positive at 34 weeks, she and her newborn were evaluated and treated empirically for GBS infection. Although all cultures for GBS were negative, a diagnosis of dengue was entertained because the women had had 4 days of fever and thrombocytopenia and Puerto Rico was in the midst of a large dengue epidemic. The diagnosis of dengue in the mother was confirmed by RT-PCR for DENV-1 one week after delivery. The same day, the newborn, who had been well and about to be discharged from the hospital after completing empiric treatment for GBS, became hypoactive and thrombocytopenic. Serum was sent to CDC for DENV testing and RT-PCR was positive for DENV-1. Later the infant developed ascites, DIC and anemia, requiring fresh frozen plasma and packed red blood cell transfusions. During hospitalization, the infant acquired a nosocomial urinary tract infection. This case highlights that dengue should be included in the differential diagnosis for pregnant women with AFI living in dengue endemic areas. This case also highlights that during the neonatal period; signs other than fever may indicate DENV infection. Recognition of dengue in the mother is important to provide optimum management for both mother and infant.

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CLINICAL FEATURES THAT DIFFERENTIATE DENGUE FROM OTHER FEBRILE ILLNESSES AMONG CASES PRESENTING TO AN ACUTE CARE FACILITY IN A CARIBBEAN ENDEMIC AREA, JUNE 2009 - DECEMBER 2010

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Dengue, a mosquito-borne acute febrile illness (AFI), is endemic and reportable by law in Puerto Rico. Clinical diagnosis of the disease can be challenging because of its non-specific presentation and similarities with other AFIs. We examined clinical features of laboratory-positive (LP) and laboratory-negative (LN) dengue cases reported to a hospital-based enhanced dengue surveillance system (EDSS) from June 2009 to December 2010. AFIs that met World Health Organization criteria for dengue or severe dengue were reported to the EDSS via submission of a report form and serum sample. LP dengue cases had either detectable dengue virus (DENV) by RT-PCR or anti-DENV IgM by an enzyme-linked immunosorbent assay (MAC ELISA). LN cases had no evidence of DENV by RT-PCR and no detectable anti-DENV IgM. Cases with no DENV detected by RT-PCR and no convalescent specimen submitted for MAC ELISA were laboratory-indeterminate. Clinical and laboratory features that distinguish between LP and LN cases were evaluated. During the study period, 1634 suspected dengue cases were reported; 810 (50%) were LP, 327 (20%) were LN, and 497 (30%) were laboratory-indeterminate. LP cases were more likely than LN cases to have headache (82% vs.71%, p <0.0001), retro-orbital pain (63% vs. 48%, p <0.0001), body aches (79% vs. 66%, p <0.0001), joint pain (66% vs. 49%, p <0.0001), rash (38% vs. 28%, p =0.0015), petechiae (30% vs. 19%, p=.0005), hemorrhagic manifestation (41% vs. 33%, p=0.0224), thrombocytopenia (76% vs. 70%, p=0.0334), and leucopenia (66% vs. 57%, p=0.0044). In contrast, upper respiratory tract symptoms were more likely to be reported among LN cases than LP cases (63.5% vs. 36.5%, p< 0.0001). We plan to conduct a sensitivity analysis and generate receiver operator characteristic curves to determine the combination of clinical and laboratory features that best predict LP dengue cases among adults and children presenting with AFI by day of presentation. Findings will help to improve clinical detection of LP cases and guide clinical management.

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ETIOLOGY OF FEBRILE ILLNESSES IN NEPAL

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Acute febrile illness is a common reason for seeking medical care in Nepal. However, with a general unavailability of diagnostic tests, cases are frequently treated empirically with the underlying illness remaining undiagnosed. The determination of accurate year-round epidemiologic data for febrile patients and information regarding predominant symptoms for different diseases will assist clinicians in their diagnoses and subsequent therapeutic interventions even when laboratory resources are lacking. To this end, the Armed Forces Research Institute of Medical Sciences (AFRIMS) and the Walter Reed/AFRIMS Research Unit Nepal (WARUN) initiated a febrile illness etiology study at 4 hospitals in 3 cities in Nepal. Study methods included taking a standardized medical history and definitive diagnostic testing of acute and convalescent samples as appropriate for typhoid and paratyphoid fever, Japanese encephalitis (JE), dengue fever, chikungunya, West Nile virus, malaria, leptospirosis, rickettsiosis, influenza, brucellosis, hepatitis A, B, C and E, and bartonellosis. From May 2009 to December 2010, we enrolled 2,046 patients presenting with an undifferentiated febrile illness with no known etiology. The average age was 26 (range 2-96 years). Testing results to date have demonstrated 69 infections with *Salmonella typhi*, 73 *Salmonella paratyphi* A, 13 malaria, 204 leptospirosis, 15 hepatitis A, 1 hepatitis B, 1 hepatitis C, 62 brucellosis, 1 chikungunya, 47 primary dengue, 47 secondary dengue, 12 JE, 7 murine typhus, 1 Thai tick typhus, 52 scrub typhus, 130 influenza A/H1N1, 6 A/H3, 167 influenza B, 2 *Bartonella henselae* and 5 *B. quintana*. These are the first known reports of both Chikungunya and Bartonella human infections in Nepal. In addition, although only documented in Nepal since 2004, dengue infections are now being seen in the cities of Kathmandu and Pokhara located at higher altitudes further from the Indian border than initial cases. Characterization of the infections and correlation with clinical symptoms is continuing in order to provide information to Nepalese healthcare providers to assist with empirical diagnosis and treatment and priorities for future diagnostic needs.

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THE ETIOLOGY OF ACUTE FEBRILE ILLNESS IN PATIENTS PRESENTING TO GARISSA PROVINCIAL HOSPITAL IN NORTHEASTERN PROVINCE, KENYA

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Acute febrile illness (AFI) is a common clinical syndrome among patients in North Eastern Kenya. The non-specificity of the signs and symptoms of these illnesses leads clinicians to assign a presumptive malaria diagnosis in the absence of supportive laboratory testing and alternative diagnostic considerations. We report on other etiologies that should form a basis for differential diagnosis of fever in this region. The study population came from a cross sectional observational study of 304 patients presenting with non-malarial fever of ≥ 38°C at the Garissa Provincial Hospital in North Eastern Province, Kenya for 12 months during 2009-2010. Malaria was excluded by examining thick and thin Giemsa stained blood smears. Total nucleic acid (RNA and DNA) were extracted and assessed for Salmonella, malaria, Brucella, Leptospira and Rickettsia by a PCR strategy (RT-qPCR) that amplifies total nucleic acid following reverse transcription. Of the 304 AFI cases, 107 (35%) had identifiable pathogens: *Brucella* spp were found in 45 (14.8%), *Salmonella* spp in 39 (12.8%), and *Rickettsia* spp in 20 (6.57%). No leptospira infection was detected. Co-infections were observed in 12 (3.9%) patients while triple infections were observed in 2 (0.7%) patients. Additionally, despite the samples being negative for malaria by microscopy, 18 patients (5.9%) tested malaria positive by real-time RT-qPCR. Other diseases such as brucellosis, salmonellosis and rickettsiosis should be considered in cases of AFI. Testing for more etiologies such as Rift Valley fever, Coxiella spp, and arboviruses are important as less than half of the AFI cases were laboratory confirmed.

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HANDHELD POINT-OF-CARE CEREBROSPINAL FLUID LACTATE TESTING PREDICTS BACTERIAL MENINGITIS IN UGANDA

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Bacterial meningitis (BM) contributes to a high burden of morbidity and mortality in resource limited settings. Diagnosis may be delayed in part due to lack of human and material resources. Therefore, we validated a handheld point of care lactate (POCL) monitor's ability to measure lactate in cerebrospinal fluid (CSF) and diagnose BM in Uganda. Using a handheld POCL monitor we prospectively evaluated (in duplicate) 98 consecutive CSF samples submitted for standard laboratory lactate (SLL) testing at the University of Virginia. After the validation step, 145 patients with suspected BM were evaluated in Mbarara, Uganda. Probable BM was defined as a CSF white blood cell count of 100 cells of which 50% were neutrophils in the absence of an alternative diagnosis. Proven BM was defined by positive CSF Gram's stain or isolation of bacteria from CSF or blood. The ability of CSF POCL to diagnose BM was assessed by receiver operating characteristic (ROC) curves. Statisitcal significance was set at $p < 0.05$. There was a strong linear correspondence between CSF POCL and SLL test results ($R^2 = 0.86$; $p < 0.001$). There was a slightly higher CSF SLL (mean = 2.89 mmol/L, SD ± 1.91) compared to POCL average concentration (mean = 2.33 mmol/L, SD ± 1.92; mean difference, 0.56 mmol/L; $p < 0.001$). A CSF POCL of ≥ 7.7 mmol/L provided 94% sensitivity and 90% specificity for BM [AUROC = 0.95, 95% CI (0.9-1.0), $p < 0.001$]. The same value provided 100% sensitivity and 88% specificity for proven BM [AUROC = 0.96, 95% CI (0.91-1.0), $p < 0.001$]. CSF POCL was not helpful in the diagnosis of cryptococcal meningitis [AUROC = 0.48, 95% CI (0.39-0.58), $p = 0.73$]. As CSF POCL values increased, the likelihood of a tuberculous meningitis diagnosis decreased [AUROC = 0.338, 95% CI (0.24-0.44), $p = 0.005$]. In conclusion, a CSF POCL concentration of ≥ 7.7 mmol/L differentiated BM from other causes of meningitis with high sensitivity and specificity in a quick and easily obtainable manner. Use of CSF POCL testing may improve management of patients with suspected meningitis where laboratory infrastructure is limited.

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RISK FACTORS FOR DEATH AND SEVERE SEQUELAE IN MALAWIAN CHILDREN WITH BACTERIAL MENINGITIS, 1997-2010

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Acute bacterial meningitis causes significant death and disability in children worldwide and HIV is an established risk factor for acquiring meningitis and suffering negative outcomes. We investigated risk factors associated with death and severe sequelae in Malawian children with bacterial meningitis. A retrospective database review of three previous studies of acute bacterial meningitis was conducted on 1,784 children less than 15 years of age who attended Queen Elizabeth Central Hospital in Blantyre, Malawi during 1997--2010. Multivariate logistic regression was used to estimate the effects of HIV seropositivity, impaired consciousness, and causative organism on death and severe sequelae after adjusting for additional risk factors, including nutritional status, age, anemia, and *Plasmodium falciparum* infection. Impaired consciousness or coma at the time of admission was strongly associated with death [Coma: OR = 14.4, 95%CI (9.42, 22.1)] and severe sequelae [Coma: OR = 3.27, 95%CI (2.02, 5.29)] in multivariate logistic regression models. HIV seropositivity was significantly associated with increased odds of death [OR = 1.65, 95%CI (1.20, 2.26)] but no association was observed for developing severe sequelae [OR = 0.88, 95%CI (0.56, 1.38)]. After adjustment, infection with Salmonella spp was associated with increased odds of death [OR = 2.11, 95%CI (1.06, 4.08)] and pneumococcal meningitis was associated with increased odds of severe sequelae [OR = 1.84, 95%CI (1.03, 3.29)]. Resistance to commonly used antibiotics was not associated with increased risk of death after adjustment for causative organism and HIV serostatus, but the proportion of Streptococcus pneumoniae and Hemophilus influenzae type b strains resistant to co-trimoxazole increased over the period of study. Based on these findings, we conclude that impaired consciousness and HIV infection are major risk factors for death from ABM in Malawian children. Use of the pneumococcal conjugate vaccine could greatly reduce the burden of ABM in Malawi.

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BIOMPHALARIA GLABRATA PLASMA PROTEINS WITH BINDING AFFINITY TO A MEMBRANE-ENRICHED FRACTION OF SCHISTOSOME PRIMARY SPOROCYSTS: A PROTEOMIC ANALYSIS

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Upon entry of infective *Schistosoma mansoni* miracidia into its snail intermediate host *Biomphalaria glabrata*, these larval stages are immediately exposed to hemolymph, comprised of both soluble plasma and circulating hemocytes. Aside from providing an environment conducive to transformation to the primary sporocyst, hemolymph also plays an important role in ultimately determining the immune compatibility between snail host and establishing larval infection. Both

plasma and hemocyte components are involved, although the complex interaction between the parasite and these immune elements is still poorly understood. In order to understand more clearly this interaction at the molecular level, we have employed an enriched fraction of biotinylated *S. mansoni* sporocyst membrane proteins immobilized to avidin-conjugated beads as an affinity matrix, for the purpose of isolating sporocyst-binding proteins from the plasma of a susceptible (NMRI) and resistant (BS-90) strain of *B. glabrata*. Isolated samples were subjected to "in liquid" digestion and nanoLC-MS/MS analysis using the Agilent 1100 nanoflow system connected to a hybrid linear ion trap-orbitrap mass spectrometer equipped with a nanoelectrospray ion source. Raw MS/MS data was searched against the *B. glabrata* supercontig genome database (ver. 4.0.1) translated into 6-reading frames using an in-house Mascot search engine. Fibrinogen-related proteins (Freps) were the predominant protein group identified including Frep2 (2.13, 2.19, 2.22, 2.25, 2.29), Frep 3 (3, 3.2, 3.3, 3.3pre, 3-2pre), Frep7 (7, 7.1), Frep 12 (12.1, 12.1pre), and Frep 13 (13.1, 13.1pre). All putative Frep sequences were detected in both *B. glabrata* strains except Frep2, which was only recovered from NMRI snail plasma. Other putatively identified plasma proteins included dermatopontin2, a selectin and hemoglobin types1 and 2 in both snails, and Ca-binding protein2 in NMRI plasma. This approach not only provides putative identification of host plasma proteins interacting with the sporocyst membrane, but also will contribute to ongoing efforts to annotate the current *B. glabrata* genomic sequence database.

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HIGH-THROUGHPUT RNA-SEQ OF *SCHISTOSOMA MANSONI* TRANSCRIPTOME

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Schistosoma mansoni is one of the agents of schistosomiasis, a chronic and debilitating disease. In the past seven years, two sequencing projects have contributed a considerable amount of molecular information on the parasite, covering a significant portion of both the transcriptome and the genome, as published previously. The most recent estimate of the number of genes is 13,207. However, the fragmented nature of the data is still apparent, as 2,836 genes predicted in the genome have no evidence of transcription in the available EST databases, while approximately 7,000 EST contigs in the public databases do not map to the genome sequence and/or to the predicted genes. Recent advances in next-generation sequencing technology promise to accelerate the acquisition of sequences and diminish the cost of sequencing of large and complex genomes as well as of transcriptomes. In the present work, we used Roche 454 pyrosequencing to explore the *S. mansoni* adult male transcriptome (RNA-seq). A total of over 1.6 million high-quality ESTs were obtained from adult males with average length = 232 nt (40-1,433 nt) from the 3'-end of messages, resulting in a 26 % higher coverage of genome bases than that of public ESTs available at NCBI. With a 15 X-deep coverage of transcribed genomic regions, our data were able to (i) confirm for the first time 990 predictions without previous evidence of transcription; (ii) correct gene predictions; (iii) identify 11 new Micro-exon Genes (MEGs); (iv) discover 989 and 1196 RNA-seq contigs that map to intergenic and intronic genomic regions, respectively, where no gene had been predicted before. These contigs could represent new protein-coding genes or non-coding RNAs (ncRNAs). High-throughput RNA-seq of *S. mansoni* adult males helped uncover the parasite transcriptome complexity.

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USING COMPARATIVE FUNCTIONAL GENOMICS TO IDENTIFY NOVEL THERAPEUTIC TARGETS IN *SCHISTOSOMA MANSONI*

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Schistosomiasis is a tropical disease caused by flatworm parasites, *Schistosoma*, that affects hundreds of millions of people in the developing world. Although only a single drug (praziquantel) is available to treat this disease, the complicated life cycle of this parasite, that involves both mollusc and vertebrate hosts, impedes efforts to uncover and validate novel therapeutic targets. Thus, we are exploring the utility of the planarian *Schmidtea mediterranea*, a free-living relative of *Schistosoma*, to serve as an experimentally tractable model to identify and characterize new anthelmintic targets. We previously reported that a peptide hormone, NPY-8, is required for the maintenance of reproductive organs in the planarian and showed that a close relative of this hormone is present in the genome of *S. mansoni*. Since the major cause of the pathology associated with schistosome infection is a result of their prodigious reproductive output (100-3000 eggs/day), we are exploring whether this class of hormones functions similarly in these parasites. Additionally, we used comparative genomics to identify genes highly conserved between the planarian and the schistosome that are not found in the genomes of mammals. This analysis uncovered hundreds of genes, many of which encode "drugable" targets including G protein-coupled receptors, ion channels, and enzymes. We are using high-throughput in situ hybridization and RNA interference to characterize these genes in the planarian; follow-up analyses for a subset of genes will be performed in the parasites. Together, our data highlight new opportunities for translating knowledge gained from studying planarians to understand and control parasitic disease.

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GENETIC KNOCKDOWN OR PHARMACOLOGICAL INHIBITION OF *SCHISTOSOMA MANSONI* MULTIDRUG RESISTANCE TRANSPORTERS DISRUPTS PARASITE EGG PRODUCTION

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P-glycoprotein (Pgp) and multidrug resistance-associated protein 1 (MRP1) are members of the ATP-binding cassette (ABC) superfamily of proteins involved in transport of toxins and xenobiotics from cells. These transporters are associated with development of multidrug resistance (MDR) in mammals, and have been implicated in resistance to antiparasitic drugs, including anthelmintics. They likely also play key physiological roles in the parasite's excretion of wastes and metabolites, and provide attractive candidate targets for novel antischistosomal agents. We have previously shown that expression of *Schistosoma mansoni* Pgp (SMDR2) and MRP1 (SmMRP1) is altered in worms exposed to praziquantel (PZQ), the current drug of choice against schistosomiasis, and that higher expression is associated with reduced susceptibility to PZQ. We have also shown that PZQ inhibits SMDR2, and is also a likely substrate of SMDR2. We are currently using molecular genetic and pharmacological approaches to define the physiological roles played by these transporters and to dissect the mechanisms by which they interact with PZQ and may modulate responsiveness to the drug. RNA knockdown of SMDR2 or SmMRP1 in adult *S. mansoni* results in disruption of egg production by worms in culture. Exposure of adult worms to a variety of Pgp and MRP1 inhibitors, including tariquidar, a highly selective, third generation Pgp inhibitor, also produces significant disruption of egg production, as does exposure to MK 571, a MRP1 inhibitor. Treatment of *S. mansoni*-infected mice with MDR inhibitors results in reduced liver egg burden. We are currently examining the mechanism underlying this disruption of egg

production. Our findings indicate that these transporters may be excellent candidate targets for new anthelmintic strategies, either on their own or as an adjunct to currently available therapeutics.

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EFFECT OF HUMAN TGF-SS ON THE GENE EXPRESSION PROFILE OF *SCHISTOSOMA MANSONI* ADULT WORMS

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Schistosoma mansoni is responsible for schistosomiasis, a parasitic disease that is a major cause of morbidity worldwide. Molecular mechanisms of host-parasite interaction are complex and involve a crosstalk between host signals and parasite receptors. Transforming Growth Factor Beta (TGF-ß) is a cytokine that regulates many process central to life of metazoans such as growth and differentiation, developmental patterning, tissue repair and cell death. TGF-β signaling pathway has been shown to play an important role in *S. mansoni* development and embryogenesis. In particular human (h) TGF-β has been shown to bind to a *S. mansoni* receptor, transduce a signal that regulates the expression of a schistosome target gene. In spite of evidence of a TGF-ß effect on schistosome biology, limited information is available on which genes are affected at the transcriptional level. In this work we present the effect of human TGF-ß on the gene expression profile of adult worms by identifying 2167 parasite genes whose expression levels are affected by *in vitro* treatment of adult worms with hTGF-β. Among these differentially expressed genes, we highlight genes related to development, cell cycle and embryogenesis that could be players of hTGF-β effects on the parasite. We confirm by qPCR the expression changes detected with microarrays for 6 out of 8 selected genes. We also highlight a set of non-coding RNAs transcribed from the same loci of protein-coding genes that are differentially expressed upon hTGF-β treatment. These datasets offer potential targets to be explored in order to understand the molecular mechanisms behind the role of hTGF-β effects on parasite biology.

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CHARACTERIZATION OF NOVEL GLUTAMATE RECEPTORS IN *SCHISTOSOMA MANSONI*

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Cys-loop ligand-gated ion channels (LGIC) are instrumental for nervous system modulation, both in vertebrates and invertebrates. Very little is known about the LGICs in the parasitic platyhelminth *Schistosoma mansoni*, even though several LGIC gene sequences are predicted from its genome. Our work focuses on 3 of these LGIC, which we have previously cloned and identified as glutamate-gated ion channel (GluCl) subunits. Further characterization of these GluCl subunits by two-electrode voltage clamp (TEVC) in X. laevis oocytes reveals that the pharmacological and biophysical properties of these GluCls is distinct from their counterparts in other invertebrates, particularly with regard to their sensitivity to agonists and modulators. In addition, confocal laser microscopy analyses show that these SmGluCl subunits are distributed throughout the central and peripheral nervous system of the worm. These new findings have important implications for the fundamental comprehension of the key roles played by neuronal modulation in the parasite life style. More importantly, these SmGluCl channels constitute a very attractive novel drug target and could be used for screening and development of new anthelmintics.

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A NEW GOLD STANDARD FOR DIAGNOSIS OF *SCHISTOSOMA HAEMATOBIUM*

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Inconsistent sensitivity and specificity among current diagnostic procedures has made it difficult to set a gold standard for the definitive diagnosis of *Schistosoma haematobium* (Sh) in people with low level or chronic infections. These people are often missed because they often pass few eggs in the urine. Our study explored an alternative diagnostic method based on the presence of Sh-specific *Dra*1, 121 bp repeat DNA fragments in human urine and introduced a novel method of collecting and filtering urine specimens using Whatman No. 3 filter paper and drying them in the field for easy transport to the laboratory and subsequent examination. Research was performed in two sets: 1) Three diagnostic tests were used to examine 89 urine specimens from school children in Kollo District, Niger: dipsticks to detect hematuria (DM) in urine, microscopic detection of Sh eggs (ME) on the paper surface, and PCR for detection of Sh *Dra*1 extracted from the paper (PCR). In all 52 (58.4%) showed hematuria, 44(49.4%) showed eggs and 51(57.3%) showed Sh-specific DNA. 2) Latent Class (LC) modeling was used to compare the performance of the three tests in 401 filtered urine specimens from unselected adults (aged 20 - 59 years) from six endemic villages in Ogun State, Nigeria: PCR was superior with specificity of 1.000 and sensitivity of 1.000, ME had specificity of 1.000 and sensitivity of 0.701, while DM had specificity of 0.857 and sensitivity of 0.955. DM also showed a difference between males and females. LC modeling enabled the evaluation of specificity and sensitivity of a test when the actual prevalence of the pathogen is unknown. In Kollo and Ogun several persons had DNA detected in urine in the absence of detectable eggs; PCR product was not dependant on the egg count; the schistosome-specific DNA was undetectable in 61 previously positive people treated and re-examined 14 days later. Filter paper samples remained potent for at least four months at room temperature. This makes field collection of urine convenient and simple.

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SAFETY AND EFFICACY OF PRIMAQUINE WHEN COMBINED WITH QUININE OR DIHYDRO-ARTEMISININ PLUS PIPERAQUINE FOR RADICAL CURE OF *VIVAX* MALARIA IN INDONESIA

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The efficacy of primaquine against relapse has not been reliably assessed since the drug was developed in clinical trials in American prisoners during the 1950s. Two linked problems compound the difficulty of assessment of therapeutic efficacy of primaquine in endemic zones: 1) the long duration between primary infection and risk of relapse; and 2) the inability to distinguish relapse from reinfection among recurrent parasitemias in subjects under long-term follow-up. We screened Indonesian soldiers returning to their base in malaria-free East Java after serving 11 months in heavily malarious northeastern Papua, Indonesia. Among 143 found positive for *Plasmodium vivax*, 116 were randomized to three treatment groups: 1) artesunate alone; 2) quinine + primaquine (0.5mg/kg/dayX14d); or 3) Dihydro-artemisinin/piperaquine (DHA-PP) + primaquine (0.5mg/kg/dX14d). Treatment was directly observed, and subjects will be followed until first recurrence of parasitemia, or for 12 months. At submission of

this abstract, subjects had been under observation for 8 to 147 days. Relapses had occurred among 30 of 41 subjects given artesunate alone, and the median day of relapse was day 21 post-patency (range 17 to 70 days). This provides a measure of the natural rate of relapse and permits calculation of primaquine efficacy against it. Relapses occurred among 5 of the 39 subjects given quinine + primaquine, and the median day of relapse was day 70 post-patency (range 35 to 104 days). Two relapses had occurred among the 36 subjects randomized to DHA-PP +primaquine, on day 82 and day 126 post-patency. The efficacy against relapse of DHA-PP +primaquine appears superior to quinine + primaquine. This study may demonstrate good safety and efficacy of an adult dose of 30mg primaquine daily for 14 days against relapse when administered with DHA-PP against the acute attack. The observed difference in efficacy of the same dose of primaquine against relapse when given with quinine or DHA-PP emphasizes the apparent impact of that a blood schizontocide may have on primaquine activity against hypnozoites.

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WHAT IS THE APPROPRIATE SECOND LINE REGIMEN IN THE ERA OF ARTEMISININ COMBINATION THERAPY: EFFICACY OF QUININE, ARTEMETHER-LUMEFANTRINE AND DIHYDROARTEMISININ-PIPERAQUINE FOR RECURRENT UNCOMPLICATED MALARIA IN UGANDAN CHILDREN

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Falciparum malaria therapy poses unique challenges in sub-Saharan Africa, where recurrent infections are common, especially in children. Though in several countries quinine is the recommended treatment for these patients, it is unclear whether this is the best approach. The study was a nested, randomized, open label, three-arm clinical trial of rescue therapy among patients who developed recurrent malaria within 28 days following treatment of the primary episode with an artemisinin-based combination treatment (ACT) in a related main study. Consecutive patients aged 6 to 59 months with recurrent uncomplicated malaria were randomised to receive either quinine or one ACT, i.e. artemether-lumefantrine (AL) or dihydroartemisinin-piperaquine (DHAPQP), and actively followed up for the next 28 days. Among 220 patients enrolled, 217 (98.6%) were assigned an efficacy outcome and 218 (99.1%) were assessed for safety. Risk of recurrent infection was significantly higher for quinine (70% [74/110], HR 3.9, 95%CI 2.4-6.7, p<0.0001) and AL (60% [21/35] HR 3.3, 95%CI 1.8-6.3, p<0.0002) as compared to DHAPQP (25% [18/72]). When adjusted by genotyping, risk of treatment failure was lower in the DHAPQP group (1% [1/72]) compared to quinine (7% [8/110]) and AL (5 % [2/35]) group, though not statistically significant. No serious adverse events were reported. A recurrent infection following an ACT treatment can be successfully treated with an alternative ACT instead, than with quinine, the current recommended second line regimen in Uganda and in 29 other African countries. An ACT rather than quinine should be used as second line treatment.

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ASSESSMENT OF THE EFFICACY, TOLERABILITY AND EASE OF ADMINISTRATION OF DIHYDROARTHEMISININ PLUS PIPERAQUINE AND ARTESUNATE PLUS SULFAMETHOXYPYRAZINE PLUS PYRIMETHAMINE COMPARED WITH SULPHADOXINE-PYRIMETHAMINE FOR PREVENTING MALARIA IN GHANAIAN CHILDREN

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Seasonal administration of intermittent preventive treatment for malaria given to children under five years old (IPTc) involves administration of a pre-defined number of treatment courses of antimalarial drugs at specified time intervals during the high transmission season. Recent reports indicate that IPTc is safe and can reduce the burden of malaria in West Africa. Using different drug combinations for IPTc will minimize the development of resistance to first line drugs. Factors such as side effects, ease of administration, duration of the treatment, become important, when selecting the appropriate treatment for IPTc. A combination of antimalarial drugs with efficacy lasting over 42 days would be of great importance for IPTc. We investigated the efficacy, longevity, tolerability and ease of administration of dihydroarthemicinin plus piperaquine (DHA+PQ), artesunate plus sulphamethoxypyrizine plus pyrimethamine (Co-Arinate FDC®) 12 hourly over 24 hours, Co-Arinate FDC® daily for three days and compared with Sulphadoxine-pyrimethamine (SP) in children aged 6-59 months with asymptomatic malaria in Ghana. An open labelled, active, controlled, randomized Phase III trial with four arms was used. A total of 590 children from 28 villages were randomly assigned to the four arms. One arm (148) received DHA+PQ daily for three days, the second arm (143) received Co-Arinate FDC®) daily for three days, the third arm (149) received Co-Arinate FDC ® 12 hourly over 24 hours and SP the comparator arm (150) received a single dose. The children were followed up to 63 days. finger prick blood was collected for blood film and filter paper on days 0, 3, 7, 14, 28, 42 and 63 for parasite identification. Safety was assessed by visiting subjects at home from day 0 to 7, haemoglobin concentration was measured on days 0, 14, 28, 42 and 63 and venous blood for liver and renal function tests collected on days 0 and 14. Ease of drug administration was assessed by interviewing parents/guardians. Treatment failure (PCR-uncorrected) by day 42 was SP 40%, Co-Arinate daily 26.6%, Co-Arinate 12hourly 34.9% and DHA+PQ was 16.2%. Vomiting was more common among children in the Co-Arinate 12hourly arm 18.1% compared to SP 6.7%, Co-Arinate daily 11.2% and DHA+PQ 8.1%. The intervention was found to be acceptable to the community. Our findings show that DHA+PQ and Co-Arinate daily are safe and efficacious for IPTc in Ghanaian children.

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STAGE SPECIFIC CLEARANCE OF ASEXUAL *PLASMODIUM FALCIPARUM* IN CHILDREN TREATED WITH ARTESUNATE-AMODIAQUINE (AA) AND ARTEMETHER-LUMEFANTHRINE (AL)

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Delay in parasite clearance time is a recognized indicator of emerging drug tolerance. Artemether-lumefantrine (AL) and Artesunate-amodiaquine (AA) are efficacious regimens that have been widely adopted in sub-Saharan Africa. We evaluated their clearance times on the asexual stages

of *Plasmodium falciparum* using Giemsa stained thick blood films. This is preliminary to evaluating stage specific delay in parasite clearance time that may be a more sensitive indicator of parasite tolerance to the antimalarial therapies. Children aged 7 months to 12 years were randomized to receive standard doses of AA and AL for three days. Peripheral blood smears were made hourly in the first 4 hours, 8h, 16h, 24h, and on days 2-7, 14, 21, 28, 35, and 42 for microscopic identification, quantification, and morphological staging of *Plasmodium falciparum*. The appearance and ratio of the parasite nuclear chromatin and cytoplasm were the characteristics used in the staging. Parasites were classified into R1 (very young rings, 0-6 hrs), R2 (young trophozoites, 6-30 hrs), and R3 (late trophozoites, >30 hrs). Schizonts were classified as immature (Si, <8 visible nuclear chromatins) and mature (Sm, > 8 nuclear chromatins). A total of 57 (28AA, 29AL) children were evaluated. Thirty four (59.6%) of the children had multiple stages of the parasite in their blood films at enrolment. The average number of stages per child at enrolment was 2. R1 was seen in Forty seven children (82.5%), while 49.1% and 38.6% had R2 and R3 respectively. Pure R3 infection was the least common (5.3%). Low density schizontinaemia (6 - 120 Schizonts/µL) was present in 36.8% of the children before treatment (21.1%, 17.5%% immature and mature Schizonts respectively). Schizonts were more likely to be present in younger than older children [5.7 (± 2.5) vs. 8.1 (±2.7) years, p= 0.002]. Stage specific parasite clearance times (Log10 hours ± SD) for R1, R2, R3, Si, and Sm, were 1.5 ± 0.2, 0.9 ± 0.3, 0.6 ± 0.3, 0.6 ± 0.3, and 0.7 ± 0.3 for AA and 1.6 ± 0.2, 1.1 ± 0.3, 0.8 ± 0.4, 0.6 ± 0.5, and 0.7 ± 0.2 for AL respectively. All parasites were cleared by 72 hours of commencing therapy. The evaluated ACTs had more rapid actions on older stages of the parasite and Schizonts. In addition, the study revealed that low density schizontinaemia is not uncommon in children with acute uncomplicated *falciparum* malaria.

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PHARMACOKINETIC PREDICTORS OF TREATMENT OUTCOME FOR DIHYDROARTEMISININ-PIPERAQUINE IN UGANDAN INFANTS WITH UNCOMPLICATED MALARIA

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Dihydroartemisinin-piperaquine (DP) is the most recently adopted 1st line artemisinin-combination therapy (ACT) option for the treatment of malaria. We evaluated the pharmacokinetics (PK) and pharmacodynamics (PD) of piperaquine (PQ) in 107 infants, aged 6 to 24 months, within the context of a longitudinal study in the high transmission area of Tororo, Uganda. Capillary plasma samples were collected prior to the 1st dose of DP, and at variable times up to day 28 after each treatment for *P. falciparum* malaria. Children were followed longitudinally, and underwent sampling for all episodes of malaria. 218 episodes of malaria (1314 samples) occurred over the 7 month study period. Median day 7 concentrations were 41.9 ng/ml (IQR, 30.2, 56.6), Univariate and multivariate analyses revealed that day 14, 21, and 28 levels were significantly associated with the risk of recurrent malaria on day 42. The risk of recurrent infection increased 85% per log10 increase in PQ level on day 14. Those individuals with a PQ level in the lowest quartile (<10.5 ng/ml) on day 28 had a 41% risk of failure, while those above that threshold had a 20% cumulative risk of failure at 42 days (p=0.01). Notably, out of 132 children who had a prior episode of malaria treated with DP, 119 had detectable PQ at the time of diagnosis of their next episode of malaria (constituting a period of up to 4 months), with concentrations ranging from 1.5 to 41.9 ng/mL. Population PK analysis was also performed. A three compartment PK model with first order absorption of drug and age-dependent apparent clearance best described PQ disposition in infants. Additional population PK/PD analyses will be presented. Our study provides the 1st data on the disposition of PQ in children < 2 yrs of age. PQ exposure on day 7 for infants is lower than day 7 levels previously reported in older children and adults. Moreover, PK exposure correlates strongly with clinical outcomes. In addition, PQ appears to remain detectable for extended periods, and was detectable in the majority of infants upon recurrent infection.

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ARTEMETHER-LUMEFANTRINE EFFICACY IN PREGNANCY: THE PROOF IS IN THE PLACENTA

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Data on efficacy of artemisinin based combination therapy to treat *Plasmodium falciparum* during pregnancy in sub-Saharan Africa is scarce. In an antenatal cohort of women in Mbarara, Uganda, a recent open label, randomized, non-inferiority trial demonstrated that artemether-lumefantrine (AL) is non-inferior to quinine with an improved side effect profile. To determine whether AL is associated with reduced pathology compared to quinine to treat uncomplicated malaria in pregnancy, malaria pigment deposition and inflammation were assessed by histology in this cohort. Pigment deposition in fibrin and placental inflammation were scored on blinded hematoxylin and eosin and Giemsa stained placental sections. Clinical data included treatment arm, parity, gestational age of first parasitemia, level of parasitemia, and day of reinfection or recrudescence. The prevalence and amount of pigment proportionately decreased with time after treatment and this decline was greater in the AL arm. AL (n=61) was associated with decreased pigment compared to quinine (n=62), correcting for parity, time of infection, reinfection/recrudescence, and level of parasitemia (p=0.003). The prevalence of intervillous inflammation in this cohort was low (6%), reflecting the efficacy of antenatal care with early detection and prompt treatment of malaria. In conclusion, AL is associated with less malarial pigment deposition compared to quinine for treatment of uncomplicated malaria in pregnancy, suggesting that AL is more effective. This may reflect the increased rates of parasitologic clearance and activity of AL at early stages of the parasite life cycle. Histology may act as a surrogate outcome in drug efficacy trials during pregnancy with limited sample size, and would be a useful tool to evaluate malaria control policy and implementation in pregnancy.

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HIGHLY EFFECTIVE THERAPY FOR MALARIA IN PREGNANCY IMPROVES MATERNAL AND NEONATAL HEALTH OUTCOME

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Pregnant women are affected by the adverse outcomes of malaria but treatment options are limited. Dihydroartemisinin-piperaquine (DHP) is a safe and highly effective antimalarial in non-pregnant adults but limited information is available on its use in pregnancy. We report the safety profile of DHP exposures in pregnancy and the impact on pregnancy

outcomes following change in antimalarial treatment policy to artemisinin combination therapy. From April 2004 to June 2009, 6519 pregnant women were enrolled in a hospital based malaria surveillance study. All pregnant women were screened for malaria and treated. Eligible data for the safety analysis were available in 1217 pregnant women with acute antimalarials exposures on hospital admission (765 exposed to DHP) and 847 women with history of antimalarial exposures during the current pregnancy (395 with prior DHP exposures). Compared with prior quinine or chloroquine+/- sulfadoxine-pyrimethamine exposures, history of DHP treatment during the current pregnancy reduced the risk of recurrent malaria at delivery (OR=0.37 (95%CI 0.27-0.52), p<0.001), congenital malaria (OR=0.06, [95%CI, 0-0.46], p=0.001) and perinatal deaths (AOR=0.32 [95%CI, 0.12-0.85]; p=0.03) when used in the second and third trimester of pregnancy. There was no increased risk of congenital malformations and stillbirths in pregnant women exposed to DHP. The introduction of DHP was associated with a 53% fall in the overall proportion of maternal malaria at delivery and 94% reduction of congenital malaria incidence. In conclusion, DHP has an acceptable safety and toxicity profile for the treatment of malaria in the second and third trimester of pregnancy and reduces associated morbidity and mortality. Further prospective studies are required to define the role of DHP for the treatment and prevention of malaria in this high-risk group. Ensuring universal access to ACT in pregnancy through novel treatment and prevention strategies is likely to impact significantly on maternal child health.

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PHLEBOTOMINE BLOODMEALS IN A PERIURBAN LEISHMANIA-ENDEMIC AREA IN NORTHEASTERN BRAZIL

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Visceral leishmaniasis (VL) is caused by *Leishmania infantum chagasi* in northeastern Brazil. It is transmitted by *Lutzomyia* longipalpis and dogs are thought to be the major reservoirs. The study objective was to assess the role of *Leishmania*-infected humans in transmission by determining the source of sand fly bloodmeals. Study site was an *L. i. chagasi* - endemic periurban neighborhood of Natal, RN, Brazil. A subsection of 10% of this neighborhood (n=120) has had DTH and antibody testing; 38 (31.7%) were positive for one of these tests. From February to April 2011, sand flies were collected with CDC light traps in chicken enclosures, dog runs, and houses with prior but no active VL cases. Female sand flies were macerated and DNA extracted (Qiagen). *Lutzomyia longipalpis*-specific GAPDH, *Leishmania* minicircle kDNA, and species-specific cytochrome C to identify bloodmeal DNA were amplified by PCR and identified by agarose gel electrophoresis. A selection of PCR products were sequenced for confirmation. 244 sandflies were collected of which 93 were *L. longipalpis* by PCR. 19 of 139 (13.7%) sand flies were positive for *Leishmania* kDNA by PCR; 11 of 47 (23.4%) of *L. longipalpis* were kDNA-positive compared with 8 of 92 (8.7%) of non-*L. longipalpis.* Human blood alone was present in 40/233 (17.2%), both human and chicken blood in 11/233 (4.7%), chicken alone in 9/233 (3.9%), and human and dog in 5/233 (2.1%). *L. longipalpis* were nearly ten times more likely than non-*L. longipalpis* to have a human, dog, and/or chicken blood meal identified, 67.9% (57/84) vs. 7.4% (11/149), respectively. Of the 19 kDNA positive sand flies, seven had detectable human DNA. In this population, sand flies, particularly *L. longipalpis,* had fed on humans more than chickens despite the greater density of chickens compared with humans. Although kDNA positivity does not denote *Leishmania* infectivity, the presence of human blood in a greater percentage of kDNA-positive sand flies than chicken or dog blood suggests that asymptomatic human carriers may be important reservoirs of infection.

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THE INFLUENCE OF STREETS ON THE SPATIAL DISTRIBUTION OF THE CHAGAS DISEASES VECTOR, *TRIATOMA INFESTANS*, IN THE CITY OF AREQUIPA, PERU

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Urban transmission of Chagas disease is a documented problem in Southern Peru and elsewhere. While the dispersal of the vector, *Triatoma infestans,* has been well described in rural habitats, little is known about how the vector moves through the urban environment. Do city streets serve as a barrier to dispersal of *T. infestans*? And, if so, how strong is the effect? The Ministry of Health in Arequipa, Peru, in preparation for an insecticide application campaign, surveyed 7,959 of 12,069 (65.9%) households in the district of Mariano Melgar, Arequipa, Peru. 608 (7.6%) of surveyed households were infested with the vector. We carefully mapped the data from this survey, and the location of all streets in the district. We use a spatial statistic, Moran's I, adapted to a structured context, to assess separately the spatial auto-correlation within city blocks and across city blocks. We propose a simple test for statistical significance of the effects of streets on the statistic. We then perform a multivariate analysis to assess the strength of streets as barriers, controlling for co-factors. We find that decrease in the strength of the auto-correlation attributable to streets is similar to that due to an increase in distance of 40 meters. As the strong effect of streets on the distribution of *T. infestans* cannot be explained by known co-factors, these results strongly suggest that *T. infestans*'s dispersion is to some extent limited by streets. The dynamic of Chagas disease is directly linked to the spatial dynamic of the vector. Our results yield important implications for the understanding of these dynamics in urban areas. Of particular interest is the possibility to use the city blocks as units of prediction and control in further approaches.

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STATUS OF TSETSE-TRANSMITTED TRYPANOSOMIASES IN LIVESTOCK AND MAN IN THE MANAFWA-RIVER-CRESCENT DISTRICTS OF SOUTHEASTERN UGANDA

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A situation analysis of the tsetse-transmitted Trypanosomiasis problem in Uganda will update the GIS-based decision-support tool for reducing the impact of Tsetse-transmitted Trypanosomiases in livestock and man across agro-pastoral farming communities in Uganda. Mapped relationships derived from primary data (tsetse fly vector species and incidence of Trypanosomiases (nagana and sleeping sickness) from districts in Manafwa-Malaba river system in SE Uganda known to be affected by floods and landslides since 2007 shows that bovine Trypanosomiasis in all affected districts varies little between extended wet and dry seasons in September 2010 and January-February 2011. Nagana situation in September 2010 was overall for Manafwa at 16% (with Bugobero - 1%; Butiru - 25%; Bushiende - 7%; and Busiu - 38%); Mbale at 38%; Iganga at overall 4% (Ibulanku - 2%; Namungalwe - 3%; Nawandala - 0%; Nawaikoke - 2%) and Namutumba - 10% (Bulange - 5%-12%; Magada -12%). Nagana data for the above regions in 2011 showed the problem

is still heavy in the Manafwa (16%); slightly low in Mbale - 33%; Butalejja at Bunghazi -31% and Himutu - 8%). The survey was extended to Kumi - Mukongoro/Agaria area were we saw 10% infection with 8/14 being T. *vivax* 2010 survey found only biting flies in the area; Ngora - Kobuin/ Atoot had 4% Iganga-Ibulinaku had 3.8 % while Namutumba - Bulange had 10%. This data showed no reduced prevalence of nagana in districts bodering Manafwa river namely Manafwa, Busiu, Butalejja and Namutumba. Besides there is now an outbreak of sleeping sickness in Bukedea country where tsetsefly presence is now confirmed in previously un-infested communal grazing valleys in the heart of the district. This data will be related to the tsetse genetic mtDNA haplotype mapping and will be geo-processed. Reports will provide for a rational evidence-based protocol for managing Tsetse-transmitted Trypanosomiases (human and animal) in mid South-Eastern Uganda.

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EMPLOYING VALIDATED *LEISHMANIA* HIGH THROUGHPUT SCREENING ASSAYS TO IDENTIFY NOVEL ANTI-LEISHMANIAL CHEMOTYPES AND DIFFERENCES IN LIFE CYCLE CHEMOSENSITIVITIES

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We developed an automated, alamar blue-based, high throughput screening (HTS) assay for drug susceptibility of *Leishmania amazonensis* axenic amastigotes. We initially validated our screening system using the 1280 compound Library of Pharmacologically Active Compound (LOPAC) set. The assay performed robustly with average Z-factor and signal-to-background being 0.65±0.05 and 5.2±0.15, respectively. Our primary active rate for the LOPAC set using the axenic amastigote population was 3.1% at a screening concentration of 10 µM. We next used our screening assay to interrogate a diversity-based 220,335 compound library. The validated assay performed robustly with average Z-factor and signal-to-background of 0.43±0.12 and 4.6±0.1, respectively and a primary active rate of 1.7% at a 10 µM screening concentration. Comparing these statistics with those collected from a *Leishmania major* promastigote HTS drug susceptibility assay (using the same alamar blue format and screening concentrations), we found the primary active rates to be uniformly higher for the promastigote life cycle form. Specifically, screening the LOPAC set as well as a diversity-based compound library similar in size (i.e., 200,000 compounds) with nearly identical composition in the promastigote HTS assay yielded primary active rates of 10.5% and 8.9%, respectively, representing a 3-5-fold higher primary active rate than what we documented with the axenic amastigote assay. Interestingly, 1160 compounds exhibited >65% inhibition of growth against both *Leishmania* life cycles. Preliminary structural clustering of these chemotypes indicated 167 common structural clusters (ranging in size from 2 to 11) with the remaining 657 compounds being classified as singletons. We will highlight and discuss potential differences in the chemosensitivities of the *Leishmania* parasite life cycle forms and the importance of compound library selection in the search for novel anti-leishmanial chemotypes.

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TRYPANOSOMA CRUZI: SUSCEPTIBILITY OF CULTURED EPIMASTIGOTES TO SINGLE AND PAIRED TREATMENTS OF RECOMBINANT ANTIMICROBIAL PEPTIDES EXPRESSED FROM THE *RHODOCOCCUS RHODNII* SYMBIONT OF THE *RHODNIUS PROLIXUS* CHAGAS DISEASE VECTOR

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Chagas disease, or American trypanosomiasis, results from infection with the protozoan parasite, *Trypanosoma cruzi,* and can result in chronic disease characterized by cardiac and gastrointestinal dysfunction. *T. cruzi* is vectored by domiciliary triatomine insects that deliver the parasite to humans during blood meals by deposition of infected feces. Chagas control programs have focused on elimination of the vectors through pesticide applications and have been effective in the short term; however, human health concerns, emerging pesticide resistance, and overall cost of the efforts hamper long term sustainability. Paratransgenesis is an alternative method of Chagas control that functions by interrupting the cycle of parasite transmission. Bacterial symbionts of the *T. cruzi*-carrying vectors are transformed with expression plasmids whose products are toxic to the parasite. These symbionts are delivered to the vector by simulated coprophagy and reside in the hindgut near the infectious trypomastigotes. Previous *in vivo* tests using the *Rhodococcus rhodnii* symbiont of the *R. prolixus* vector expressing the cecropin A anti-microbial peptide (AMP) resulted in the complete clearance of infective *T. cruzi* in ~70% of the vectors and a decrease in parasite titers for the remainder. *In vitro* toxicity testing of multiple AMP's against *T. cruzi* identified candidate AMP's that exhibited additive and synergistic lethal concentrations. The DNA sequences for these AMP's were cloned into expression shuttle vectors and transformed into *R. rhodnii*. Media was collected and cell lysates prepared from clones whose expression was confirmed by Western blot and ELISA. Treatment of *T. cruzi* in culture with AMP-positive media and lysates for 96 hours resulted in parasite killing as measured by reduction in normal cell density changes at 600 nm, and microscopic examination of live cell numbers with Calcein-AM. Media from AMP transformants was less toxic to *T. cruzi* than the corresponding cell lysates which showed substantially greater toxicity when combined in pair-wise treatments.

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OPTIMIZING INHIBITORS OF METHIONYL-TRNA SYNTHETASE FOR TREATING LATE-STAGE AFRICAN TRYPANOSOMIASIS

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Better drugs are desperately needed to treat human African trypanosomiasis, particularly for late-stage disease when the *Trypanosoma brucei* parasites have invaded the central nervous system (CNS). This poses a particular challenge in drug development as the blood brain barrier (BBB) effectively excludes most small molecules from attaining significant levels in the CNS. In previously published work, we described inhibitors of the *Trypanosoma brucei* methionyl-tRNA synthetase with EC_{50} values as low as 4 nM on *T. brucei* cultures and that demonstrated potent activity in the murine model of acute *T. brucei* infection. Unfortunately, the described quinolone compounds were observed to have poor permeability characteristics in the MDR1-MDCKII *in vitro* model of the BBB. Variations of the scaffold were synthesized and new compounds containing a urea core were discovered with excellent permeability properties in the MDR1-MDCK11 model. In addition, the new urea compounds have greater selectivity between the trypanosome methionyl-tRNA synthetase and the human mitochondrial tRNA synthetase (~200:1) in comparison to the quinolone compounds (~5:1). However, the urea compounds have

higher EC50 values (~150 nM) against *T. brucei* cultures, thus more potent analogs are needed. To aid the compound design process, crystal structures of the *T. brucei* methionyl-tRNA synthetase have been solved in complex with several urea compounds to define the binding mode and opportunities for improving affinity to the enzyme. We will report on progress on improving anti-*T. brucei* activity while optimizing CNS permeability and metabolic stability of new lead compounds.

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CO-INFECTION OF KALA-ZAR AND FLAVIVIRUSES: A CASE REPORT OF A PATIENT FROM NORTHERN KENYA WHO WAS SEROLOGICAL POSITIVE FOR KALA-ZAR AND FLAVIVIRUSES, 2010

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In mid September 2010, a 50 year old male patient from Wajir town, North-eastern province, Kenya was seen at a private clinic in Nairobi with complaints of fever, epistaxis, joint pain and myalgia. He had been unwell since mid April 2010 and reported having had jaundice, which subsided after one month, and marked weight loss. Clinical laboratory investigations showed pancytopaenia with mild deranged liver function tests. A blood sample was collected after clinical tests and sent to the viral haemorrhagic fever laboratory at Kenya Medical Research Institute (KEMRI) for arbovirus tests. IgM, IgG ELISA and RT-PCR tests were conducted for Yellow fever (YFV), Dengue (DEN), West Nile (WNV), Chikungunya (CHIK), Rift Valley fever (RVFV) and Crimean Congo Haemorrhagic fever (CCHF) viruses. Both IgM and IgG ELISA tests were positive for Flaviviruses but negative for other arboviruses tested, suggesting that the patient could have possibly had a prior exposure to one of these flaviviruses. RT-PCR was negative for both group primers for Flaviviruses and specific primers for YFV, DEN and WNV. Later the same month, the patient was admitted at Kenyatta National Hospital with a fever of 39.9°C and massive hepato-splenomegally. Further tests were conducted at KEMRI, to rule out Kala-zar, using four different rapid detection kits: Diamed IT-Leish Kit, Signal KA kit, rK39 and InBios Kalazar detect kit. The sample was positive for Kala-zar by all the kits. Ministry of Public Health and Sanitation sent a team to Wajir County to review the hospital records and establish the burden of Kala-zar. A total of 600 patient records from Wajir met the case definition, of which 237 patient records had been positive by rK39. The results show that Kala-zar is endemic in Wajir County. The positive results for flaviviruses indicate the possibility co-circulation of Kala-zar with these viruses, such as YFV, DEN and WNV. Systematic studies need to be conducted to determine sero-prevalence levels and factors associated with Kala-zar and arboviruses.

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THE WHO THRESHOLD OF 50% SCHISTOSOMIASIS PREVALENCE AMONG SCHOOL-AGED CHILDREN TO EXPAND PRAZIQUANTEL MDA TO ADULTS IS NOT USEFUL IN CENTRAL NIGERIA

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WHO guidelines for preventive chemotherapy (PCT) call for praziquantel (PZQ) treatment of adults, in addition to treatment of school aged children, where schistosomiasis (SCH) prevalence in school-aged children is ≥50%. The purpose of this study was to ascertain the value of the 50% threshold in predicting higher infection rates in adults, and so justify the additional cost of expanded PZQ treatments. We evaluated urinary SCH prevalence in adults in hyperendemic communities (where ≥50% in children were heme dipstick positive), compared to SCH prevalence in adults in mesoendemic communities (where heme dipstick positivity ranged from 20-49% in children). The study was conducted in Plateau and Nasarawa states of north-central Nigeria. From baseline mapping of SCH among school-aged children in 2008, 7 hyperendemic and 12 mesoendemic communities across 4 districts were selected for evaluation. The prevalence of hematuria (reagent stick) and the presence and intensity of *Schistosoma hematobium* eggs in urine was determined among adults aged 20 years and older from randomly selected households in each community. A total of 1,164 adults were examined out of 1,287 registered; 505 in hyperendemic communities (where mean hematuria among children in 2008 was 70.4%) and 659 in mesoendemic communities (where mean hematuria among children in 2008 was 26.6%). The prevalence of hematuria was similar among adults in hyperendemic communities (18.2%, 95% CI 11-25%) and mesoendemic communities (17.8%, 95% CI 8-27%). Similarly, the prevalence of infection was 21.0% (11-31%) in hyperendemic communities and 17.0% (7-27%) among the mesoendemic communities. The prevalence of intense infections (defined as egg density of ≥10 eggs/5 ml) was 1.2% (0.1-2.3%) and did not differ by community group. We concluded that in this setting there was no evidence to implement an expanded treatment program that would include adults only where the prevalence of micro-hematuria in school-age children was ≥50%.

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TREATMENT OF SCHISTOSOMIASIS IN INFANTS AND PRESCHOOL-CHILDREN WITH PRAZIQUANTEL: CORRECT DOSE, SIDE-EFFECTS AND CURE RATES

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In large-scale interventions for control of schistosomiasis, use of the World Health Organization dose pole is favoured for mass-drug administration of praziquantel to school-aged children and adults. Application of this simple tool has enabled pragmatic tablet dosing using patient height as a proxy for body weight, allowing control programmes to expand into resource-poor settings. New evidence advocates the immediate inclusion of preschool aged children (≤5 year olds), also at high risk for disease and morbidity, in future control campaigns; therefore, the current WHO pole needs updating. Height and weight data were measured during several epidemiological surveys conducted in Angola (N=1067), Mali (N=405), Uganda (N=3303), Sudan (N=137), Zanzibar (N=470) and Zimbabwe (N=104) to establish and validate an extended PZQ dose pole, which now includes two new height-intervals: 60-84cm for ½ tablet and 84-99cm for ¾ tablet divisions. Anthropometric data from other African countries (Demographic Health Surveys) are now also available and will be analysed in the near future. Treatment has been given to different child cohorts and results show that while treatment cure rates can vary significantly between cohorts (25-100%), side-effects tend to be mild and transient. Theoretical application of the updated dose pole results in >95% of children receiving an acceptable dose (30-60 mg/kg). Using this pole, we suggest that mass-drug administration can be better optimized, streamlining general treatment to reduce drug wastage which could lead to significant programmatic savings and allocation of treatments to younger children with minimal additional cost.

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PREVALENCE AND INTENSITY OF *SCHISTOSOMA* SPP TWO YEARS AFTER A PRAZIQUANTEL TREATMENT AMONG SCHOOL-AGE CHILDREN FROM A RURAL VILLAGE FROM AN IRRIGATION SCHEME SUBJECTED TO MULTIPLE TREATMENTS WITH PRAZIQUANTEL, MALI

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Schistosomiasis remains a major public health problem in developing countries with praziquantel (PQZ) being the only treatment available for prolonged use to prevent associated morbidity. The central issue remains about the optimal interval between PQZ treatment rounds to achieve significant long term decrease in worm loads. We evaluated the impact of a single dose of PQZ treatment on the prevalence and infection intensity of *Schistosoma mansoni* and *S. haematobium* in a rice irrigated village in Mali. Two cross-sectional parasitological surveys of children (6-14 years old) were carried out in 2005 and 2007 in a single village in Mali. Stool and urine samples were examined for *S. mansoni* and *S. haematobium* eggs. Difference in prevalence and infection intensity between the surveys was tested adjusted for age and sex by logistic, negative binominal (NB) and zero-inflated negative binominal (ZINB) modelling. 1948 single *S. mansoni* parasite were genotyped. At 2 years post-treatment, the overall prevalence of *S. mansoni* infection was stable, 93% and 88% [OR 0.55, CI95 0.26-1.10], while *S. haematobium* infection fell significantly from 74.5% to 28.0% [OR 0.12, CI95 0.07-0.20]. Geometric means of *S. mansoni* and *S. haematobium* infections decreased significantly from 179 to 83 eggs/gram of faeces [egg count ratio (ECR) 0.58; CI95 0.42-0.78] and 12.3 to 1.8 eggs/10 ml urine [ECR 0.074, CI95 0.044-0.127]. The proportion of children with heavy infections decreased significantly from 42% to 26% for *S. mansoni* and 26% to 0.9% for *S. haematobium*. *S. mansoni* molecular epidemiology identified closely related strains. In conclusion, the ZINB model was effective for the analysing egg count data due to excess zero observations. Praziquantel appeared to have a long term effect on *S. haematobium* but not on *S. mansoni* thought this might also suggest species-specific differences in praziquantel treatment. Sufficient reduction of schistosomiasis infection was not attained and requires additional control measures specific to the 'Office du Niger' irrigation scheme to synergise chemotherapy.

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TRANSCRIPTIONAL RESPONSES OF *SCHISTOSOMA JAPONICUM* EXPOSED *IN VIVO* TO SUB-LETHAL DOSAGES OF PRAZIQUANTEL

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The central reliance of praziquantel (PZQ) for the treatment and control of schistosomiasis is a concern from a public health perspective. PZQ displays a bimodal spectrum of activity being active only against very young schistosomules and the sexually mature blood flukes. Adult schistosomes exposed to PZQ respond with muscle contraction, paralysis, membrane depolarization and the influx of extracellular Ca^{2+}. Molecular characterisations of Ca^{2+} homeostasis, while providing some insights on drug resistance, have been based on initial *in vitro* observations only. Despite these useful studies the precise identity of the molecular targets and mechanisms of detoxifying PZQ are unknown. We have used a murine model to administer *in vivo* sub-lethal dosages of PZQ to adult *Schistosoma japonicum*. Differential gene expression of parasites was followed between 30min and 24h post-PZQ administration, using a whole transcriptome microarray platform. Differential gene expression was considered separately for male and female worms. In males up-regulated genes were associated initially with functions such as "Tegument/Muscle Repair" and "Lipid/Ion Regulation", while later responses included "Drug Resistance" and "Ion Regulation". In contrast, in females, a different sub-set of genes were initially up-regulated including those involved with "Ca^{2+} Regulation" and "Drug Resistance". Genes associated with "Detoxification" and "Pathogen Defense" functions were more prominently upregulated during the later response of female worms to drug treatment. The unique combination of chemotherapy together with the host immune response, provides a more biologically relevant insight into the effects of PZQ on adult schistosomes. Following on from the microarray analysis, we used qPCR to validate a sub-set of genes with either putative drug resistance/detoxification roles or Ca^{2+}-dependant/ modulatory functions. The functional importance of these genes for parasite survival after PZQ treatment was corroborated using RNAi and *in vitro* PZQ assays.

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TOWARD THE ASSESSMENT OF PHYTOCHELATIN SYNTHASE AS A DRUG TARGET FOR SCHISTOSOMIASIS

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Schistosomiasis is a parasitic disease caused by blood flukes of the genus *Schistosoma*, responsible for more than 280,000 deaths annually. The treatment of the disease relies on a single drug, praziquantel. Because it is a cost-effective drug, it has been disseminated through control programs; hence, it is likely that resistance of the parasite to the drug emerge. Therefore, there is an urgent need to identify new targets and drugs for schistosomiasis treatment. We are currently investigating the potential of phytochelatin synthase (PCS) as a drug target in *S. mansoni*. This enzyme is of particular interest since humans do not have a PCS gene in their genome. PCS is a cysteine protease-like enzyme that catalyzes the production of glutathione-derived peptides, the phytochelatins (PCs), with a structure (γGlu-Cys)nGly (n=2-11). These peptides are known to be involved in heavy metal detoxification and accumulation (Pal and Rai 2009). Initial analyses of *S. mansoni* PCS indicated that it protects yeast from metal toxicity and that its expression in cultured worms is increased in response to the presence of metals (Ray and Williams 2011). To assess the function of this protein in *S. mansoni*, studies on the recombinant enzyme have been carried out. Recent work with the purified recombinant *S. mansoni* enzyme showed evidence for the production *in vitro* of PCs (γGlu-Cys)nGly, with n=2-7) from glutathione. Enzyme activity was measured by fractionation of PCs using HPLC, identification by MALDI-TOF, and quantification by derivatization of the PCs with Ellman's reagent. Interestingly, using glutathione-S-bimane as a substrate, *S. mansoni* PCS is capable of cleaving the glycine residue yielding the corresponding γGlu-Cys-S-conjugate. These data attest to the role of PCS in potential detoxification pathways, for both heavy metal scavenging and xenobiotic-glutathione conjugate degradation, making this enzyme likely to be necessary for the parasite, particularly in stress conditions. We are currently developing a biochemical assay that could be used in a high throughput manner that would allow us to screen for inhibitors. Identification of PCS inhibitors will help us to understand the function of the protein in the parasite and will be used to assess its potential as a drug target in a mouse model of infection

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SCHISTOSOMA MANSONI HISTONE-MODIFYING ENZYMES AS DRUG TARGETS

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Enzymes that modify histones (HME) are under increasing scrutiny as therapeutic targets in a number of pathologies, ranging from cancer to parasitic diseases. In particular, inhibitors of histone deacetylases (HDACi) induce cell cycle arrest and/or apoptosis in cancer cells. Treatment of schistosomula or adult worms with HDACi induces the death of both larval (schistosomula) and adult worms and this is preceded in the larvae by the induction of apoptosis as measured by TUNEL staining and the increase in the activity of caspase 3/7. Moreover, such treatments induce a rapid increase in the general level of histone acetylation, particularly of H4. This in turn correlates with the overexpression of certain genes, including those encoding caspases 3 and 7. Finally, qChIP analysis shows that the proximal promoters of both these genes show hyperacetylation of histone H4 after HDACi treatment. These results led us to consider schistosome HDACs, as well as other HMEs, as promising targets for the development of new drugs against schistosomiasis. To this end, a project (SEtTReND) supported by funding from the European Commission has been initiated with the aim of developing specific inhibitors against selected schistosome HMEs that could be candidates as lead compounds for drug development. All HMEs encoded in the *S. mansoni* genome involved in histone acetylation/deacetylation and methylation/demethylation have been identified. Using a phenotypic screen we have shown that inhibitors of all these enzyme classes induced apoptosis and death in schistosomula. Among the schistosome HMEs chosen for further study, SmHDAC8 is particularly promising. Its catalytic domain is more divergent from the human orthologue than are those of other schistosome HDACs and its validity as a therapeutic target was confirmed by RNAi. A combination of high-throughput and in silico screening is being applied to identify potential specific inhibitors of SmHDAC8. In parallel, other HMEs are also being investigated as potential targets.

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CHARACTERIZATION OF FARNESYL DIPHOSPHATE SYNTHASE AND GERANYLGERANYL DIPHOSPHATE SYNTHASE IN *SCHISTOSOMA MANSONI* AND THEIR ROLE AS POTENTIAL DRUG TARGETS

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Schistosomiasis affects over 260 million people worldwide with over 200,000 deaths annually. There is currently only one drug available for disease treatment, praziquantel. We report here that *Schistosoma mansoni* farnesyl diphosphate (FPP) synthase (SmFPPS) and geranylgeranyl diphosphate synthase (SmGGPPS), essential enzymes in many eukaryotes involved in protein prenylation and the generation of sterols and non-sterol products of mevalonate, could serve as drug targets for the treatment of schistosomiasis. In humans, FPPS is a target for the bisphosphonate drugs widely used in bone resorption therapy. Validation of FPPS and GGPPS as drug targets may allow the repositioning of bisphosphonates for schistosomiasis treatment. SmFPPS and SmGGPPS have 35% identity to human FPPS and 53% identity to human GGPPS, respectively. We successfully expressed active, recombinant SmFPPS and SmGGPPS. Recombinant SmFPPS was found to be a soluble 44.2 kDa protein while SmGGPPS was a 38.3 kDa soluble protein. Characterization of the substrate utilization of the two enzymes showed that, unlike the human enzymes, which display strict substrate specificity, both worm enzymes were able to couple isopentenyl PP with three allylic acceptors (dimethylallyl diphosphate, geranyl diphosphate, and FPP). This indicates that the schistosome enzymes have overlapping substrate specificities, making their actions appear to be redundant. Against SmFPPS, several bisphosphonates had IC50s in the low nanomolar range; these inhibitors had significantly less activity against SmGGPPS. While hydrophilic bisphosphonates had no activity against cultured adult parasites, a lipophilic bisphosphonate at 50 µM was active against ex vivo adult worms with worm death occurring over 4-7 days.These results indicate that FPPS and GGPPS could be important targets in the search for new drugs for the treatment for schistosomiasis.

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SINGLE NUCLEOTIDE POLYMORPHISM-BASED SELECTIONS IN THE ß-TUBULIN GENE OF *ONCHOCERCA VOLVULUS*: A NEW STEP IN FILLING THE GAP OF THE POSSIBLE IVERMECTIN FAILURE

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The control of onchocerciasis or river blindness with ivermectin (IVM) has been a great success until now, so that in certain foci its elimination was found to be feasible. However, after more than 21 years of IVM repeated treatment, the disease still persists in many endemic countries. Though sub-optimal responses and genetic changes have been reported in *Onchocerca volvulus* populations under high IVM pressure, unequivocal evidence of resistance has yet to be established. This situation must therefore be urgently clarified to preserve the achievements of onchocerciasis control programs. In this study, *O. volvulus* adult worms were collected from the same patients, before IVM exposure and following three years of annual or three-monthly treatment at 150 µg/kg or 800 µg/kg. Four single nucleotide polymorphisms (SNPs) occurring in the β-tubulin gene of these parasites were investigated. We found multiple nucleotide changes in *O. volvulus* β-tubulin gene associated with the dose and the annual frequency of IVM. Among the SNPs investigated, three showed a high level of selection and nonrandom allelic associations after treatment. Therefore, they may be relevant markers to follow selection for IVM resistance in the field. These results strengthen the warning that selection for IVM resistance could emerge in some *O. volvulus* populations.

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A LATERALLY TRANSFERRED FERROCHELATASE GENE IS FUNCTIONAL AND ESSENTIAL IN FILARIAL NEMATODE PARASITES

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Species in the phylum Nematoda lack a heme biosynthetic pathway and require extraneous heme. Many filarial nematodes contain an obligate endosymbiont, *Wolbachia,* which has a functional heme biosynthesis pathway. Sequencing of the human filarial nematode *Brugia malayi* revealed a genomic ferrochelatase (BmFc) gene, the terminal step in heme biosynthesis. The BmFc gene contains 9 exons spanning ~ 4.5

kb and includes a mitochondrial-targeting domain. The ferrochelatase is functional based upon enzyme assay, complementation to an *E. coli* hemH$^-$ mutant, inhibitor studies with a ferrochelatase-specific inhibitor in *B. malayi* and as a transgene in *Caenorhabditis elegans*. RNAi inhibition experiments also provide evidence that BmFc is functional. While the mitochondrial targeting domain is required for mitochondrial location, it is not required for enzyme activity. FISH reveals the BmFc gene is almost universally expressed in both male and females tissues, except for female late-stage embryos and male late stage sperm cells. Orthologues have been identified in several other filaria, as well as from non-*Wolbachia* containing species and a non-filarial nematode. Phylogenetics suggests a non-Wolbachial, but α-proteobacterial, origin with the lateral transfer acquisition predating the split of the Rhabditida into the Spirurina and Rhabtinia clades. This is the first reported functional LGT gene in animal or human filarial nematodes and its requirement for worm viability suggests it could play a role in the symbiotic relationship between the filarial nematode host and its symbiont and be a potential target for drug discovery against filariasis.

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CLONING AND CHARACTERIZATION OF THE TREHALOSE-6-PHOSPHATE PHOSPHATASE FROM *BRUGIA MALAYI*, A CANDIDATE ANTIFILARIAL DRUG TARGET

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Approximately 120 million people are infected by either *Brugia malayi* or *Wuchereria bancrofti*, the parasitic nematodes responsible for lymphatic filariasis. Although there are several drugs available to treat this disease, there remains a need for additional pharmacological therapies. A draft sequence of the *B. malayi* genome is available and it shares significant similarity to the genome of the well-characterized free-living nematode *Caenorhabditis elegans,* as reported previously. The wealth of information about and the robust genetic tools of *C. elegans* can be used to aid the search for new antifilarial drug targets. One study ranked approximately 600 predicted drug targets from *B. malayi* without human homologs based, in part, on the strength of the RNAi knockdown phenotype in *C. elegans*, as reported previously.We focused our studies on one gene ranked in the top 40 of the predicted targets, the homolog of the *C. elegans gob-1* (gut-obstructed) gene. Consistent with its high ranking in this list, this gene is both essential in *C. elegans* and there is no homolog in the human genome. This gene encodes a trehalose-6-phosphate phosphatase and is required for the biosynthesis of trehalose (as reported previously). We cloned the *B. malayi gob-1* gene (Bm_GOB-1) and expressed it in *E. coli.* We purified Bm_GOB-1 and biochemically characterized its phosphatase activity. Using *C. elegans* we confirmed the observation that the accumulation of trehalose-6-phosphate (T6-P), rather than the reduction of trehalose, is likely responsible for the observed lethality (as reported previously). We are currently examining biochemically whether T-6P inhibits the activity of *B. malayi* hexokinases in an effort to understand the mechanism of the T-6P toxicity. Further characterization of both Bm_GOB-1 and the T6-P toxicity may aid in the development of new antifilarial therapies.

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TARGETING THE *WOLBACHIA* CELL DIVISION PROTEIN FTSZ AS A NEW APPROACH TO ANTIFILARIAL THERAPY

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The use of antibiotics targeting the obligate bacterial endosymbiont *Wolbachia* of filarial parasites has been validated as an approach to controlling filarial infection in animals and humans. The availability of genomic sequences for the *Wolbachia* present in the human filarial parasite *Brugia malayi* enables genome-wide searching for new potential drug targets. FtsZ is such a target protein as it is essential for bacterial cell and absent from humans. In the present study, we have cloned, expressed and purified *Wolbachia* and E. coli FtsZ protein. We determined that *Wolbachia* FtsZ protein possesses the GTPase activity using the spectrophotometric coupled enzymatic assay. We demonstrate that the *Wolbachia* FtsZ GTPase activity was inhibited by berberine and validated the berberine's antibacterial effect using E. coli as a model organism. A library of naphthalene-, quinoline- and biphenyl-based compounds, which was constructed using Ugi multicomponent reaction chemistry, was examined for the discovery of antagonists of E. coli and *Wolbachia* FtsZ. From screening efforts, the (6-{butylcarbamoyl-[(aryl)-(butylcarbonyl)-amino]-methyl})-naphthen-2-ol scaffold emerged as a potent antagonist of both E. coli and *Wolbachia* FtsZ. Interestingly, from structure-activity relationship studies it appears that modification of the aryl substituent on the scaffold may afford selectivity for *Wolbachia* FtsZ. Additional compounds are currently being prepared to examine this possibility. Our results will facilitate the discovery of selective inhibitors of FtsZ as a novel anti-symbiotic approach to controlling filarial infection.

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HSP90 AS A TARGET IN FILARIAL NEMATODES

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In order to identify novel inhibitors of Hsp90 in filarial worms, we adapted a fluorescence polarization assay that was originally designed for screening inhibitors of Hsp90 in tumor cells. The assay relies upon the ability of small molecules to inhibit the binding of fluorescently labelled geldanamycin to Hsp90 and has the advantage of using extracts of worms rather than requiring recombinant protein. The assay was validated using known inhibitors of Hsp90 that compete with geldanamycin for binding to Hsp90, including members of the synthetic purine-scaffold series, and was sufficiently sensitive to differentiate between binding of PU-scaffold compounds to human and *Brugia* Hsp90. The assay was then used to screen a focused kinase library and identified several hits, two of which were tested on adult worms *in vitro*. One of these molecules has a significant effect on adult worms *in vitro* and thus provides a scaffold for further structure based drug activity studies.

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CLONING AND OVEREXPRESSED STUDIES ON HUMAN LUNG MAST CELL RECOMBINANT CARBOXYPEPTIDASE A IN *SACCHAROMYCES CEREVISIAE*

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Mast cell carboxypeptidase A (MC-CPA) is a highly conserved secretory granule protease. It specifically catalyzes the hydrolysis of the peptide bond adjacent to the C-terminal end of a polypeptide chain. Little is known about the function of this enzyme. It has been established however, its ability to cleave the substance angiotensin I into angiotensin II. This suggests that the human mast cell carboxypeptidase A might play a role in the hypertension disease. Normally, the biological actions of proteases are controlled by specific interactions with proteinaceous inhibitors. So far, however, only a few protein inhibitors have been identified for this type metallocarbopeptidases. To shed more light to the function of Human Mast Cell Carboxypeptidase A (hMCCPA) to screen for novel inhibitors for this enzyme we cloned and overexpressed the gene in *E. coli.* The recombinant protein however was expressed in the form of inclusion bodies. To overcome this problem we cloned the gene in *Saccharomyces cerevisiae*. The aim of this project was to produce a soluble active form of CPA in *Saccharomyces cerevisiae.* Gene cloning

was carried out using the vector pYES2 (Invitrogen). The results showed that the gene has been successfully overexpressed in the yeast. Our study also optimized the growth conditions to produce soluble recombinant CPA. The overexpressed soluble CPA was then purified using the one step purification technique.

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EXPRESSION OF PUTATIVE MOLTING-ASSOCIATED GENES IN POST-INFECTIVE FILARIAL L3

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Nematode molting is a complex process that requires synthesis of new cuticle coordinated with other developmental changes plus shedding of the old cuticle. Prior studies have shown that tetracyclines inhibit molting of *Brugia malayi in vitro* and *in vivo*. The *B. malayi* genome contains orthologues for many genes that are required for molting in *Caenorhabditis elegans* based on RNAi results. The purpose of this study was to compare expression profiles of orthologues of putative molting genes in pre- and post-infective larvae of *B. malayi*. L3 were obtained from mosquitoes (vL3) and post infective larvae were recovered from jirds 3 and 6 days after ip injection. We used qRT-PCR to assess relative expression levels for 56 putative molting genes. 73 and 66% of these genes were differentially expressed on day 3 and day 6 (>2 fold change relative to vL3). 13 were up-regulated and 28 were down-regulated on day 3, and 21 were up-regulated and 16 down-regulated on day 6. 57% of these genes were also differentially expressed (25 were up-regulated and 7down-regulated) in day 6 larvae compared to day 3 larvae. Up-regulated genes in post-infective L3 encode homologues of proteases (*nas-37*, *nas-36*), protease inhibitors (*mlt-11*), peroxidase (*bli-3*), DNA binding and sterol sensing domains (*nhr-23, ptr4, ptr23*), extracellular matrix (*noah-2*), signaling and novel genes. Some genes required for molting may also be essential for growth and development. Three days of ip doxycycline (25 mg/kg) had no significant effect on expression of putative molting genes in day 3 larvae. Additional studies are in progress to assess effects of longer exposure to Doxy on gene expression prior to the L4 molt. This research has provided new information on changes in expression of putative molting genes in post-infective *B. malayi* L3. Additional studies will be needed to verify whether these genes are essential for molting in filarial worms.

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ASSESSMENT OF THE VIABILITY OF FILARIAL PARASITES USING MOLECULAR MARKERS

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Many major investigative activities, such as assessing the effects of macrofilariacides and the immune system on these complicated parasites, depends most commonly on assessment of their morphological status. The morphological assessment of the viability and the stages of degeneration of mature filarial worms however, is a difficult and often very subjective process. The use of in situ molecular markers by immunocytochemistry has been used by a number of investigators often with reagents that are directed against mammalian antigens, rather than using those that have been clearly defined as nematode constituents. We have identified markers that appear to have homologous presence and functions in both mammals and nematodes. These immunocytochemical reagents directed against putative nematode components of cellular metabolism and replication have been used to reflect the effects of ivermectin on adult Onchocerca volvulus. The presence of these markers can be quantitated and provide more objective data as to the status of adult worms than has been previously used. The results from the use of these markers suggests that the long term use of ivermectin has a general depressive effects on the health and in all likelihood the longevity of this worm. This approach to assessing worm viability and degenerative status is believed to be suitable for general use and allows this important assessment activity to be carried out by a wider range of scientists than only those with extensive parasitological knowledge.

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DIFFERENTIAL EXPRESSION OF CYS-LOOP LIGAND-GATED ION CHANNEL GENES IN *BRUGIA MALAYI* ADULT WORMS AND MICROFILARIAE

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Nematode cys-loop ligand gated ion channels (CLGIC) are important targets for anthelmintics such as macrocyclic lactones (MLs) and nicotinic agonists. Different parasite species and stages within species vary with respect to sensitivity to drugs that target CLGIC. For example, MLs that target glutamate-gated chloride receptors (GluCl) are highly effective against some gastrointestinal nematode species and filarial L1 larvae (microfilariae, or Mf), but they are less effective against filarial adult worms. Drug sensitivity may be related to CLGIC expression levels. Therefore, we used qRT-PCR to assess relative transcription levels for 32 CLGIC genes in Mf and adult worms of the filarial nematode *Brugia malayi*. These genes encode different classes of GLGIC including GluCl, nicotinic acetylcholine receptors (nAChR), and gamma amino butyric acid (GABA) receptors. Interestingly, transcription levels of GluCl subunits (Bma-AVR-14 A and B) were highest in females, intermediate in Mf, and lowest in males. Most genes encoding nAChR had higher expression in males than in Mf or females, while most genes encoding orphan group channels were most highly expressed Mf. These results show that expression of GLGIC genes varies between life cycle stages and sexes in filarial worms. These differences may reflect stage-specific differences in neurobiology and explain in part the variable sensitivity of filarial stages to anthemintic compounds that target neurotransmission.

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A COMPARATIVE ANALYSIS OF NEMATODE EXCRETORY-SECRETORY PRODUCTS

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Excretory-secretory products (ESP) from parasitic nematodes are thought to be involved in a series of processes that determine their fate, to succeed or fail in host infection. Although the identification of these products has often been limited by the typically low amount of recovered material from *in vitro* incubations, developments in mass spectrometry-based protein identification, along with genomic and transcriptomic sequencing platforms, offer a new way to overcome this limitation and to investigate the roles of components of ESP at the host-parasite interface. To gain a deeper understanding of how parasitic nematodes survive, and the multiple strategies that they may employ to adapt to each particular host and niche therein, we initiated a comparative analysis of ESP from several parasitic species, including *Brugia malayi*, *Meloidogyne incognita* and *Heligmosomoides polygyrus*, as well as the free-living species *Caenorhabditis elegans*. ESP were collected and analyzed through 1D-SDS PAGE and LC-MS/MS. Strategies for MS-to-peptide assignation included database searches on either protein models datasets assessed from their respective genome projects or the deduction of 6 open reading frames from next-generation sequencing transcriptomic assemblies. Comparisons were carried out in terms of protein homologues identified as well as

their functional annotation inferred using bioinformatic tools. Differences in the suite of ESP from parasitic and non-parasitic species reveal specific patterns that may be associated with different niches and host locations.

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THE ROLE OF MELATONIN IN REGULATING NOCTURNAL PERIODICITY OF AVIAN MICROFILARIAE (*CHANDLERELLA QUISCALI*) WITHIN ITS NORMAL HOST, THE COMMON GRACKLE

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Lymphatic filariasis is a debilitating mosquito-borne disease affecting millions of people throughout the tropics. It is caused by filarial nematodes (*Wuchereria bancrofti* and *Brugia malayi*) that inhabit the lymphatic system. To perpetuate their life cycle, female worms produce millions of microfilariae (mf) that enter the blood stream, in the hopes that some will be ingested by mosquito vectors. Throughout most of their range, *W. bancrofti* and *B. malayi* exhibit nocturnal periodicity - i.e., mf only appear in peripheral blood at night. During the day, mf are sequestered in the alveolar capillaries of the lungs and are virtually absent from the peripheral blood. The cue or "pacemaker" responsible for synchronizing mf with the circadian rhythms of their human hosts has never been elucidated. We hypothesize that the pacemaker may be melatonin, a bioactive amine that is secreted from the pineal gland at a regular circadian periodicity. To test this, we used a locally-available avian system - i.e., *Chandlerella quiscali* mf in the Common Grackle. To determine the pattern of mf periodicity in this system, venous blood was taken every 26 hours from 7 microfilaremic grackles. Despite a wide range of mf intensities, the patterns of nocturnal periodicity were similar. Microfilaremias started at 2200h, peaked at 0200 to 0400h and subsided at 0600h. To determine if melatonin would cause mf to appear in peripheral blood, exogenous melatonin was injected into infected grackles during mid-afternoon when they are not microfilaremic. One bird received saline (control) and two received melatonin. The control bird remained amicrofilaremic. A high dose of melatonin (100ng) provoked an earlier appearance of mf than did a low dose (25ng), but at the end of the 3 hour post-treatment observation period, the low dose produced a higher microfilaremia, closer to that of a normal nighttime microfilaremia. Studying nocturnal periodicity of mf from the perspective of hormonal regulation of circadian rhythms may lead to a new approach to block transmission of lymphatic filariasis.

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IN VIVO DUAL TRANSCRIPTOME ANALYSIS OF FILARIAL WORM-MOSQUITO INTERACTIONS

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Filarial worms that cause lymphatic filariasis have a complex life cycle involving both human and mosquito hosts. Within mosquito tissues, nematodes undergo life cycle changes from microfilariae (mf) to infective-stage larvae (L3). Although critical for transmission and completion of the life cycle, molecular processes underlying parasite development in, and interaction with, the mosquito host tissue remain largely uncharacterized. In this study, we simultaneously profiled both the parasite and the host transcriptome over the course of parasite development from mf to L3. *Aedes aegypti* thoracic tissues infected with *Brugia malayi* were collected, in replicate, for 8 consecutive days at 24-hr intervals, and the *in vivo* dual transcriptomes were analyzed using RNA-seq. Tissue samples from mosquitoes fed on uninfected blood were analyzed in parallel. In addition, we evaluated the parasite-host interactome using a strain of *A. aegypti* that fails to support *B. malayi* development. Parasite transcripts ranged from 0.1 to 9.0% of the total transcripts recovered from thoracic tissues during the course of parasite development, but because our dataset is comprised of ~1 billion reads that mapped to the two reference genomes, this experimental approach provides an unprecedented view into parasite development within the mosquito host. In addition, the use of longitudinal and cross-sectional comparisons, both within and across the two organisms, enable a unique analysis of parasite-host interaction involved in this symbiotic relationship.

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THE COMPLETE MITOCHONDRIAL GENOME SEQUENCE OF FILARIAL NEMATODE *WUCHERERIA BANCROFTI*

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Wuchereria bancrofti (Wb) is the primary causative agent of lymphatic filariasis (LF), a deforming and debilitating disease estimated to affect 120 million people in 83 countries. Despite constituting a major public health problem in many tropical and subtropical regions, this mosquito-borne parasitic nematode remains poorly understood with respect to its mitochondrial (mt) genome sequence. To address this knowledge gap, the complete mt genome of Wb was sequenced following amplification and cloning of 15 overlapping mt fragments from a Papua New Guinean isolate. The resulting reads were assembled into a single contiguous sequence and annotated with reference to the complete mitochondrial genome sequence published for the filarial nematode *Brugia malayi*. We find that the Wb mt genome is 13,637 nucleotides in length and contains 36 genes that are typically found in metazoans. Encoding 2 ribosomal RNAs, 22 transfer RNAs, and 12 protein-coding genes, this genome is characterized by a high AT content (74.6%). In addition to using start codons identified previously in the mitochondrial protein-coding genes of other filarial nematodes, Wb mt DNA appears to be unique in its use of TGT as a start codon. Similarly, use of incomplete stop codons in mt protein-coding genes appears to be more common in Wb than in other human filarial parasites. The mt gene order for Wb is identical to that reported for O. volvulus, D. immitis, S digitata and *B. malayi* but is distinctly different from the other nematodes compared. In conclusion, the complete mt genome sequence reported here provides new genetic markers that may be used to monitor the progress of public health interventions aimed at control and elimination of this important human parasite. This data has facilitated preliminary exploration into Wb diversity and will be helpful for future studies aimed at population genetic and molecular epidemiology of LF.

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MITOCHONDRIAL CYTOCHOME OXIDASE I (COXI) SEQUENCE POLYMORPHISM REVEALS POPULATION GENETIC DIVERSITY OF *WUCHERERIA BANCROFTI* IN PAPUA NEW GUINEA

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Wuchereria bancrofti (Wb) is the primary causative agent of lymphatic filariasis (LF), a deforming and debilitating disease estimated to affect 120 million people in 83 countries. A global chemotherapeutic program to eliminate LF by mass drug administration has been introduced. This large-scale chemotherapeutic approach is likely to result in changes in the genetic structure of Wb populations. In this study, blood samples were collected from individuals from 4 villages in the Dreikikir district, East Sepik Province in Papua New Guinea (PNG). These villages represent high and moderate transmission areas (High: Peneng, Kilmangleng, Albulum; Moderate: Moilenge). Wb-positive samples were identified using a post-PCR LDR-FMA described previously. One positive sample from each of the four villages was used in this study, in which a portion of the cytochrome oxidase I gene (680 bp) was amplified, cloned, and sequenced to study

polymorphism and determine the extent of genetic heterogeneity. Among the resulting 38 sequences, 35 haplotypes (Haplotype diversity [Hd]= 0.98) and 99 polymorphic sites (101 mutations) were observed. Diverse populations of Wb were detected in both high (Peneng, Hd= 0.956; Kilmangleng, Hd= 0.9; Albulum, Hd= 0.923) and moderate (Moilenge, Hd= 0.957) transmission villages. Two haplotypes were shared between villages; one haplotype between individuals in Peneng and Kilmangleng, and one shared among individuals in Kilmangleng, Albulum and Moilenge. Additionally, one haplotype was represented twice in individuals from Kilmangleng. The present study suggests that genetically diverse Wb populations exist within and between the four PNG villages studied, as well as within the four individuals studied. A better understanding of the genetic structure of Wb populations may provide important insights into patterns of transmission, disease outcome, and anthelmintic drug resistance, and may thus inform the design and implementation of public health interventions aimed at eliminating LF.

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USING MICROFLUIDIC DEVICES FOR METABOLITE PROFILING OF *BRUGIA MALAYI* HOST-PATHOGEN INTERACTIONS

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Parasitic filarial nematodes such as those that cause lymphatic filariasis (*Brugia malayi, B. timori, Wuchereria bancrofti)* and river blindness (*Onchocerca volvulus*) put nearly 2 billion people at risk in some of the world's poorest countries. These parasites can survive innate and adaptive immune challenge for over a decade despite occupying immune-rich niches such as the skin and lymphatic vessels. A number of immunomodulatory effects have been attributed to filaria, including deficient antigen presentation, induction of regulatory cell populations, suppression of Th1- and Th2- associated cytokine production and altered effector cell recruitment. While some macromolecules with immunomodulatory properties have been isolated from filarial secretions, there has been little effort directed towards studying small molecules secreted by filarial parasites. Building upon our previous studies using LC/MS metabolomics to identify a serum biomarker set for onchocerciasis infection, we have designed and built a 'lab-on-a-chip' system to facilitate the general study of parasite/host interactions. These devices are simple to construct and allow for uni- and bi-directional signaling between immune cells and parasites, facilitating both phenotypic assessment as well as periodic sampling for metabolomic studies. Using these devices, we have studied the interaction between components of the human immune system (e.g., eosinophils, neutrophils) and *B. malayi* by LC/MS-based metabolomics. In this lecture, the molecular profiles of exposing immune cells to the parasite secretome, and vice versa, will be discussed.

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FROM GENE TO VACCINE FOR *BRUGIA MALAYI*: APPLYING IMMUNOINFORMATICS TOOLS TO NEGLECTED TROPICAL DISEASES

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Lymphatic filariasis (LF), which causes the well-known clinical manifestation of elephantiasis, afflicts over 120 million individuals worldwide with another 1.34 billion at risk of infection due to living in endemic regions. While current treatments are effective at lowering parasitemia within infected individuals, no treatment is available that prevents infections from occurring. To effectively prevent infection and to meet the goals of disease elimination set by the WHO, a vaccine would be very helpful. In this study, we used oligonucleotide microarrays to study changes in gene expression during the important transition of *Brugia malayi* from the infective stage in the mosquito vector (L3 mosquito) to the infective stage following entry into the mammalian host (L3 jird). Then, through use of the matrix algorithm EpiMatrix, we identified T cell epitopes in proteins that are upregulated during this transition. To identify possible vaccine candidates, we adopted the genome-to-vaccine design described by Arditto et al. (2010). This approach hinges upon two main principles: "(i) a minimal set of immunogens capable of inducing a robust and sustained immune response to a pathogen can be discovered using immunoinformatics, and (ii) administration of these immunogens, in a suitable delivery vehicle together with adjuvant, will result in protection from disease." This method of vaccine design delivers only what is needed to obtain protection, eliminating unnecessary material and the potential for adverse reactions. Out of 178 genes seen to be up-regulated, 23 were predicted to be highly immunogenic (e-value ≥50). Of these, 9 are predicted to be secreted, making them even better vaccine candidates. As the number of proteins that are being considered for vaccine development expands, rapid, inexpensive and accurate tools are in great demand. The approach described here may significantly accelerate the development of a vaccine for lymphatic filariasis.

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MOLECULAR DIAGNOSIS OF SCHISTOSOMIASIS FOR EPIDEMIOLOGICAL STUDIES

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Demonstration of parasite eggs in urine samples by microscopy is still the method of convenience in most epidemiological studies of schistosomiasis despite its numerous disadvantages including most especially low sensitivity. Recently an ITS-based real-time PCR for the detection of *Schistosoma* species DNA in both urine and faeces samples has been developed which shows a high sensitivity and specificity for schistosoma DNA amplification. This protocol was tested in the field in schistosomiasis endemic areas in Ghana using a total of 730 urine samples collected from school children in comparism with results of microscopy analysis. The samples analysed for the presence of schistosome eggs and DNA using microscopy and real-time PCR respectively. A high proportion of samples without detected eggs by microscopy were included to obtain a more precise estimation of the test sensitivity. Out of the total samples analysed, 8.9% (57) were found to contain schistosoma egg by microscopy detection where as PCR amplification revealed the presence of *Schistosoma* species DNA in 20.8% (152) of the samples. Taking into consideration true positives as microscopy positives and/or PCR positives, positive predictive value calculated was 100% for each school sampled an indication of correct diagnosis of *Schistosoma* positive sample. Of the 152 PCR positives, 59 (38.8%) had Ct values above 35 cycles majority (94.9%) of which were egg negatives. In 102 (15.1%) of the samples testing negative with microscopy, PCR detected DNA amplification with most of these samples exhibiting Ct values less than 35. A good correlation was observed for high intensity infections (egg counts of >50 eggs per 10ml urine), for which PCR samples tested positive for all with low Ct values (<30) indicating higher DNA loads. Results of this study show PCR to be significantly sensitive than microscopy for the detection of the presence of the parasite in the population and evaluating the intensity of infection, which is an important aspect of epidemiological studies. ITS-based multiplex real-time PCR can as such serve as a powerful tool in epidemiological surveys of schistosomiasis in providing more precise results than microscopy as well as eliminating many of the negative drawbacks associated with parasitological analysis.

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EOSINOPHIL CATIONIC PROTEIN AS A POTENTIAL PROGNOSTIC MARKER FOR INFECTION INTENSITY DETERMINATION IN A *SCHISTOSOMA*-ENDEMIC COMMUNITY IN GHANA

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There is increasing demand for more accurate and non-invasive methods in determining infection intensity in schistosomiasis. Recent studies have shown the urine filtration and Kato-katz techniques to grossly underestimate intensity of infection with *S. haematobium* and *S. mansoni* respectively. Accuracy in infection intensity determination by these methods improves only with increasing number of samples collected per participant. This however is tedious and time-consuming, hence increasing chances of experimental errors. This study sought to determine any association between levels of Eosinophil Cationic Protein (ECP) and infection intensity both in *S. haematobium* and *S. mansoni* single and co-infections.The study was conducted in Pakro, a peri-urban community in the Akuapem-South district of the Eastern Region of Ghana. A total of 254 participants: 124 males and 130 females; aged 6 to 96 years, were involved. Each participant provided up to 50ml urine samples and at least, 2g stool samples, which were processed using the filtration and Kato-katz techniques respectively, and examined by microscopy. Aliquots of urine from 73 participants, aged 6 to 40 years were analyzed for ECP levels using the Mesacup ECP-ELISA kit (MBL International). Thirty-nine were *S. haematobium* egg-positive, 2 were positive for both parasites, and 32 were egg negative. Of the 254 urine samples examined, 59 (23.23%) were positive for *S. haematobium*, 1 for *S. mansoni*, and 2 for both parasites. Also, ECP positively correlated with infection intensity by egg count ($p < 0.001$). Higher levels of infection intensity was observed among males ($p < 0.05$). Mean ECP levels were found to be significantly higher in *S. haematobium*-positive, than in *S. haematobium*-negative samples ($p < 0.001$); and almost twice as high for mixed, than for single infections. The ECP ELISA technique showed high sensitivity, but lower specificity. Further research is needed to improve specificity and ascertain ECP levels for mixed infections in schistosomiasis.

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HUMAN ANTIBODY RESPONSE TO THIOREDOXIN PEROXIDASE-1 AND TANDEM REPEAT PROTEINS AS IMMUNODIAGNOSTIC ANTIGEN CANDIDATES FOR *SCHISTOSOMA JAPONICUM* INFECTION

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Since its discovery in 1851, schistosomiasis has continued to be a public health problem in many tropical and subtropical countries and is far from being eradicated in spite of national control programs implemented in endemic areas. Schistosomiasis diagnosis plays a major role in evaluating the efficacy of such control programs. Improving therefore the diagnostic tools for surveillance and monitoring in areas which have reached elimination level will help hasten the possible elimination of this disease. In this study, we assessed the immunodiagnostic potential of thioredoxin peroxidase-1 (SjTPx-1) and 4 tandem repeat proteins (Sj1TR, Sj2TR, Sj4TR, Sj7TR) using human samples. This study therefore aims to develop ELISA through the use of these recombinant proteins. Cut-off values were calculated using 20 serum samples from healthy Japanese volunteers. Eighteen schistosomiasis-confirmed human samples were used to assess these antigens. Results showed that SjTPx-1 and Sj7TR both have 77.8% sensitivity which may be further improved by complementing these 2 antigens. Furthermore, these antigens were also tested against *Plasmodium falciparum*, *P. vivax* and *Entamoeba histolytica* positive sera and showed no cross-reaction between these parasitic infections. These results suggest the potential of these defined antigens for development of a more reliable diagnostic test for schistosomiasis.

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DEVELOPMENT OF A SEROLOGICAL DIAGNOSTIC ASSAY FOR *SCHISTOSOMA MANSONI* USING RECOMBINANT SM25

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The microsomal antigens (MAMA) of *Schostosoma mansoni* have been used as diagnostic antigens with great success for twenty years. Tests using MAMA are accepted as one of the most reliable assays for the accurate detection of low-burden infections, exposure, and identification of cases in endemic areas with a low rate of transmission. There are two diagnostic bands in MAMA, Sm25 and Sm29. Antibody reactivity with either of these proteins indicates a current or previous infection with *S. mansoni*. Because of the cost of adult worms and the complexities of preparing MAMA, it is critical to develop recombinant proteins of these diagnostic antigens. We purified, sequenced and cloned the integral membrane protein, Sm25. A hydrophilic stretch of 90 amino acids from Sm25 protein sequence was expressed in a baculovirus expression system with a GST tag (rSm25). In this study, we developed and evaluated an enzyme-linked immunoelectrotransfer blot (EITB) using rSm25 for laboratory identification of schistosomiasis. We analyzed a panel of 374 sera composed of 189 sera from parasitologically confirmed cases, 110 sera from persons with other parasitic infections and 75 from persons with no documented illnesses (normal sera). The optimized assay has a sensitivity of 91.2% and specificity of 97.2%. Our results suggest rSm25 EITB assay may be valuable in detecting *S. mansoni* infections and may make this assay more widely available.

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GLYCAN BASED DIAGNOSTIC ANTIGENS DISTINGUISH ACTIVE FROM FORMER INFECTIONS IN EXPERIMENTAL SCHISTOSOMIASIS MANSONI

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Like many other neglected tropical diseases, the control strategy for schistosomiasis consists of mass drug administration (MDA). However, current assessment of MDAs for schistosomiasis is limited because the available serologic diagnostic tools lack the capacity to distinguish current from former infections, even after successful chemotherapy. This limits the ability to rapidly monitor program impact or clearly indicate whether or not there is a need for repeat MDA. Because the world's current production of praziquantel, the only drug available for schistosomiasis MDA, is much less than the number of persons who need treatment, judicious use of drug is of utmost importance. A sensitive and specific diagnostic tool that can rapidly detect active infection is critically needed to evaluate the efficacy of control programs and to help determine the most cost effective approaches for schistosomiasis control. Antibody responses in schistosome-infected mammalian hosts are directed primarily against carbohydrate epitopes on schistosome worm surfaces and their secreted products. This suggests that carbohydrate antigens could be useful as sero-diagnostic tools for schistosomiasis. However, the evaluation of schistosome carbohydrate antigens has been limited by the challenges associated with generating large quantities of specific glycan structures for testing. In a previous study, use of glycan arrays demonstrated that epitopes terminating with beta (1, 2)-xylose (core-xylose) and alpha (1, 3)-fucose (core-fucose) were strongly recognized by serum of infected mice, rhesus monkeys, and humans. We have now generated these glycan

epitopes from naturally occurring plant and animal products, conjugated them to specific carrier proteins, and tested their utility as sero-diagnostic tools by ELISA. In longitudinal studies on rhesus macaques, they are recognized during active infection but the antibody response disappears after clearance of schistosomes. Preliminary data suggest a similar temporal recognition pattern in humans with active schistosomiasis and following treatment.

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THE DEVELOPMENT OF A NOVEL MULTI-CHANNELED SEROLOGICAL ASSAY FOR THE DETECTION OF IGG4 ANTIBODY LEVELS VERSUS MORE TRADITIONAL METHODS TO ESTIMATE THE BURDEN OF DISEASE OF URINARY SCHISTOSOMIASIS AND LYMPHATIC FILARIASIS IN A POPULATION ON THE COAST OF KENYA

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Coinfection with multiple parasites is common in the developing world. To better understand the impact of polyparasitism in a population in coastal Kenya, we developed a novel, serum sparing, multi-channel fluorescent serological assay that simultaneously measures serum or plasma IgG4 against *Brugia malayi* antigen (BMA) and *S. haematobium* soluble worm antigen (SWAP). IgG4 was chosen for diagnosis because it indicates active or recent infection and represents the most specific IgG isotype response against BMA or SWAP. Our new IgG4 bead assay was compared to ELISAs for anti-SWAP and anti-BMA IgG4 and to standard parasitologic diagnoses for lymphatic filariasis (ICT antigen detection card) and urinary schistosomiasis (urine filtration). Bead assay cut-off values were determined using control sera from a different area of Kenya that is endemic for multiple parasites but not lymphatic filariasis or urinary schistosomiasis, i.e., positive samples had fluorescence 3 SD above mean values for control sera. Compared with IgG4 ELISA as a gold-standard diagnostic, the novel multi-channeled assay had a sensitivity of 100% and a specificity of 58% with regard to BMA antibody response and a sensitivity of 98% and specificity of 57% with regard to SWAP. However, the IgG4 fluorescent bead assay was more sensitive and specific when compared to the filarial card or urine filtration. Overall, IgG4 by multiplex assay also correlated well with infection-associated morbidity at the population level. Measuring antibody levels by fluorescent bead microassay may not only increase the sensitivity of testing for these diseases, but could provide more useful epidemiological information than standard parasitologic tests.

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APPLICATION OF GOOGLE EARTH AND WEB-BASED GEOGRAPHICAL INFORMATION SYSTEMS (WEB GIS) ON THE REAL-TIME MONITORING PLATFORM FOR SCHISTOSOMIASIS TRANSMISSION

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The application of geographic information systems (GIS) on epidemiological study of schistosomiasis are rapidly growing since the late 1980s in China. However, there are still many obstacles for furthermore application and development. The technology of Internet and virtual globe technologies Google Earth enable scientists, professionals in disease control or decision makers early access to the GIS servers to share their data and findings in a visually attractive without the need for highly sophisticated GIS or much technical assistance. This study elaborated the basic framework and structure in fast risk assessment of schistosomiasis transmission based on Web-based geographical information system (Web GIS), Google Earth, which initially designed with functions in information collection, search and evaluation and analysis of risk factors, dynamic prediction and dynamic early-warning and functions of guidance and management in this system. The design of this system provided evidence based and real-time information to understand the endemic status of schistosomiasis transmission, release real-time information and properly take quick response to transmission foci. The evaluation from running system showed that the combination of two venues, e.g. Google Earth and Web GIS, will have tremendous potential to strengthen overall monitoring and evaluation capacity and facilitate the support system to strengthern the capacity of schistosomiasis control.

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ASSESSING THE POTENTIAL OF *MACROBRACHIUM VOLLENHOVENII,* A LARGE FRESHWATER PRAWN, TO CONTROL SCHISTOSOMIASIS TRANSMISSION IN THE SENEGAL RIVER BASIN

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Schistosomiasis is one of the most common parasitic diseases of humans, and Senegal contains some of the highest transmission sites for schistosomiasis in the world. In Senegal, schistosomiasis outbreaks are thought to have been exacerbated by the building of the Diama Dam in 1986, since the dam increased freshwater habitat for aquatic snail intermediate hosts of schistosome parasites. Controlling snail populations, therefore, is a logical step in the control of schistosomiasis transmission. However, the drug praziquantel has become the predominant tool of schistosomiasis control programs, and biological control of snails has received little attention in comparison. One of the most promising candidates for biological control of schistosome-hosting snails in West Africa is the native prawn, *Macrobrachium vollenhovenii*. We hypothesize that prawns were a common snail predator in the Senegal River, but the Diama Dam posed a barrier to prawn reproduction, and hence, prawn populations have declined, contributing to the emergence of schistosomiasis. In this study, we assess the current population dynamics of free-ranging *Macrobrachium* spp. prawns in the Senegal River Basin, as a first step towards assessing the potential of prawn re-introduction as a snail control strategy. We examine 11 sites, surveyed bimonthly over a period of one year, and record water quality, relative snail abundance, and prawn distributions. We find that freshwater snail abundances are high and seasonally variable, and schistosome intermediate host species predominate at most sites. Prawn abundances are low at all sites but are highest at downstream sites near the river mouth and below the dam. Water quality parameters pH, temperature, nitrate, nitrite, phosphate, alkalinity, and Ca^{++} hardness are within the ranges reported acceptable for *Macrobrachium* growth and survival at most sites. Anecdotally, local fishermen report that native prawns had been a viable fishery. However, catches have declined dramatically in the river basin over the last few decades. Our results suggest that aquaculture of *M. vollenhovenii* prawns warrants serious consideration as a biological control strategy for schistosomiasis. In addition to their potential as snail predators, *M.* spp. prawns are a valuable global commodity, and thus, prawn aquaculture may be a promising tool for both disease control and poverty alleviation.

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POPULATION GENETICS AND EPIDEMIOLOGY OF *SCHISTOSOMA MANSONI* IN SYMPATRIC HUMANS AND NON-HUMAN PRIMATES IN THE GOMBE ECOSYSTEM TANZANIA

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Increased contacts between humans and wildlife around Gombe National Park in western Tanzania have raised the risk of disease sharing amongst them. Although both humans and non-human primates in the area have schistosomiasis, it is not known whether strains of their schistosomes are epidemiologically and genetically distinct. The genetic ecology of Biomphalaria snails, the intermediate hosts for schistosomes in the area is also not well known. This study investigated the infection patterns of schistosomiasis in humans and non-human primates in Gombe and surrounding villages of Mwamgongo, Bugamba, Kiziba and Mtanga. It also examined if there is genetic mixing of snails between village streams and whether they can spread parasites between them. Snails were collected using a scoop and exposed to light for shedding schistosome larvae. Representative snails were dissected and preserved in RNA-later for molecular analysis. Faecal samples obtained from 16 vervets and 110 baboons were examined for parasites using formol-ethyl acetate technique. Stool samples from 55 people in Gombe National Park and about 80 people from each village were examined for parasites using the Kato-Katz technique. Additional stool obtained from 41 people and 9 baboons was analyzed using molecular methods to characterize the genotypes of their schistosome parasites. Overall, the prevalence of *Schistosoma mansoni* in humans was 45.05% and 11.24% in baboons, with no such infection in vervets. Other parasites diagnosed in humans included Trichuris trichiura (1.49%), *Ascaris lumbricoides* (0.99%), and Taenia sp. (0.25%). Molecular analysis of human and baboon faecal eggs based on sequencing of the small subunit rRNA region confirmed the parasites to be *S. mansoni*. More variable genetic markers will be screened to establish the genetic relationships between human and baboon strains of schistosomes. Based on shedding, none of the snails from Gombe and Bugamba had schistosome larvae while 22.64% from Mwamgongo, 22.58% in Kiziba and 16.62% from Kigoma town were infected. PCR-based tests will be conducted to determine whether snails that did not shed parasites could be infected. The implication of these infections to human and animal health in the Gombe area will be explored. Information obtained will help to understand the epidemiology of schistosomiasis and facilitate control programmes for the disease both in humans and wild animals in the area.

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SCHISTOSOMIASIS IN CATTLE HERD BOYS: POSSIBLE IMPACT ON SCHOOL-BASED CONTROL STRATEGIES IN THE DANGME EAST DISTRICT OF GHANA

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The fundamental strategy of controlling the morbidity of schistosomiasis is through mass administration of praziquantel, in school-aged children. This strategy may not achieve the desired goals if it is limited to a school-based mass treatment approach that excludes children who are not enrolled in school. The out of school children could act as sources of re-infection. We evaluated the possibility of cattle herd boys as reservoirs of *Schistosoma* species that could lead to re-infection in treated communities. The study was undertaken in the Dangme East District of Ghana where in a previous cross sectional study in 2006, 1,030 school children (aged 6-17 years) screened indicated a prevalence of 8.4% (86/1030) and 0.3% (3/1030) with the intensity of infection ranging between 4-493 eggs per 10ml and 1 to 6 epg in *S. haematobium* and *S. mansoni* respectively. We conducted a four-month (September-December 2010) study on 17 cattle herds boys aged 8-18 years. This involved stool and urine examination by kato-katz and urine-filtration techniques, and confirmed with real time PCR. We used GPS to map the trail of the cattle herdsmen and water sites frequented during their activities. An infection prevalence of 35.3% (6/17) and 11.8% (2/17) *S. haematobium* and *S. mansoni*, with egg counts between 2 to 212 per 10ml and 4 to 6epg respectively were observed. The map of the trail used by the cattle herd boys during their activities indicated that the grazing happens in close proximity to the communities and schools with the water sites (for the cattle) utilized by the herd boys, school children and the communities. The infection level in the cattle herd boys and the proximity of schools to the grazing trail and water sites could influence infection and re-infection among school children in the communities studied. These preliminary findings could be followed up using a larger population of cattle herdsmen over a larger area.

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MULTI-COMPONENT INTEGRATED CONTROL FOR THE ELIMINATION OF SCHISTOSOMIASIS FROM CHINA: STUDY DESIGN AND BASELINE RESULTS

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Zoonotic schistosomiasis japonica is a major health risk for more than 40 million Chinese with a million people and several hundred thousand livestock infected. Major endemic foci occur in the lake (Dongting and Poyang) and marshlands along the Yangtze River basin; elimination of transmission has proved difficult. For the past 50 years the Chinese government has made great strides in controlling a disease regarded as a public-health priority, but this is predicted to change as a consequence of the completion of the Three Gorges Dam (TGD), which crosses the Yangtze River. The environmental and ecological impacts will result in exponential expansion of the habitat for the intermediate snail host *Oncomelania hupensis*, increasing the risk of human and bovine infection, resulting in potentially severe consequences for control. We have shown that bovine infections are responsible for the persistence of human schistosomiasis transmission in China. Schistosomes debilitate infected domestic livestock which are used for food and as work animals, consequently adding to the economic burden and suffering of endemic communities. Transmission reduction is a key step in eliminating schistosomiasis, but current praziquantel-based control programs are unable to achieve this due to the inability of praziquantel to prevent re-infection. We propose that a multi-component integrated approach (incorporating praziquantel chemotherapy, molluscicding and bovine vaccination) targeting the various transmission pathways is required for sustainable control and elimination in response to the changing environment as a result of the TGD. In 2010 we commenced a 5-year intervention trial to determine the impact of multi-component integrated control on schistosome transmission. Here we present the study design and baseline results.

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SCHISTOSOMIASIS IN MOTHERS AND INFANTS (SIMI) FROM UGANDA: KEY FINDINGS FROM A CLOSED COHORT LONGITUDINAL STUDY ADMINISTERING PREVENTIVE CHEMOTHERAPY

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In certain parts of Uganda, the transmission of intestinal schistosomiasis can be particularly intense such that very young children (< 5 years of age) can be evidently infected with *Schistosoma mansoni*. Indeed, in many lakeshore communities, young children come into daily contact with schistosome cercariae owing to the domestic use of freshly drawn lake water by their respective mothers; the prevalence of egg-patent infections can be in excess of 50% in children under 3 years of age. From a public health perspective, treatment of these younger children with praziquantel is being explored as international guidelines need to be revised if better access to medications for this group is required. Therefore convincing evidence from on-the-ground studies is essential to demonstrate a clear need and likely benefit for revision of international policies for control. The schistosomiasis in mothers and infants (SIMI) project, funded by The Wellcome Trust, has now completed 18-months of close epidemiological surveillance and supervision of praziquantel treatment of over 1,500 young children in 6 shoreline villages of Lakes Albert and Victoria, Uganda. Using a combination of new diagnostic tools, epidemiological monitoring and clinical markers the key findings of this project will be presented. Foremost, hurdles and solutions will be discussed but, above all, the pressing need for roll-out of control to this ageclass will be strongly advocated as, not doing so, is increasingly untenable.

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APPLICATION OF MICROSATELLITE GENOTYPING TO CERCARIAE IN THE INVESTIGATION OF URBAN SCHISTOSOMIASIS

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Historically, schistosomiasis has been described as a rural disease, however, urban transmission is more and more commonly observed in cities of Brazil. Our goal was to determine the utility of cercariae shed from collected snails for assessing genetic relationships between urban populations of parasites. In an ongoing malacologic study of all major collections of water in the city of Salvador (total 158), 7 were positive for infected snails. Cercarial DNA from 5 sites was extracted and quantified by qPCR. Jost's D differentiation index was determined based on genotypes from 14 microsatellite markers. Worm and cercarial DNA from laboratory strains maintained at Case Western Reserve University and at Oswaldo Cruz Foundation, respectively, were genotyped for comparison and as positive controls. Eggs collected from 9 infected children in the neighborhood of São Bartolomeu were also genotyped. The total number of alleles observed for all markers and samples was 120 (range 44 - 91). The average effective allele number (Ae) was similar across all cercarial samples (mean 2.31), but largest in stool eggs (Ae = 3.96). A pairwise comparison of the Jost's D values of all cercarial collections showed a high degree of differentiation between them (mean 0.411), statistically no different than comparing field collected cercariae and controls (mean 0.395). Comparing the cercariae collected from snails on the main river of São Bartolomeu to infected children gave a Jost's D value of 0.505, indicating very different populations. Only two collections suggested potential gene flow between them, the laboratory strain Feira de Santana and cercariae from a small lake near São Bartolomeu (Dique do Cabrito' mean Jost D = 0.017). This, however, is spurious since they are reproductively isolated from each other. Therefore, there was no correlation between geographic location and genetic similarity. While examination of snails for infection may be an important tool for evaluation of transmission, it may not be useful to assess parasite population structure and dynamics in the human host.

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REGULATORY T CELLS IN HUMAN SCHISTOSOMIASIS BEFORE AND AFTER TREATMENT WITH PRAZIQUANTEL

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Human schistosomiasis, one of the most common parasitic infections worldwide, is associated with down-regulation of host immune responses. It has been suggested that regulatory Tcells are induced by schistosomes to allow their long term survival within an immunocompetent host. Here, we study the frequency of CD4+CD25hiFoxp3+ cells in peripheral blood of subjects with and without *Schistosoma haematobium* infection and assess their suppressory activity by comparing total and CD4+CD25hi -depleted PBMCs. Proliferation and cytokine production was measured in response to schistosome egg antigens (SEA) and the vaccine-antigen BCG. Infected children were treated with praziquantel and regulatory T cells were assessed 6 week post treatment. Higher numbers of peripheral blood CD4+CD25hiFoxp3+ Treg cells were found in *S. haematobium* infected children compared to non-infected control subjects. Six weeks after treatment, proliferative responses to antigens tended to increase while there was a significant enhancement of Th2 cytokines in response to schistosome antigens and Th1 cytokines in response to BCG. The number of regulatory T cells decreased by 50%, and their suppressive activity on proliferation as well as on IL-5 to SEA and TNFα to BCG, diminished after treatment. Taken together these data suggest that *S. haematobium* infection is associated with upregulation of regulatory T cells and downregulation of certain antigen specific responses.

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AGE-STRATIFIED SERUM CYTOKINE PROFILE (IL-6, IL-10, TNF-A) IN KENYAN CHILDREN WITH EARLY *SCHISTOSOMA HAEMATOBIUM* INFECTION

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In a study of children with polyparasitic infections in a *Schistosoma haematobium* (Sh) endemic area, we examined the hypothesis that infection-associated inflammation precedes detection of *Sh* infection by standard urine filtration. Children 5-18 yr old were surveyed in August - October 2009, and tested for *Plasmodium falciparum* by ICT card and for *Sh* both by urine filtration and anti-SWAP detection. IgG4 anti-SWAP positive children (n=221) were compared to anti-SWAP-negative children (n=62) for levels of pro-inflammatory cytokines IL-6, TNF-α, and down-regulatory IL-10. In the α-SWAP positive children, regardless of age, there were higher serum IL-6 levels compared to α-SWAP negative children, with