Introducing rapid diagnostic testing for malaria into the private sector
evidence from a cluster-randomized trail in registered drug shops in Uganda
Mbonye, Anthony K.; Chandler, Clare; Hansen, Kristian S.; Lal, Sham; Cundill, Bonnie;
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particularly environment. For instance the higher prevalence E. histolytica / E. dispar and Blastocystis spp., during the rainy season suggests they are waterborne and perhaps less resistant to drier conditions. The fact that Ancylostoma spp. was most frequent in the dry season is consistent with the reproductive cycle of geohelminths. Its life cycle is more effective in moist soils but the intensity of the rains during the wet season could be having a wash off effect. Besides seasonal variations in the weather other factors also moderate transmission such as the indigenous population’s life style in which villagers live in communal homes of more than six individuals. For instance in the wet season they spend more time in their dwellings. This linked to their hygienic habits contributes to increased person-person transmission. Besides this environmental degradation leads to changes in habits which is another factor effecting intestinal parasite transmission in this indigenous community.

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A NOVEL THERAPEUTIC OPTION FOR BALAMUTHIA MANDRILLARIS INFECTION

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Balamuthia mandrillaris infection is an uncommon disease characterized by involvement of the skin with subsequent extension to the central nervous system, where it causes granulomatous encephalitis which is almost invariably fatal. No optimal therapy is available for this lethal condition. To report the outcomes of seven patients with B. mandrillaris infection treated with a combination regimen of miltefosine, fluconazole and albendazole. A case report is presented. Indirect immunofluorescence staining and PCR using the primer mitochondrial 16S rRNA gene were used to identify B. mandrillaris from tissue biopsies. Seven patients are included in this report. Four had granulomatous encephalitis (range of age: 8 to 46 years-old; three of them had in association skin lesions (Two on one of their knees and the other on his nose), and the fourth had rhinosinusitis. The skin lesion was one extensive violaceous plaque, which preceded the neurological involvement (range: 4 - 60 months). The brain MRI features were ring enhancing lesions (one or multiple). A combination regimen including miltefosine (2mg/kg/day), fluconazole (8mg/Kg/day) and albendazole (800mg/day) was initiated after observing compatible histopathology features. Five patients received in addition amphotericin B deoxycholate (total cumulative dose of 25mg/kg); and two patients had a surgical resection of a skin lesion in addition to medical therapy. Four patients had significant improvement and are currently alive with no evidence of active disease after receiving treatment for 6 to 18 months, only one developed neurological involvement. Three patients died after three weeks to 6 months on treatment. Two had extensive centrofacial lesion and granulomatous encephalitis with multiple lesions. Although the prognosis of B. mandrillaris infections is still ominous, it seems that is not invariably fatal. The combination regimen of fluconazole, albendazole and the amebicidal drug miltefosine may be included in the limited existing armamentarium for treating free living amebic infections.

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EVALUATION IN VITRO OF THE ANTI ENTAMOEBA HISTOLYTICA EFFECT OF TWO FLAVONOIDS: EPICATECHIN AND KAEMPFEROL

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Entamoeba histolytica is the parasite that causes amebiasis, a parasitic infection commonly treated efficiently with metronidazole. However, it has been reported that some ameba strains have become resistant to the drug. Research about new therapies to eliminate E. histolytica is an important health priority. We evaluated the in vitro anti-amebic activity of two flavonoids, epicatechin and kaempferol, at different times of incubation by spectrophotometric assay. Control samples were incubated with different concentrations of metronidazole and a vehicle. The viability of amebas incubated with epicatechin at 69 µmol/L was diminished by 10, 20 and 30% at 2, 3 and 4 h, respectively. At the same incubation times, the reduction of amebic viability with epicatechin at 1379 µmol/L was 25, 30 and 45%, respectively, and with epicatechin at 2068 µmol/L the reduction was 30, 50 and 53%, respectively. On the other hand, kaempferol at 698 µmol/L diminished amebic viability by 30, 33 and 50% at 2, 3 and 4h, respectively. At the same incubation times, the decrease of amebic viability with kaempferol at 1397 µmol/L was 50, 53 and 55%, respectively, and with kaempferol at 2096 µmol/L the decrease was 60, 70 and 75%, respectively. At similar times, metronidazole at 698 µmol/L reduced the amebic viability by 52, 65 75%, respectively, and at a concentration of 1392 µmol/L, the reduction was 70 and 78%. The highest dose of metronidazole (2096 µmol/L) diminished amebic viability by 73, 75 and 78% at 2, 3 and 4 h. In the present work we demonstrated the anti-amebic effect of epicatechin and kaempferol, as well as showing that such an effect is dose- and time-dependent, evidenced by the fact that amebic viability decreased with increasing doses and with a greater time elapsed.

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GIARDIA LAMBLIA GENOTYPING IN CHILDREN AND DOGS FROM A HIGHLY ENDEMIC AMERINDIAN COMMUNITY IN PANAMA

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Giardia lamblia, is the etiologic agent of giardiasis, a gastrointestinal parasitic disease of humans and animals. Giardiasis is an important public health concern among children living in rural and indigenous population in Panama. Genetic characterization of G. lamblia isolates has revealed the existence of two groups (assemblages A to B) which are found in humans and in other mammals including domestic dogs. However, the role of these pets in the epidemiology of human infection is still unclear, despite the fact that the zoonotic potential of Giardia has been recognized. The present work aimed to evaluate the genetic identity of human and dog G. lamblia isolates from fecal samples collected in the indigenous community of Ipetí Choco, Ditrict of Chepo, Panama. After obtaining an informed consent from parents and dog owners, 81 fecal samples from children less than 10 years old and 76 dogs were examined for intestinal parasites by microscopy (formalin-acetate concentration procedure). Of the human and dogs evaluated, 42% (34/81) and 11.8% (9/76) were positive for Giardia cysts respectively. DNA was extracted from these positive samples. Genotyping was performed using a PCR-RFLP analysis based on the polymorphisms of the tpi and β-giardin genes. Additionally, a real time PCR of the SSU rRNA gene was used. According to the tpi and β-giardin genes analysis, the most frequent human genotype was assemblage B (76,6%, 23/30). Assemblage A and mixed infections (AB) were present in one sample (3.3%, 1/30) each one. In dogs assemblage B was found in 2.6% (2/76) and assemblage A in 1.3% (1/76). Using the real time PCR analysis, mixtures of assemblages in individual isolates were commonly observed. Human isolates were identified as AB (46.6%, 14/30); B (36.6%, 11/30) and A (3.3%, 1/30). While dog Giardia isolates were characterized as AB (33.3%, 3/9) and B (11.1%, 1/9). Apparently, the frequency of canine giardiasis is low in Ipetí Choco community. However, the zoonotic potential of giardiasis under the observed epidemiological scenario needs further studies.

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ROLE OF REACTIVE OXYGEN SPECIES AND ANTIOXIDANT ENZYME CAPACITY DURING EXPERIMENTAL AMOEBIC LIVER ABCESS

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In an amoebic liver abscess (ALA), polymorphonuclear cells surround Entamoeba histolytica (E. h) amoebas to impede their contact with hepatocytes. Although amoebas in a necrotic area of ALA are usually not viable, parenchymal cells and leukocytes generally suffer damage. This suggests that necrosis could be caused by toxic molecules, including reactive oxygen species (ROS). A decrease in antioxidant enzyme activity may contribute to the extension of parenchymal damage. The present study was undertaken to determine the role of ROS as well as the status of antioxidant enzymes during ALA development. Hamsters were inoculated with E. h. and sacrificed at 12h, 48h and 7d of ALA evolution. Control animals were not infected. We determined the percentage of liver lining with lesion, and in liver homogenates measured (by spectrophotometric methods) oxidative stress (TBARS), total antioxidant capacity (TAS), and the enzymatic activity of superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (Cat) in the necrotic area and normal parenchyma. Compared to the control group, the following significant differences were observed in infected animals: a rise in TBARS (P=0.001) and a decrease in TAS (P=0.01104) in the necrotic area at 7 d, an increase (at 12h) and a decrease (at 48h) in SOD in the abscess in both the lesion area (P=0.003) and hepatic parenchyma (P=0.009), an elevated (at 12h; P=0.03) and a diminished (at 7d; P=0.048) CAT production in the lesion. In necrotic areas, high ROS levels suggest an important role by these molecules in the pathogenesis of ALA. The reduction of TAS and antioxidant activity indicates a failure in defense systems, contributing to the extension of ALA.

ASSESSING SEROLOGICAL TESTS OF TOXOPLASMOSIS IN PREGNANT WOMEN FROM 2002 TO 2010 IN SENEGAL

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In many African countries including Senegal, toxoplasmosis is not subject of a real understanding. The purpose of this study was to update data on toxoplasmosis antibody prevalence based on antenatal surveillance tests in pregnant women in Dakar, Senegal. The test has been performed in 1310 pregnant women at the laboratory of parasitology and mycology at Le Dantec teaching hospital from 2002 to 2010. Immunoenzymatic method in solid phase has been used. To accomplish this evaluation, two serological tests (S1 and S2), using venous blood at 3 weeks of interval, are carried out among these pregnant women. The second serology will allow confirming a toxoplasmosis from an immune response, or a non specific antibody fixation. From the 1255 patients tested, we found a prevalence of 8.7%, and 0% for (IgG+IgM+) respectively at serology S1 and S2; 24% and 27.1% for (IgM+IgG+), 13.8% and 11% for (IgM+IgG+). 37% of pregnant women present toxoplasmosis antibody, a progress from previous data collected in Senegal. These data confirm the presence of toxoplasmosis among pregnant women in Dakar.

INDICATION OF HIGH RISK OF MOTHER-TO-CHILD TOXOPLASMA GONDII TRANSMISSION IN GHANA

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Toxoplasmosis is a parasitic disease caused by Toxoplasma gondii which can be acquired by ingestion of infective stages of the parasite or congenitally from mother to child. Infection can be acute with tachyzoites in circulation or chronic with formation of cysts in muscles and organs. Acute infection may be primary or as a result of re-activation of chronic infection. Congenital infection of infants is known to result in ophthalmic disorders later in life. Recent research in Ghana revealed high sero-prevalence among pregnant women and eye patients. This study sought to determine the risk of transmission of T. gondii infection from mother-to-child among women at delivery in a hospital facility. The study involved 76 pregnant women aged 20 to 45 years who consented to participate. Blood and tissue samples were taken from the maternal side of each placenta after delivery. Umbilical cord blood samples were also taken after they were separated from the infants. Finger-prick blood was taken from infants from participating mothers two to six weeks post-natal. ELISA was used to detect IgG and IgM antibodies against T. gondii in all blood samples while Nested-PCR was used to detect T. gondii DNA extracted from placental tissue. Data collected were analysed using SPSS. Results showed 42.1% (32/76) of maternal blood to be positive for anti-T. gondii IgG-ELISA (CTK Biotech, Inc.) with 42% of corresponding umbilical cord blood samples being positive for IgG. 32.5% (13/40) of post-natal infant blood was positive for anti-T. gondii IgG. All of the same blood samples were negative for IgM. Nested-PCR detected T. gondii DNA in 40.2% of placental tissue. 3 (3.4%) maternal blood were positive for IgG but the corresponding placental tissue samples were negative for PCR. The presence of anti-T. gondii IgG antibodies only and T. gondii DNA in placental tissues which could be from cysts indicate the women might have had the infection during the pregnancy. In addition, detection of anti-parasite antibodies in umbilical cord and post-natal infant blood suggests a high risk of congenital transmission of the infection to the infants. These results provide baseline data for future work to ascertain the rate of mother-to-child transmission in Ghana.

EVALUATION OF INDICATORS OF BOVINE BABESIOSIS IN TICKS RHICIPESPHALUS (BOOPHILUS) MICROPLUS AND CATTLE FROM THREE TO NINE MONTHS OF THE COLOMBIAN MIDDLE MAGDALENA

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Babesia sp. is a parasite transmitted by ticks affecting livestock in the world. The Magdalena Medio region of Colombia is enzootic for babesiosis, but no studies that demonstrate the behavior of its transmission cycle. The objective was to evaluate entomological and parasitological indicators of infection with Babesia sp. in cattle and ticks in the region, through direct microscopic and molecular techniques. We designed a descriptive study of representative non-probability, the number of calves sampled was 237, to which was extracted from blood and adult female ticks for analysis by direct microscopic and molecular techniques to detect infection Babesia sp. We obtained a positive for B. bigemina of 59.9% and mixed infection (B. bovis + B. bigemina) of 3.4% was not found positive for B. bovis as a single agent. In ticks, a total of 770 specimens captured, analyzed for hemolymph, the total percentage of ticks positive for B. bigemina was 79.2% and 9.4% mixed infection. The total infestation area was 3.2 ticks per calf. The calves of 6-7 months
had the highest degree of infestation with 4.7 ticks per calf. A positive correlation was established between the frequencies of the bath over time and parasite burden in cattle. In conclusion, monitoring is needed of the disease in pastoral areas, with new entomological and parasitological indicators that account for the complexity of fenómeno. These results infer that a higher frequency of acaricide treatments 90 days in stable areas is an abiotic factor that favors the acquisition of protective immunity in calves, a positive influence on the natural control of infection and the absence of disease over time. CODI

DEGRADATION AND UTILIZATION OF COMPLEX CARBOHYDRATES BY TRICHOMONAS VAGINALIS

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*Trichomonas vaginalis* is a protozoan parasite that is the causative agent of trichomoniasis, a widespread sexually transmitted disease that affects millions worldwide. Several reports suggest that infection with this protozoan correlates with a decrease in the glycogen content of the vaginal epithelium. Most studies of *T. vaginalis* include the maintenance of parasites in media containing either glucose or maltose as carbohydrate sources. Here, we demonstrate that *T. vaginalis* grows equally well in media containing the glucose polymers amylopectin or glycogen as the principal carbon source. Having demonstrated the ability of *Trichomonas* to grow and utilize these polymers to support growth, we sought to analyze cell pellets and culture supernatant for hydrolytic activity towards amylopectin. We hypothesized that *Trichomonas* utilizes glucose polymers by first degrading the polymers into smaller subunits. Our data indicate that *T. vaginalis* possess both cell-associated and secreted hydrolytic activity towards glucose polymers and that activity accumulates in the medium during growth. Furthermore, carbohydrate limitation triggers an increase in both activities. Our initial analysis of the secreted activity reveals enzymatic properties consistent with those of an α-amylase. Collectively, our data provide evidence for a potential role of glucohydrolases in the growth of *T. vaginalis*.

PENTATRICHOMONAS HOMINIS IS ASSOCIATED WITH DIARRHEAL EPISODES IN CAPTIVE-BRED OWL MONKEYS (*AOTUS NANCYMAEAE*)


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Owl monkeys (*Aotus nancymaeae*) are small New World, non-human primates found in Brazil, Colombia and Peru. This species has been extensively used in biomedical research in the areas of infectious diseases, glomerulonephritis, atherosclerosis, immunology, and vision research. They have also been established as animal models for diarrhea caused by enterotoxigenic *Escherichia coli*, *Campylobacter jejuni*, and *Shigella flexneri*. Protozoan organisms such as *Giardia intestinalis*, *Cryptosporidium* spp., and *Entamoeba histolytica* are known to cause gastroenteritis in New World primates. On the contrary, Trichomonads and Blastocystis spp. are commonly found in these species but normally no clinical signs are observed and treatment is not routinely warranted. However, after a small increase in diarrhea rates at our laboratory facility, we investigated the prevalence of intestinal protozoa in captive-bred owl monkeys and its association with abnormal stool consistency. Four hundred sixty-one stool samples were collected irrespective of stool consistency at the NAMRU-6 Laboratory Animal Facility between 2009 and 2012. Identification of intestinal protozoa was performed by microscopy. Trichomonad species was determined by sequencing the ITS locus. Diarrhea was defined as non-formed stool and association with protozoan presence was analyzed with regression methods. Overall, trichomonads and Blastocystis spp. were found in 54% and 39% samples, respectively. Two hundred fifty-eight (56%) samples were classified as diarrhea. The prevalence of diarrhea in animals with and without trichomonads was 72% and 38%, respectively (ratio: 1.91, 95%, p<0.001). Similarly, the prevalence of diarrhea in animals with and without Blastocystis spp. was 72% and 46%, respectively (ratio: 1.56, 95%, p<0.001). In regression analyses, both protozoa had highly significant and independent effects (trichomonad ratio: 1.76, p<0.001, Blastocystis ratio: 1.34, p<0.001). Sequencing analysis of trichomonads organisms found showed 99% homology to *Pentatrichomonas hominis*. While intestinal trichomoniasis is normally non-pathogenic, infection due to *P. hominis* in research non-human primates may result in diarrhea and could influence the outcome of gastroenteritis research studies. Careful evaluation of research animals should be instituted and treatment alternatives should be considered to treat and prevent diarrhea due to trichomoniasis.

CONGENITAL TOXOPLASMOSIS IN BRAZIL: MODELING THE COST OF MATERNAL SCREENING

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Toxoplasma gondii is a protozoal parasite infecting a high proportion of the world’s population, although infection is generally asymptomatic in immunocompetent people. Congenital infection can result in fetal death or mild to profound visual, cognitive, and hearing impairment. A decision-analytic model applying the European protocol of universal maternal screening/treatment to the low-prevalence US population found cost saving of $1 billion and prevention of avoidable injury in thousands of children every year. Using TreeAge Pro Suite software, we constructed a decision-analytic model to estimate costs of untreated toxoplasmosis and costs of screening, treatment, and follow-up for 3 high-prevalence Brazilian states. The model includes probabilities of maternal and fetal infection, fetal loss due to congenital toxoplasmosis (CT), post-natal infection, distribution of visual, hearing, and central nervous system injury, treatment efficacy, and non-probabilistic variables, such as costs of screening tests and treatment. Brazil has very high prevalence of toxoplasmosis, from 30% to 80% in different states, with different ecologies and quality of water and sanitary infrastructure. High adult prevalence is associated with high incidence during pregnancy due to acquisition in adolescence and young adulthood. High incidence of CT is compounded by a more virulent strain than found in Europe. The Brazilian strain affects 1 in 500 births and also can produce blindness when acquired post-natally, even in immunocompetent persons. Clinical experience in Brazil indicates that the local strain, if untreated, produces more profound injuries than the European strain, but that prenatal treatment is equally effective in preventing or mitigating injury. High levels of exposure, including from the water supply, make pre-natal and post-natal incidence a serious public health problem. In this high-incidence population, maternal screening is found to be cost-saving. Universal screening also has spillover benefits in community education, reducing post-natal infection and visual injury.
ASSESSMENT OF CRYOPROTECTANT TOXICITY ON THE VIABILITY OF CRYPTOSPORIDIUM PARVUM AND EVALUATION OF A CRYOPRESERVATION METHOD FOR SPOROZOITES

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Cryptosporidium parvum is an obligate intracellular parasite that can cause life-threatening infections among immunocompromised individuals. Successful cryopreservation and recovery of viable C. parvum has not been achieved in spite of multiple studies. This constitutes an obstacle to the establishment of standardized isolates or cloned stocks. Compounds used as cryoprotectants may have detrimental effects on parasite viability, thus toxicity studies should be pursued before developing any cryopreservation method. The present study assessed the effects of different cryoprotective agents on the viability of C. parvum and evaluated the infectivity of sporozoites following cryopreservation. Treatment of C. parvum oocysts with ethylene glycol (EG), propylene glycol (PG), dimethyl sulfoxide (DMSO), glycerol, or sucrose did not result in incorporation of propidium iodide at cryoprotectant concentrations ranging from 0.5 to 4 M. In addition, excystation of sporozoites was not impaired by the treatment of oocysts with these compounds. Treatment of excysted sporozoites with EG, PG, DMSO, or sucrose did not affect the infectivity of C. parvum as determined by the presence of intracellular meront stages in infected HCT-8 cells. Moreover, the expression of the C. parvum genes TRAP, CWOP, and 18S rRNA in infected HCT-8 was unaffected by prior treatment of sporozoites with cryoprotectants. Following cryopreservation of sporozoites in a cocktail of DMSO and sucrose, infectivity of HCT-8 cells was observed up to 4 days of infection. In summary, oocysts are highly resistant to potentially toxigenic concentrations of cryoprotectants, an observation that is in all likelihood attributed to the low permeability of the thick cyst wall. In addition, sporozoites are able to withstand concentrations of cryoprotectants commonly used in freezing protocols and are amenable to cryopreservation as assessed by an in vitro infection assay. We have established a framework to monitor the concentrations of cryoprotectants and cooling parameters as work continues towards developing an optimal freezing method for the long term storage of C. parvum.

OPPORTUNISTIC INTESTINAL PARASITES AND MALNUTRITION IN MADAGASCAR: HOW TO DESIGN STUDIES?

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Opportunistic intestinal parasites (cryptosporidias, microsporidias, etc.) were mostly described in Europe after emergence of the HIV outbreak but their prevalence fall down dramatically with effective antiretroviral therapies. Some of these parasites could affect immune-competent hosts and are described in travellers coming back from their trip with diarrhea. In tropical countries prevalences of opportunistic parasites seem to be higher than in Europe, but are not well documented. Moreover, in tropical areas other causes of immune-depression can occur like malnutrition or tuberculosis. In Madagascar chronic malnutrition concerns 50% of children fewer than 5 years. Chronic malnutrition could induce immunodepression and opportunists have thus to be intensively researched in these children during diarrhea. To address this link between malnutrition and opportunists, we started studies on four pathogens: cryptosporidias, microsporidias, Isospora belli and Cyclospora cayetanensis. However the difficulty is to define a technical procedure usable i) to analyse large set of stools collected in the same time on the field, ii) sensitive, specific and at low cost and iii) which do not require trained personal. Quantitative PCR could be the best solution for epidemiological campaigns. However pitfall in definition of PCR is the low number of genes potentially targeting due to the low number of sequences available to do multiple alignment. Moreover in tropical countries water and vegetables can be a huge source of parasites in transit. Overall we choose a three steps procedure for epidemiological studies: Q-PCR / microscopy / sequencing, using ribosomal small subunit sequences. In the same time we setup studies in two hospitals to target under-fed children. A third study was conducted on samples already collected to analyse causes of diarrhea in children leaving in the suburban area of Moramanga (Madagascar). Studies are still in progress but associations of cryptosporidias and microsporidias have been already found in several patients.

ELECTRON MICROSCOPY CHARACTERIZATION OF DIENTAMOEBA FRAGILIS VIRUS LIFE CYCLE

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Dientamoeba fragilis is a pathogenic trichomonad parasite found in the gastrointestinal tract of humans and is implicated as a cause of diarrheal disease. The objective of this study was to describe, by transmission electron microscopy, the presence and morphological details of the virus population found in different clinical isolates of D. fragilis growing in xenic culture. These virus populations comprise different sizes ranging from 40-200 nm. Their most common shape was spherical, enclosing a dense core, a middle electron-lucent layer and an outer coat. In addition, these VLP populations have anicosahedral capsid structure. The D. fragilis VLPs were found in the cytoplasm closely associated with the Golgi complex, with some VLPs budding from the Golgi while other VLPs were detected adjacent to the plasma membrane. These VLPs attach and penetrate into D. fragilis by endocytosis and are maintained within vacuoles during batch culture for several daily passages and excreted through exocytosis. Virus-like particles are abundant in the growth media of stationary-phase D. fragilis cultures. This is the first study to describe in detail the ultrastructural characteristics of a Dientamoeba fragilis virus (DFV) and its mode of replication in different cultured isolates of D. fragilis.

PERKINSUS MARINUS, THE AGENT RESPONSIBLE FOR DERMO DISEASE IN OYSTERS, DOES NOT INDUCE PATHOLOGY IN HUMANIZED HLA-DR4 MICE AND STIMULATES ORAL IMMUNITY

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Perkinsus marinus (Phylum Perkinsozoa) is a marine protozoan parasite closely related to dinoflagellates (responsible for harmful algal blooms) and apicomplexans (e.g. Toxoplasma, Plasmodium) that has devastated natural and farmed oyster populations in some areas of the USA, significantly affecting the shellfish industry and the estuarine environment. P. marinus range extension in the northeastern USA has been associated with global warming, and currently Dermo disease is under surveillance by the World Organization for Animal Health (http://www.oie.int/). The infection prevalence has been estimated in some areas to be as high as 100% often causing death of infected oysters within 1-2 years post-infection. Human consumption of infected oysters is thus likely to occur,

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but to our knowledge it has not been investigated in humans or other mammals whether *P. marinus* induces gut pathology or whether oral immunization occurs upon consumption. Here we used a humanized mouse model expressing HLA-DR4 molecules and at the same time lacking expression of mouse MHC-II molecules (C57/BL6 background) to address these questions. Oral feeding with live *P. marinus* PRA240 did not induce pathology as manifested by histological examination of the gastrointestinal tract, lungs, and kidneys. Furthermore, PCR testing showed absence of the oyster parasite in fecal material, indicating that *P. marinus* cannot replicate and/or infect cells in the gastrointestinal tract. Interestingly enough, the humanized mice elicited strong humoral (IgG) and cellular responses to the oyster parasite. Our results thus demonstrate that *P. marinus* does not induce pathology and stimulates gut immunity in HLA-DR4 humanized mice. Ongoing studies are aimed to address whether anti-*P. marinus* immunity can protect humanized mice against malaria.

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**A STUDY OF MUSSELS Perna perna infected with Cryptosporidium spp. intended for human consumption indicating environmental contamination**

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Sources of contamination such as water drainage of the animal faeces, the use of organic fertilizers and the release of part or untreated sewage contamination favor of various aquatic environments by this parasite since the oocysts are eliminated in the faeces of the host. In the seas the presence of *Cryptosporidium* spp. directly affects the quality of fish such as mussels present in the Brazilian coast and is therefore limiting the consumption of food. The study aimed to diagnose and characterize genetically type (s) and/or genotype (s) of *Cryptosporidium* in mussels taken from rocky shores at two locations, Lage Preta and Saco’s Beach, in the Mangaratiba city, State of Rio de Janeiro, performing the sequencing and phylogenetic analyzes, including the deposit of *Cryptosporidium* sequences from GenBank, to correlate the presence of the parasite with the index of rainfall in the region and to establish possible risks of eating mussels, by identifying the genotype (s) and or species with zoonotic potential. Mussels were collected monthly from March 2009 to February 2010 totaling 12 samples. During data collection, 30 animals were separated from each location and divided into three groups of 10 animals each, totaling 72 samples. For the analyzes, the DNA extracted from tissues of mussels was used in the amplification of sequences 18S SSU rRNA by nested-PCR technique. Results: For species identification, the amplicons were sent for sequencing. During all the study samples was possible to diagnose mussels *Cryptosporidium* positive at least one of the study sites. It was possible to identify three species *C. andersoni*, *C. meleagridis* and *C. parvum* in samples obtained from two locations of mussels, by observing the similarity of 99% when compared to existing sequences in GenBank. It is possible the occurrence of human cryptosporidiosis by the consumption of mussels, raw or partially cooked, from the city of Mangaratiba. Statistical analysis showed no influence of rain in positivity of the samples of mussels for *Cryptosporidium*. With these results we conclude that there is likelihood of human exposure through ingestion of mussels from the region studied.

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**EPIDEMIOLOGIC ASPECTS OF INFECTION CRYPTOSPORIDIUM SPP IN CALVES DAIRY AND GENETIC CHARACTERIZATION OF SPECIES AND SUBTYPES**

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The bovine cryptosporidiosis is caused mainly by four different species, *Cryptosporidium parvum, C. bovis, C. ryanae* and *C. andersoni*. The first one is of great concern for both livestock and public health. With regard to public health, the species is the subject of several studies due to its high zoonotic potential. The study aimed to: Perform the genotypic characterization of *Cryptosporidium* species and subtypes obtained from fecal samples from calves under one year of age, from dairy farms in the State of Rio de Janeiro, Brazil, establishing the potential for zoonotic species *C. parvum* through diagnosed subtype. Methods: The aim of this study is to determine the occurrence of *Cryptosporidium* species and subtypes in calves up to one year of age, throughout PCR technique usin 18S and GP60 as gene target. The occurrence of *Cryptosporidium* species in calves up to 1-year-old was determined for 143 animals on three dairy farms on the state of Rio de Janeiro, Brazil. A fecal samples collected directly from each calf rectum was processed to concentrate oocysts using the centrifugal flotation technique in saturated sugar solution before being evaluated microscopically. Results: Of the 28 positive samples in microscopy, 23 were confirmed by Nested-PCR using gene 18S rRNA. After each PCR-positive specimen was sequenced, the presence of three species of *Cryptosporidium* was observed infecting calves at different ages. Pre-weaned calves were infected with *C. parvum* (7%), whereas post-weaned calves were infected with *C. andersoni* (15%) and *C. ryanae* (1%). All positive samples are being submitted to a second Nested-PCR using gene GP60 as target. A new sequencing will be made for *C. parvum* positive samples, to observe the most prevalent subtype in the area. Conclusions: Were diagnosed by means of molecular techniques *C. parvum* and zoonotic subtypes, *C. andersoni*, species of importance for dairy production and *C. ryanae*, this species is the first report infecting calves in the state of Rio de Janeiro and the second description of the species in Brazil.

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**MALNUTRITION IS ASSOCIATED WITH INCREASED MORTALITY IN ADULT MEDICAL INPATIENTS AT A REGIONAL REFERRAL HOSPITAL IN SOUTHWESTERN UGANDA**

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The contribution of malnutrition to the course of acute illness and hospital-based mortality in adults in sub-Saharan Africa (SSA) is not fully described. To determine if malnutrition is associated with increased mortality in hospitalized patients in SSA we conducted a prospective observational study of 318 adult (age ≥ 18 years) medical inpatients admitted to the Mbarara Regional Referral Hospital in southwestern Uganda. For each patient, we calculated body mass index (BMI) and a mini-nutritional assessment short form (MNA-SF) score. We followed patients until death or 30 days from admission. The cohort included 152 (48%) women and the mean (± SD) age was 42 (± 8) years. There were 144 (45%) HIV infected patients and 132 (42%) had suspected tuberculosis (TB). Other diagnoses included severe anemia with Hb ≤ 7 g/dl (89, 28%), diarrhea (52, 16%), pneumonia (44, 14%), kidney disease (24, 8%) and stroke (11, 4%). Malnutrition (MNA-SF ≤ 7) occurred in 187 (59%) patients and 149 (47%) patients had a BMI of <18.5 kg/m2. The in-hospital mortality was 18% (57 of 318). Of the 261 patients discharged, only 27 (10%) were lost to 30 day follow-up. The 30 day mortality was 40% (117 of 291). In the univariate analysis, malnutrition, an abnormal temperature (≥38 °C or <36 °C), HIV infection, and presence of suspected
95% (231/243) were isolated from vaginal swab and 5% (12/243) between,
and an auxanogram. Then identification by PCR targeting the hyphal
identified by the germ tube test, the chlamydospore production test
strains isolated in Dakar. Oropharyngeal and vaginal
pathophysiology of malnutrition in acutely ill hospitalized
in SSA and ways to mitigate its effect.

TOXOPLASMA MYOCARDITIS IN IMMUNOCOMPETENT
REQUIRING CARDIAC TRANSPLANTATION

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Toxoplasma gondii is a common protozoan parasitic zoonosis with varying
prevalence. Toxoplasmosis is predominantly of concern in pregnant
women and in immunocompromised hosts either as a primary infection
or reactivation. It presents as a self limiting illness in immunocompetent
persons but rarely presents as chorioretinitis, encephalitis, polyomatis,
pneumonitis, hepatitis and myocarditis. Severe infections are rare
because the parasitism is short lived due to the transformation of
tachyzoites into bradyzoites. There were fewer than 50 reported cases of
severe toxoplasmosis and 15 cases of myocarditis in immunocompetent
subjects worldwide. We report the case of an 18 year old Caucasian
immunocompetent female living in tropical Australia who was diagnosed
with Toxoplasma myopericarditis causing fulminant heart failure and
cardiogenic shock requiring urgent BIVAD implantation as a bridge
to cardiac transplantation. Patient did not have a significant travel or
exposure history other than to a cat. Prodromal symptoms were very
nonspecific but in 4 weeks, patient developed pulmonary oedema and
ECG showed widespread ST elevation. Echocardiogram revealed a non
dilated left ventricle with an ejection fraction of 20 percent. Toxoplasmosis
was diagnosed by EIA levels and the low avidity index of IgG indicating
an acute infection. Trimethoprim and Co-Trimoxazole were commenced
and an extensive negative test panel excluded other causes. Viruses were
not isolated on biopsied ventricular tissue and histopathology did not
reveal any micro organisms on special stains. CD3 positive T lymphocyte
predominant lymphocytic myocarditis with extensive myocyte necrosis
was reported. Patient developed progressive cardiogenic shock requiring
urgent BIVAD implantation. Patient underwent cardiac transplantation and
device removal 61 days after presenting to hospital. To our knowledge,
this is the first reported case of severe myopericarditis secondary to
Toxoplasma infection in an immunocompetent host requiring a ventricular
assist device and cardiac transplantation.

IDENTIFICATION OF THREE CANDIDA AFRICANA STRAINS IN SENEGAL

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Frequency of candidiasis has increased dramatically in recent years.
Candida albicans is the most common species. However, other species
which are pathogenic and resistant to usual antifungal agents beginning
to emerge. Among theme C. dubliniensis and C. africana are the most
frequent. These two species presented morphological similarities. Thus,
it is important from epidemiological point of view to identify the fungal
isolates in Senegal. This study was undertaken to identify new species
among Candida strains isolated in Dakar. Oropharyngeal and vaginal
swabs were performed at Fann Hospital in Dakar. The strains were
identified by the germ tube test, the chlamydospore production test
and an auxanogram. Then identification by PCR targeting the hyphal
wall protein 1 (hwp1) gene, was carried out in order to discriminate C.
albicans between, C. dubliniensis and C. africana. Among the 243 yeasts,
95% (231/243) were isolated from vaginal swab and 5% (12/243)
from oropharyngeal swab. Species identified by phenotypic methods
are C. albicans which is the most frequent, C. tropicalis, C. glabrata, C.
dubliniensis, C. krify and C. lusitaniae. Of the 150 strains analyzed by
PCR, 75% (112/150) were positive. Among the 112 strains of C. sp PCR
positive, 97% (109/112) were identified as strains of C. albicans and 3%
(3/112) as C. africana. No strains of C. dubliniensis was not found in our
study. In conclusion, this study isolates C. africana for the first time in
Senegal. Further studies on a larger sample will better know the actual
proportion of these three species among the isolated yeasts.

ENTAMOEBA HISTOLYTICA MENINGOENCEPHALITIS IN A SAMOAN TODDLER WITH ACUTE ONSET SEIZURES

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Invasive amebiasis causes extraintestinal disease via hematogenous
spread to the liver, lung, brain and rarely other organs. The medical
literature reports amebic brain abscesses, which represent advanced
infection that is often fatal, but to our knowledge Entamoeba histolytica
meningoencephalitis has not been described in the modern literature,
which is distinct from that caused by free-living amebae. We report what
may be the first case of E. histolytica meningoencephalitis diagnosed in
American Samoa in a 16 month old Samoan boy. Without brain or liver
abscesses on CT scan, he developed acute onset seizures while receiving
appropriate dose oral metronidazole for intestinal amebiasis and, CSF
exam revealed 2+ motile trophozoites. He was treated with intravenous
metronidazole for 2 weeks during a complicated ICU course, and had
apparent full recovery with no further seizures to date. E. histolytica
is endemic on the island and is the most common intestinal parasitic
infection diagnosed in our lab in the past 2 years. Hence, in American
Samoan and other areas endemic for E. histolytica, where similar cases
might be escaping diagnosis, pediatricians should have a high index of
suspicion for CNS amebic infection in a child with intestinal amebiasis
and new onset seizures.

ATYPICAL RABIES: A CASE REPORT FROM A RABIES OUTBREAK IN KENYA

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An outbreak of canine and human rabies occurred in the Kisumu East
district of Western Kenya between December 2011 and March 2012,
resulting in 369 human exposures, primarily through dog bites. Of these,
20135.3% persons began post-exposure vaccination. Two human deaths
were reported. Seven (41.2%) of 17 dogs were diagnosed rabid via the
direct fluorescent antibody test. One of the cases was a 4½ year-old
boy that was admitted at a district hospital on 7th February, 2012 with a
direct fluorescent antibody test. One of the cases was a 4½ year-old
child with intestinal amebiasis and new onset seizures.

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vaccination, administered on days 0, 3 and 12, but without rabies immune globulin. The patient's condition deteriorated on day 7 with bouts of vomiting. The patient subsequently developed paraparesis and dyspnea before dying on day 13. Postmortem specimens were obtained from the brain stem, cerebrum and cerebellum. Rabies virus-specific antigens were detected using a direct rapid immune-histochemistry test (dRIT). Although the patient did not have access to intensive care, he survived for two weeks in the hospital whereas typically rabies cases do not survive more than a week without intensive care. This is the first human rabies case to be diagnosed using the dRIT in a developing country. Rabies vaccine administration to the patient after illness was in conflict to existing recommendations and confounded ante-mortem diagnostic testing. The WHO recommendation is to administer the post-exposure vaccine on days 0, 3, 7, 14 and 28. Immune globulins should also be administered.

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A STUDY TO ASSESS INITIAL PARENTAL RESPONSE TO FEVER IN CHILDREN IN MBARARA, UGANDA, A MALARIA ENDEMIC REGION

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Malaria remains one of the leading causes of morbidity and mortality in the developing world, with the greatest burden of which being in sub-Saharan Africa, especially in countries like, Uganda. Traditionally, development of fever in endemic areas has been correlated with malaria, and several cross sectional studies performed in sub-Saharan Africa, have shown that fever is one of the most recognized signs of malaria within the community. It is important to examine parental initial responses to fever as delaying seeking out treatment for malaria and other common causes of childhood fever can be associated with worse outcomes. Our objective was to determine the initial response to fever among parents presenting with febrile children to the pediatric ward at Mbarara Regional Referral Hospital, in southwestern Uganda. We also aimed to identify factors that determine a parent’s initial response to fever. A questionnaire was administered to 74 parents of sick children who presented with a chief complaint of fever. The questionnaire included questions on demographics of the parents and children, and assessed initial parental decisions in response to their child’s fever. Despite the fact the majority of the study participants had little formal education and had poor socioeconomic status, the most favored parental response to fever was to utilize a nearby clinic. However only 40% of the respondents reported seeing a healthcare worker within 24 hours of onset of fever. Almost 30% of the respondents reported waiting more than 72 hours to seek out a healthcare worker. The majority of the respondents who did not go to the clinic first reported that they didn’t feel the child was sick enough or reported that transportation was a barrier. Given these findings, parents in this region, should be counseled not to delay seeking assistance when their child becomes febrile to help aid in the diagnosis and treatment of malaria, and other potentially life-threatening non-malarial causes of fever such as pneumonia and gastroenteritis.

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ESTIMATION OF THE RIFT VALLEY FEVER BURDEN OF DISEASE IN THE 2006/2007 OUTBREAK IN KENYA

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Rift Valley Fever (RVF) virus causes severe epidemics in livestock and humans resulting in considerable economic losses from disruption of livestock production and market chain and morbidity and mortality in humans. This study estimated the burden of RVF disease in humans using disability adjusted life years (DALYs), assessed human health RVF epidemiological parameters and private and public health costs during the last RVF epidemic in the 2006/2007 in Kenya. We interviewed family members that cared for an infected person in eligible household and key informants in the public sector in Garissa and Kilifi districts that were heavily affected by the epidemic and at the public health leaders at the national level to assess the private and public health costs. An eligible household was household that had an RVF cases during the 2006/2007 outbreak as identified from the line list. Secondary data from the Ministry of Health and published literature were reviewed for epidemiological parameters including age and sex categorized incidences, proportions of disease manifestation, and mortality rates in order to compute DALYs using methods developed by the World Health Organization. A total of 127 eligible households were enrolled in to the study with one member interviewed in each household. Those interviewed in these households included 54% males and ranged from 19 to 81 years old with 40 and 45 years as mode and median age, respectively. The RVF virus predominantly infected males during the outbreak with an annual incidence of 0.7 per 1,000 population compared to females at 0.5 per 1,000 population. The burden of RVF during the 2006 and 2007 outbreak was 3.4 DALYs per 1000 population, representing 1% of the total DALYs and estimated household costs of USD120 for every human case reported. In comparison, the total burden of HIV/AIDS and malaria in Kenya is the highest at 24.2% and 7.2% DALYs, respectively. Our results provide vital data on burden of RVF for use by the Government and other institutions to guide in health policy making and resource allocations for prevention and control.

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BURDEN OF PODOCONIOSIS IN EAST AND WEST GOJAM ZONES, NORTHERN ETHIOPIA

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Podocentosis is geochemical entaliosis of the lower legs that affects barefoot individuals exposed to red clay soil of volcanic origin. Podocentosis can be prevented, early forms of the disease can be treated, and disease progression can be curbed. Podocentosis is an important public health problem in Africa, Central America and northern India. The aim of this study was to assess the burden of podocentosis in East and West Gojam Zones, northern Ethiopia. A cross-sectional household survey was conducted in two districts covering all 17,553 households in 20 randomly selected kebeles (administrative subunits). Following this, detailed structured interview was conducted on all cases identified. The prevalence of podocentosis in the population aged 15 years and above was found to be 3.3% (95% CI, 3.2% to 3.6%). 87% of cases were in the economically active age group (15-64 years). On average, patients sought treatment five years after the start of the leg swelling. Most subjects had second (42.7%) or third (36.1%) clinical stage disease, 97.9% had mossy lesions, and 53% had open wounds. On average, patients had five episodes of acute ALA per year. The commonest treatment facilities visited were health centers (28.7%) and traditional healers (29.4%). The most common coping measures employed against ALA were staying in bed (55.6%), resorting to less laborious work (44.2%), use of antibiotics (25.8%) and local herbs (20.5%). The median prevalence of podocentosis in the population aged 15 years and above was found to be 3.3% (95% CI, 3.2% to 3.6%). 87% of cases were in the economically active age group (15-64 years). On average, patients sought treatment five years after the start of the leg swelling. Most subjects had second (42.7%) or third (36.1%) clinical stage disease, 97.9% had mossy lesions, and 53% had open wounds. On average, patients had five episodes of acute ALA per year. The commonest treatment facilities visited were health centers (28.7%) and traditional healers (29.4%). The most common coping measures employed against ALA were staying in bed (55.6%), resorting to less laborious work (44.2%), use of antibiotics (25.8%) and local herbs (20.5%). The median age of first use of shoes and socks were 22 and 23 years, respectively. More men than women owned more than one pair of shoes (61.1% vs. 50.5%; χ2= 11.6 p=0.001). At the time of interview, 23.6% of the respondents were barefoot, of whom about two-thirds were women. This study showed high prevalence of podocentosis and associated morbidity such as ALA, mossy lesions and open wounds in northern Ethiopia. Predominance of cases at early clinical stage of podocentosis indicates the potential for reversing the swelling and calls for disease prevention interventions.
ACCESS TO TREATMENT FOR CHAGAS DISEASE IN MEXICO: A POLICY ANALYSIS

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The most recent prevalence estimates from the World Health Organization indicate that as many as 1.1 million people in Mexico are infected with Trypanosoma cruzi, the etiologic agent of Chagas disease. However, limited information is available about access to treatment for this disease. The aims of this study were to assess the current extent of access in Mexico, analyze the national and state barriers to access, and suggest strategies to overcome them. Morelos was used as a state case study and data were collected from this state and the national Chagas program. Semi-structured in-depth interviews were conducted with 16 key informants and policymakers at both levels. Government policy documents about Chagas disease treatment in Mexico were collected, analyzed to assess treatment access and used to triangulate interview data. Interview responses and information from policy documents were analyzed according to the health systems “control knobs”, as defined in the Flagship Framework for Pharmaceutical Policy Reform: regulation, financing, payment, organization, and persuasion. The data showed that 2,847 new cases of Chagas disease were registered nationally from 2007-2011 and in each year but one, the number of new cases was below the national program’s target by 11-36%. The Morelos case study revealed that this state made a concerted effort to increase access by purchasing benznidazole, consistent with state responsibility for medicine procurement. The national program mainly coordinated donation of nifurtimox from the WHO Nifurtimox Donation Program. The procurement process used by Morelos was complex and reflected important obstacles at the national level such as exclusion of antitrypanosomal medicines from the national formulary (regulation), exclusion of Chagas disease from the Seguro Popular social insurance package (organization) and limited understanding of the disease by providers (persuasion). The study proposes strategies to overcome these barriers, including adding these medicines to the national formulary and increasing education about the disease.

RELATIONSHIP BETWEEN NUTRITIONAL STATUS AND THE PREVALENCE OF MALARIA AND ANEMIA AMONG CHILDREN IN THE KASSENA-NANKANA DISTRICT OF GHANA

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Under nutrition, malaria and anemia are significant public health concerns in northern Ghana, especially among children under five years. There is growing interest in the effects of nutritional status on clinical malaria outcomes in pre-school age children. The study objectives were to characterize the effects of indicators of dietary risk on clinical malaria during both the wet and dry seasons. Cases of clinical malaria (wet season n=40, dry season n=42) and matched controls (wet season n=47, dry season n=37) were identified from records of the longitudinal Birth Cohort Study (BCS). Clinical indicators (Hgb, malaria parasitemia, weight) were extracted from study records, and retrospective household questionnaires captured indicators of household hunger, dietary diversity, household food security, and malaria risk behavior. Children from households classified as having very low food security had an increased risk of being clinical malaria cases compared to children from households not classified as having very low food security. (OR=1.78, 95% CI (.92-3.45), p=.087)

CHRONIC LIVER DISEASE IN HEMODIALYSIS

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The functional integrity of the liver is crucial to vitality in normal people and end stage renal disease (ESRD) patients; the prevalence of chronic liver disease (CLD) in hemodialysis (HD) patients differs according to the country or even the region in the same country. Hepatitis C virus (HCV) was recognized as an important cause and consequence of chronic kidney disease. We aim to investigate the prevalence of CLD among HD patients in Tabuk, Saudi Arabia. All HD patients were offered to participate in the
study, interview, medical records including; demographics, family history, and investigation, were the main source of information. Blood samples were taken, before start of HD to detect HBsAg, HCV antibodies and biochemical tests. Abdominal sonar was performed to scan liver, spleen, portal vein and to become aware of ascites. The prevalence of ESRD was 350 per million populations, mean HD duration 40±15 month, mean age 44±18 year, BMI 23±2 kg/m², serum albumin 3.2±0.5 (g/dl), males 52 %. Etiology of ESRD was vague in 33% of HD patients, HCV +Ve in 31%, HBsAg +Ve in 6% and 1% +Ve for both, 4% had nonalcoholic steatohepatitis, 3% had compensated liver cirrhosis, 1% ascitis. Most of the CLD patients did not have symptoms. Older age, low education, living in pastoral areas and longer duration of HD appear to be risk factors for CLD in HD patients. Despite no evidence suggests that HD patients are more prone to suffer from hepatic toxic effects than people with normal kidney function, but HD patients usually receive multiple medications; that may have a role in the pathogenesis of drug induced liver disease in this population. Chronic hepatitis C and B among HD patients are mild in disease activity, and are not progressive, perhaps due to immunological abnormalities in HD patients. CLD were more prevalent in HD patients with vague etiology of ESRD so, more extensive investigations are required as HCV, or any CLD may be incriminated in the development of ESRD with vague etiology.

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**OUTPATIENT TREATMENT OF SEVERE ACUTE MALNUTRITION AFTER THE PORT-AU-PRINCE EARTHQUAKE: A SINGLE-CENTER REVIEW OF OUTCOMES FROM 2010 AND 2011**

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Malnutrition is a major contributor to preventable child mortality in Haiti. Pneumonia and diarrheal disease cause nearly half of all child mortality under age 5 in Haiti, and malnutrition is well-established as a primary risk factor for death from these infections. We conducted a single-center retrospective review of admission and discharge data for children ages 6-60 months who presented to an outpatient treatment program for severe acute malnutrition between April 1, 2010 and December 31, 2011. Recorded data included age in months at entry; neighborhood; gender; grade of edema; mid-upper arm circumference (MUAC); admission weight, height and weight/height z-score; criteria for entry into the program; corresponding anthropometric data at the time of discharge and co-morbid conditions. The records of 1,695 patients were reviewed. Eight patients were excluded due to incomplete discharge data. There were 851 (50%) male patients, 1,441 (85%) were between the ages of 6 and 24 months, 234 patients (14%) were referred to a malnutrition clinic closer to their residence and 132 (8%) were admitted for inpatient malnutrition treatment. Of those enrolled, 1,083 (82%) children successfully completed the outpatient treatment program, while 45 (3%) did not respond to treatment, 31 (2%) abandoned the program, 18 (1%) were transferred to a cholera treatment center, and 131 (10%) were transferred to unspecified medical facilities. Patients identified 21 distinct geographic zones as their current location of residence, the most common being Cite Soleil, Petionville, Croix-des-Bouquets, Delmas, and Tabarre (all within 10km of the program). Among these localities, Croix-des-Bouquets had the highest rate of program completion (84%). Cite Soleil had a lower completion rate than the four other common zones combined (75% vs. 83%, p=0.015). In the two years after the earthquake, this outpatient treatment program achieved recovery rates similar to those previously reported from diverse settings. Root causes for neighborhood differences in rate of program completion are likely multifactorial and include geographic and socioeconomic obstacles to care. These results demonstrate that outpatient treatment for pediatric severe acute malnutrition can be successfully completed in a complex post-disaster setting.

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**USING LONGITUDINAL STUDIES TO ESTABLISH NORMAL REFERENCE RANGES FOR HEMATOLOGIC AND IMMUNOLOGICAL PARAMETERS IN HEALTHY PREGNANT WOMAN AND YOUNG CHILDREN IN MALI**

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Accurate assessments of hematologic safety profiles and immunological responses to investigational products such as vaccines are often complicated by a lack of normal reference ranges (RR) in the target population, and a poor understanding of how age, gender, genetics, diet and underlying medical conditions influence these parameters. Two specific populations that lack normal RR in Mali are pregnant women and children under five years. We performed a RR study on peripheral whole blood collected from healthy participants enrolled in longitudinal studies, and compared these data to established RR from Uganda and the U.S. We analyzed leukocyte, erythrocyte, and platelet profiles for hematologic RR; and B-cell, T-cell and monocyte subsets for immunological RR. Hematologic profiles varied with pregnancy status, age, gender and geographic location, and the latter suggests a possible genetic effect. Immunological profiles also varied with these parameters, but to a lesser degree. Site-specific normal RR are necessary to accurately establish baseline hematologic and immunological parameters in a target population. In the future, these RR will facilitate the accurate inclusion or exclusion of potential study volunteers, will make the assessment of research-related adverse events more reliable, and will improve the clinical management of patients in Mali.

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**INTEGRATING DENGUE AND DIARRHEA CONTROL IN RURAL SCHOOLS IN COLOMBIA: A CLUSTER RANDOMIZED CONTROLLED TRIAL**

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Diarrheal diseases and dengue fever are major global health problems. Where provision of clean water is inadequate, water storage is crucial. Fecal contamination of stored water is a common source of diarrheal illness, but stored water also provides breeding sites for dengue vector mosquitoes. Poor household water management is therefore a potential determinant of both diseases. Little is known of the role of stored water for the combined risk of diarrhea and dengue, yet a joint role would be important for developing integrated control efforts. Even less is known of the effect of integrating control of these diseases in school settings. This trial investigates whether interventions against diarrheal disease and dengue can reduce diarrheal disease and dengue risk factors in rural primary schools...
in Colombia. This is the first trial investigating the effect of integrating dengue and diarrhea control interventions and it is also the first trial to do this in school settings. A 2x2 factorial cluster randomized controlled trial is being carried out in rural primary schools in La Mesa and Anapoima municipalities, Cundinamarca, Colombia. Schools were randomized to one of four study arms: diarrhea interventions (DIA), dengue interventions (DEN), combined diarrhea and dengue interventions (DIADEN), and control (C). Schools were allocated publicly in each municipality at the start of the trial. The objective of the trial is to investigate whether these interventions will significantly reduce diarrhea incidence and dengue entomological risk factors. The primary outcome for diarrhea is incidence rate of diarrhea in school children and for dengue entomological risk, Aedes aegypti adult density per school. A total of 873 pupils from 34 schools are enrolled in the trial. Here we report results from baseline and the first follow-up data collections.

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A CAMBODIAN DERMATOLOGY NEEDS ASSESSMENT

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There are no epidemiological studies published in the English literature describing the common skin diseases found in Cambodia. Dermatology services are at an early stage of development and, in order to provide information for policy makers and dermatology training course designers a simple needs assessment was undertaken in the environs of Phnom Penh. The aim of the study was to identify the common dermatoses and their impact on those presenting with them. Four different sites were selected; 2 semi-rural and 2 urban. The necessary permission was obtained via Ministry of Health officials. Patients were invited to attend a clinic where they completed an administered questionnaire and were then examined by a team of dermatologists and their diseases recorded. Patients were then given appropriate free treatment. 625 patients were assessed with 76 different diagnoses recorded however the 10 commonest disorders accounted for nearly 60% of the cases with acne, eczema and scabies being the top three. The majority of patients had disease classified as mild to moderate and for a median duration of 12 months. 53% of patients had previously spent an average of $10 on treating their skin disease unsuccessfully. The most likely group of patients to have previously paid to not get rid of their disease was those with scabies (65.3% of those with scabies). The study dermatologists estimated that 97.1% of patients could have been appropriately managed with treatments available in Cambodia. There are no previously published studies assessing the impact of skin disease on patient in Cambodia. Scabies, eczema and acne were the commonest dermatoses with scabies being the most costly to the patient. This supports the notion that educating communities and basic healthcare workers about simple management of common skin diseases with locally available treatment could significantly reduce the impact of dermatoses for the patient and community alike.

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ACCIDENTAL CAUSTIC SODA INGESTION IN GHANA - AN ALARMING AND INCREASING PROBLEM DEMANDING EARLY DETECTION AND INTERVENTION

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The morbidity and mortality associated with accidental chemical ingestion are preventable. A sudden increase in the numbers of children admitted to Okomfo Anokye Teaching Hospital in 2010 following caustic soda ingestion led to a detailed case note review of all admissions with poisoning from January 2009 to June 2010. The purpose of the review was to identify possible causes for the increase and then develop an effective public health strategy to reverse the trend. There was a six fold increase in the number of children suffering from caustic soda poisoning from Jan-Jun 2010 (13) compared with Jan-June 2009 (2), whilst numbers of cases of poisoning for all causes (19 vs 34) poisoning had doubled. The majority of cases were under three years old and males accounted for 50 of the 72 cases. All children received palm oil to induce vomiting. Complications included oesophageal ulceration, aspiration pneumonia and death. A number of hypotheses for these increases are postulated including a new National Health Insurance Scheme (NHIS) leading to an increase in the number of cases who present to health care facilities; a possible increase in the use of caustic soda as a domestic cleaning agent and the introduction of water bottles which are reused to sell and store caustic soda. We favour the last of these hypotheses because the introduction of these drinking bottles has occurred simultaneously to the increase in presentations and we refute the other hypotheses offered. We propose that a public health awareness campaign via radio and text message be used to spread the message of the risk to children of the use of caustic soda and the danger of palm oil as first aid. This should be accompanied by a campaign to restrict and regulate sales of the corrosive chemical.
A QUALITATIVE STUDY ON BARRIERS TO CONSISTENT USE OF FOOTWEAR IN WOLAITA ZONE, SOUTH ETHIOPIA: IMPLICATIONS FOR PREVENTION AND CONTROL OF PODOCONIOSIS

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Effectiveness of prevention and control of podocnosis depends on a community’s consistent use of footwear. However, little is known about factors impeding the use of footwear among communities at high risk of podocnosis through exposure to red clay soil. This study explored the shoe wearing practices of communities in Wolaita, Southern Ethiopia, and identified major barriers to use of footwear with the aim of informing evidence-based preventive strategies. The study was entirely qualitative involving 38 in-depth interviews, 28 focus group discussions, and 7 case studies in four selected communities in Wolaita zone. In total, 307 informants (52 children and 255 adults) participated in the study, using convenience sampling from affected and unaffected segments of the population. Data were coded and analyzed using the NVivo-9 software. Perceiving shoes to have either protective or social value facilitated use of footwear. Although shoe wearing was commonly intermittent, patients were more likely to wear shoes regularly than non-patients. Financial issues, low perceived risk of podocnosis and lack of access to higher quality protective shoes were major reasons hampering consistent shoe use in the community. Further, lack of sufficient knowledge about the cause of the disease and misconceptions about shoe wearing resulted in irregular use of footwear. Interventions must emphasize changing mindsets about footwear and improving accessibility to protective shoes in the community. Implications of the findings on preventing podocnosis in Wolaita district are discussed.

TRAUMA TRAINING COURSES AVAILABLE AROUND THE WORLD: A SYSTEMATIC REVIEW

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Injury deaths are increasing in low-, middle-, and some high-income countries. This burden of disease is greatest in economies that are least equipped to manage trauma care. The availability of trauma training courses to guide management of trauma care throughout the world is not known. We performed a systematic review of English language literature using the search terms “trauma” and “education”. In addition, professional colleagues were contacted, and a world-wide web Internet search was completed in an effort to identify all available trauma training courses. 44 courses were identified in total. 71% of all courses identified were developed in high-income countries (HIC); 67% of courses are taught in high-income countries. Of courses implemented in low-middle income countries, 60% of them were developed in HIC. Few courses (14%) are designed exclusively for physicians. Most courses (43%) include health care providers with variable levels of education and training. Trauma care training courses are given throughout the world, many for non-physician providers. It is unknown if additional courses are available yet unidentifiable via our search methodology of scientific publications, internet search, and personal communication. In view of the current global burden of injury, dissemination of training and education in the management of acutely injured patients is essential.

ANALYSIS OF MEASLES SURVEILLANCE DATA FOR DECISIONS ON SUPPLEMENTARY IMMUNIZATION CAMPAIGN IN SOUTHERN ETHIOPIA

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In 2010, Ethiopian Ministry of Health implemented measles supplementary immunization activity (SIA) campaigns targeting children 9 months-5 years. Despite the campaign, measles outbreaks continued to occur in the Southern regions of the country affecting several districts in the region. Regional epidemiologic and laboratory measles surveillance data from July 2010 to February 2011 i.e. before and after the SIA were reviewed to guide public health decisions to advise the implementation of the campaign. A total of 34,782 cases and 64 deaths were reported from 32 of the 125 districts in the region with an incidence of 863 per 100,000 populations and case fatality rate of 0.18%. About 87% (30,366/34,872) of the patients were children below 15 years of age. The incidence was 218 per 100,000 population among children <5 years and 92 per 100,000 populations among 5-14 years of age before the campaign. After the campaign, the incidence was 346 per 100,000 populations among children age <5years, and 193 per 100,000 populations among 5-14 years of age. The incidence in adults was 12 and 38 cases per 100,000 populations before and after the campaign respectively. The proportion of reported patients from targeted age group was 49% (5,732/11,808) before and 43% (9,110/22,974) after the campaign and proportion from non-targeted age group was 42% (5,017/11,808) before and 45% (10,507/22,974) after the campaign. In conclusion, the 2010 measles SIA decreased the proportion of reported patients from targeted age group after the campaign, but did not prevent further spreading of the outbreak. Changing target age group and schedule follow-up SIAs based on local epidemiology may help to control ongoing outbreaks using such campaigns. Accordingly it is recommended that the SIA started inclusive of age groups beyond 5 years.

THE ROLE OF ANGIOGENIC AND INFLAMMATORY FACTORS IN THE PATHOGENESIS OF PREECLAMPSIA

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Pre-eclampsia, a pregnancy complication characterized by hypertension and proteinuria is still a major cause of neonatal and maternal mortality, and acute and long-term morbidities for both mother and neonate. There is mounting evidence that an imbalance between angiogenic factors, such as VEGF (vascular endotheial growth factor) or PIGF (placental growth factor), and inflammatory factors such as interleukin 1 (IL-1) and Tumour Necrosis Factor (TNF) are closely related to the pathogenesis of pre-eclampsia. This study was conducted to determine the role of angiogenic and inflammatory factors in the endothelial dysfunction of the placenta and onward pathogenesis of preeclampsia by measuring and comparing maternal serum levels of Ang 1, Ang 2 and Tie receptor 2 with PlGF, sFlt-1 to cytokines IL-1 and TNF. Venous blood would be collected from Obstetrics and Gynaecology Department of the Korle-Bu Teaching Hospital. The study would involve healthy non-pregnant women, healthy pregnant women and pregnant women between the ages of 16 and 45. The samples would then be centrifuged at room temperature for 15 minutes at 1000 x g. Maternal serum would be analyzed by ELISA for levels of Angiopoietin 1 and 2, PIGF, IL-1 and TNF. It is expected that there would be a correlation between the angiogenic and inflammatory
cytokines in the pathogenesis of preeclampsia which would add to existing knowledge of the syndrome and aid in early diagnosis, treatment and prevention.

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USE OF TELEMEDICINE TO DIAGNOSE RINGWORM IN KENYAN SCHOOL CHILDREN

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Internet-based telemedicine has the potential to alleviate the problem of limited access to healthcare in developing countries. The Mashavu project aims to deploy kiosks that transmit health data and pictures to clinics for analysis by trained personnel. To test this principle, we investigated whether dermatophytic fungal infections (ringworm) could be diagnosed by Kenyan clinicians from pictures of lesions. Six physicians, five physician assistants, and five nurses from Nyeri Provincial Hospital took a test consisting of 15 pictures of KOH prep-confirmed ringworm lesions and 15 pictures of KOH prep-negative skin lesions affecting local children. The mean (SD) sensitivity and specificity of ringworm diagnosis for the whole group was 73% (19) and 83% (11) respectively. The physicians had the highest sensitivity and specificity, although only sensitivity reached statistical significance when compared to physician assistants. These results suggest that teledermatology can be used to diagnose simple skin conditions with reasonable sensitivity and specificity.

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HAS TANZANIA EMBRACED THE GREEN LEAF? IMPACT OF AFFORDABLE MEDICINES FACILITY - MALARIA (AMFM) ON ANTIMALARIAL PROVISION IN TANZANIA

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In Tanzania the first line antimalarial is artemisinin based combination therapy (ACT), but uptake remains low. The Affordable Medicines Facility - malaria (AMFM) was launched in 2010 in eight national-scale pilots, to increase access by subsidizing quality-assured ACTs (QAACTs), which have a green leaf logo. We conducted nationally representative surveys of public and private antimalarial outlets before AMFM implementation and one year after to assess impact on QAACT affordability, availability and market share. Here we present detailed results for mainland Tanzania, stratified by rural/urban area and outlet type. This work was commissioned by the Global Fund to Fight AIDS, Tuberculosis and Malaria as part of the AMFm Phase 1 Independent Evaluation. We randomly selected 49 wards at baseline (2010) and follow up (2011), and visited all outlets with potential to stock antimalarials, collecting data on outlet characteristics and stocking patterns from outlets with antimalarials in stock. 3,151 and 3,785 outlets were enumerated at baseline and endline respectively, of which 631 and 788 stocked antimalarials and were interviewed. Analysis of results of availability, affordability, and market share. Availability: 78.6% of public health facilities (PHFs) stocked QAACTs compared to 10.7% of private for profit (PPF) outlets. Within PPF outlets, pharmacies were most likely to stock QAACTs (65.3%), compared to less than 10% of drug stores and general stores. Affordability: 78% of QAACTs in PHFs were provided free; the rest had a median price of $0.47 per adult equivalent treatment dose. QAACTs were most costly in PPF outlets (median $4.93), especially in urban areas ($7.04). Among PPF outlets, non-artemisinin drugs, such as SP and amodiaquine, were the cheapest antimalarials. Market share: QAACTs had a market share of 49.1% in urban areas, while non-artemisinin therapy dominated the rural market (78.2%). QAACT market share was very low in PFP outlets (1.0%), compared to 96% for non-artemisinin therapies. These findings will be compared with results at follow up to assess the impact of AMFm on these key indicators.

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HEALTH INFORMATION TECHNOLOGY APPROACHES FOR CONTINUOUS QUALITY IMPROVEMENT OF ANTIRETROVIRAL PROGRAMS IN DEVELOPING COUNTRIES

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Health information technology (HIT) has potential to support continuous quality improvement (CQI) of antiretroviral ARV) programs. The fields of CQI and HIT, however, have yet to become fully integrated. To narrow this gap an assessment of various types of HIT used in CQI was conducted. A comprehensive systematic literature review of PubMed, Google Scholar, ACM Digital Library and IEEE Explore was performed to develop a compendium of HIT approaches for CQI. Resources were included if they addressed the feasibility, implementation, or evaluation of innovative HIT that directly supported CQI of ARV programs or patient outcomes in low and middle income countries. The literature review identified 379 articles addressing HIT and HIV programs or outcomes since 2002; 15% (57) used HIT for CQI. At the programmatic level (n=21), geographic information services, health information management systems and electronic medical records are methods to longitudinally track program and facility characteristics to assess overall performance, determine best practices and facilitate planning. At the clinic level (n=15); electronic medical records and cellular phones provide tools to aide clinical decision-making and more efficiently manage patient information leading to improve patient outcomes. At the patient level (n=21); cellular phones can be used to remind patients about adherence as well as clinic follow-up and electronic adherence monitors can provide a mechanism to remotely monitor and promote ARV adherence. Special consideration must be given to the local context, including the technical expertise and physical infrastructure required to implement, sustain and potentially modify the technology. HIT can facilitate CQI to better inform program planning and support clinical care. When implemented with due planning, these technologies can be powerful tools to longitudinally track the quality of HIV care and facilitate solutions for improvement.

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HEALTH WORKERS IMMUNIZATION STATUS AGAINST HEPATITIS B VIRUS IN BURKINA FASO

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The health workers are among the groups most at risk for infection with hepatitis B. Our study assessed the immunization status against hepatitis B infection among healthcare workers of two health districts of Burkina Faso. We conducted a cross-sectional survey using a self administered questionnaire followed by blood sampling of health workers during August and September 2010. The blood samples were analyzed in the IRSS laboratory to search for anti-HBs antibody. On a total of 462 health workers interviewed, only 59.5% had an immunization card, 47.7% reported receiving at least one dose of HBV vaccine, and only 15.1% had been properly vaccinated (three doses of vaccine according the vaccination schedule). Our results show a variation of the vaccination status according
to sex, age, occupational category and seniority in the profession. The search for anti-HBs antibody (biological markers of immunity against HBV) has shown that vaccinees were significantly better protected (p = 0.01) against HBV infection than those who reported never having been vaccinated (76.7% against 58%). In conclusion, results demonstrate the shortcomings of the infection prevention of occupational health in Burkina Faso. It would be desirable to define strategies that can help strengthening the prevention through routine vaccination of all workers in the health profession.

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VIRTUAL EXPERT PANELS: BRIDGING COMMUNITIES TO PROMOTE EXCHANGE IN GLOBAL HEALTH DELIVERY

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GHDonline.org Expert Panels were designed to foster knowledge exchange among multiple disciplines in an asynchronous online conference. Panels capture practical knowledge and bridge disciplines and professional groups to generate new strategies in delivering health care. Traditionally venues for professional public health exchange have included academic conferences, published literature, and other colloquia. Until recently, few alternatives existed. For global health professionals, the need for a web-based, no-fee system was paramount. In 2008, the Global Health Delivery Project at Harvard launched GHDonline.org, a virtual platform that now hosts nine public and 51 private communities for over 6,500 health professionals. Members share resources, recommendations, and experiences on diverse topics from how to scale male circumcision for HIV prevention to ventilation design in TB clinics. Each month, GHDonline hosts “Expert Panels,” virtual, asynchronous conferences led by experts that users may read and respond to during a two-week window. Given the electronic nature of the conference, GHDonline can track page views, downloads, member contributions, and other statistics. A recent panel, “Strengthening Health Systems - The Role of NGOs,” was moderated by leaders including Dr. Agnes Binagwaho, Rwanda’s Minister of Health. Over 695 participants from 87 countries representing 472 organizations joined the panel, exchanging 124 commentaries and 23 resources. The data and content from this panel, and all panels, are published in Discussion Briefs, available to all GHDonline users. Expert Panels are an innovative alternative content from this panel, and all panels, are published in Discussion Briefs, available to all GHDonline users. Expert Panels are an innovative alternative to traditional conferences for generating robust discussion, connecting diverse practitioners, and uncovering knowledge from the field. The GHDonline Expert Panel model is transforming the availability and depth of exchange among health professionals, especially those working in remote, resource-limited settings. Insights, perspectives, and experiences shared among experts, at no cost, contribute significantly to the emerging knowledge base in the field of global health delivery.

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KEUR SOCE HEALTH AND DEMOGRAPHIC SURVEILLANCE SITE SYSTEM IN SENEGAL: SITE DESCRIPTION, BASELINE FINDINGS AND POLICY IMPLICATION

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The objective of this study was to analyze baseline results from first phase Demographic and Health Surveillance in Keur Soce Subdistrict, Senegal. To compare results with national and international data and comment on their relevance to health development. Multi-round prospective community based study, Initial Census 2010. Keur Soce is located in rural areas in the region of Kaolack, in the district of Ndéliendi. The area lies between longitudes 16°00'14.8" and 16°07'13" W and latitudes 13°51'53" and 14°00'00" N. It is located at 230 km from Dakar in the Sudano-Sahelian region of Senegal and covers an area of 478 sq. km. The estimated population is 29,645 inhabitants and composed mostly of Wolof (90%) and lives mainly on agriculture and livestock. This population is distributed in 73 villages with an average density of 62.7 inhabitants/ km². Almost all of the area is not electrified, running water (from deep wells) is available in just over half the area, otherwise the water comes from traditional wells. The climate is characterized by the alternation of a long and dry season from November to June and a short rainy season from July to October. The area has 2 health post and 09 functional health huts. Baseline description of each resident including age, sex, marital status, relationship with HH, education. A full demographic profile was given. The total population is 29,645 inhabitants. Forty two were under 15 years of age. The sex ratio is more pronounced for male than female regarding all age categories, except for the reproductive age group. Over 50% of the population are not married. Thus, the married monogamous represent 20% of the population and married polygamous represent 18% of the population. A small proportion of the population

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PRELIMINARY RESULTS OF A SYSTEMATIC REVIEW OF THE EFFECTIVENESS AND COSTS OF STRATEGIES TO IMPROVE HEALTH WORKER PERFORMANCE IN LOW- AND MIDDLE-INCOME COUNTRIES (LMICS)

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Health workers (HWs) play key roles in improving quality and coverage of health interventions. In LMICs, however, HW performance is often inadequate. Existing reviews of strategies to improve performance are outdated or have important methodological limitations. To characterize the effectiveness and costs of strategies to improve HW performance in LMICs, we conducted a systematic review of 15 electronic databases, 29 document inventories of international organizations, and bibliographies of 510 articles. We focused on studies with methodologically “adequate” designs (eg, trials with comparison groups). After screening, data from relevant reports were double-abstracted and entered into a database. Effect sizes were estimated as absolute changes in performance outcomes. Outcomes included HW practices, patient outcomes, and economic measures. As studies often used different outcomes, we calculated a summary measure: the median effect size (MES) for all primary outcomes from a study. We screened >105,000 citations, and 841 reports met our inclusion criteria. Numerous performance improvement strategies have been studied, usually with multiple components. Most strategies had small MES (<10 percentage-points [%-points]), although some had large effects (>25 %-points). Among eight mutually exclusive strategy groups, MES for most (e.g., training + job aids, community activities) were similar (median MES: 7-11 %-points). Job aids alone seemed less effective (median MES = 2 %-points) and strategies that provided commodities seemed more effective (median MES = 17 %-points). Contextual and methodological heterogeneity made comparisons difficult. Preliminary results suggest that the effectiveness of strategies to improve performance varies substantially, with many strategies having small effect sizes. Standardization of methods would facilitate efforts to synthesize the evidence. Additional analyses will identify factors associated with increased effectiveness. Results from this review will inform recommendations on how best to improve HW performance in LMICs.
THE ECONOMIC IMPACT AND BURDEN OF DENGUE ILLNESS IN NICARAGUA

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Over the past two decades, the number of cases and burden of dengue has steadily risen in Nicaragua, though there have been comparatively few efforts to quantify the economic cost and burden [measured in disability-adjusted life years (DALYs)] of dengue to society. In this study, we utilize source data from the Nicaraguan Ministry of Health (MINSA) to estimate the cost and burden of dengue illness from 1996-2010, including both epidemic and endemic seasons of illness. Costs incorporated both direct costs, which included medical expenditures, prevention campaigns, and vector control costs, and indirect costs, which stemmed from lost productivity secondary to illness. Expansion factors were utilized to account for the large portions of underreported and primarily asymptomatic cases. Monte-Carlo simulations and probabilistic sensitivity analyses were conducted on key parameters in the DALY and costs calculations using primary data from MINSA and other previously published literature values. From 1996-2010, the annual burden of disease ranged from 99-805 DALYs per million, with a mean of 347 DALYs per million, and a majority resulting from classic dengue fever (DF). The total cost of dengue illness ranged from US $5.91-27.6 million per year, with the cost per case ranging from US $125-273, resulting in a per capita cost of US $0.97-5.44 over this study period. This analysis will be important for re-assessment of scarce resources for dengue control in Nicaragua and Latin America, as well as for determining cost-effectiveness of novel vaccine candidates and other therapeutics. Such a comprehensive analytic approach can be easily applied to dengue and similar illnesses in the region to yield a more complete picture of the combined costs of disease to the nation.

RESEARCH CAPACITY BUILDING IN INDIA: LESSONS LEARNED IN NETWORK COORDINATION, RESEARCH CAPACITY BUILDING AND RESEARCH WITH THE INDOX RESEARCH NETWORK

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INDOX is an academic partnership between Oxford University and eleven of the top cancer centres in India. We work under three domains of activity: network collaboration, research capacity building, and research. Network coordination is administered by a number of permanent staff and through a system of internal governance, annual network meetings, and weekly teleconferences based around 7 cancer site specialty groups made up of specialists from across all the INDOX centres to focus on the specific cancers that are more common in India. Our research capacity building work targets the creation of a network of investigators with sufficient training to conduct multicenter trials. We facilitate research opportunities and award training fellowships to scientists and clinicians from India. Over 100 members of the Network have been awarded fellowships and have attended training courses in Oxford and India. The scheme has covered several areas of clinical research including: early phase trials, protocol design, randomised controlled trials, medical statistics, and good clinical practice. Our research focus is on identifying and answering those questions which address local priorities and in trialling solutions which can be applied locally. As such we are currently conducting a case-control study to investigate the risk factors for common cancers in the India. This study is being conducted across all centres and is the biggest study to date of risk factors associated with cancer in India. Two sections of this study, in breast and colorectal cancers, have already begun and are expected to be complete in two years. The researchers will recruit a 10,000 people newly diagnosed with these two cancers, and a further 10,000 people as healthy controls. As we endeavour to exploit priorities determined from analysis of the epidemiology we have coordinated the centres to participate in investigator initiated and sponsor initiated studies. At each site there is a dedicated INDOX Site Principal Investigator and Site Coordinator, who together form the core of the network. Through the process we have sought to mediate the spend of pharmaceutical companies on trials in India and ensure that trials are aligned with local needs; designed with local ethical knowledge; and executed in such a way that capacity is built in India. We report here on the lessons learnt and the barriers encountered in delivering our tripartite mission in the Indian context.
EVALUATION OF ANTIBODY RESPONSE AGAINST GLOSSINA SALIVA IN CATTLE: A SUPPLEMENTARY OR ALTERNATIVE APPROACH TO ASSESS EXPOSURE OF TSETSE BITES

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Our study proposes a new strategy, alternative or complementary to the entomological methods based on trapping tsetse flies, to target zones at risk and evaluate tsetse flies control programs in animal African trypanosomiasis. It aims to develop a sero-epidemiological tool to assess cattle exposure to tsetse bites. IgG responses against Glossina saliva was assessed by ELISA on bovine that were experimentally exposed to tsetse flies and other bloodsucking arthropods in order to detect the cross-reactivities between Glossina spp saliva and these arthropods saliva. Only the saliva of Tabanidae spp has cross-reacted with Glossina spp saliva. In any case, antibody (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 the Ab response to tsetse saliva. Immunoproteomic screening followed by mass spectrometry and bioinformatics tools using has permitted to identify three peptides whose two of Tsal1 (Tsetse salivary gland protein1) and one of Tsal2 (Tsetse salivary gland protein2). These peptides will be produced and validated on bovine serum of CIRDES and PATTEC-Burkina (Pan African Tsetse and Trypanosomiasis Eradication Campaign) in order to develop an easy and reproducible test with higher specificity for the evaluation of Glossina exposure.

LYMPHATIC FILARIASIS MID-TERM IMPACT ASSESSMENT FOLLOWING THREE EFFECTIVE ROUNDS OF MASS DRUG ADMINISTRATION IN SIERRA LEONE

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Lymphatic filariasis (LF) mapping in 2005 using Immunochromatographic Test cards indicated that all 14 health districts (HDs) of Sierra Leone were endemic (prevalence >1%) and needed treatment for LF: 2 had low (1%-4.999%), 1 moderate (5%-9.999%) and 11 high (≥10%) prevalence. Baseline microfilaraemia (mf) studies using the thick blood film method performed in 26 sentinel sites in 2012, 9 months after the third round of praziquantel (PZQ) administration. Overall prevalence was 15.2% (95% CI: 13.3-17.3%) and arithmetic mean intensity of infection was 642 (49.9%) and female 644 (50.1%). Overall prevalence was 15.2% (95% CI: 13.3-17.3%) and arithmetic mean intensity of infection was 12389 (95% CI: 105.5-152.979pg). Compared with the baseline data collected in 2008-9, it showed a significant overall reduction of 66.3% in prevalence and 51.7% in intensity of infection (p<0.0001). In seven districts, the prevalence ranged from 0.5% (95% CI: 0.0-2.8%) in Bo to 36.0% (95% CI: 26.6-46.2) in Koinadugu. Overall 1.2% of SAC were heavily parasitized (≥400egpg) and 3.3% were moderately parasitized (100-399egpg), a significant reduction from 8.8% and 18.2% respectively. Twelve sentinel sites were highly endemic in 2008-9 and only 2 sites, Bumbuna in Tonkolili and Sinkunia, in Koinadugu were still highly endemic in 2012. The results suggest that there had been a significant reduction in S. mansoni endemicity level across Sierra Leone following 3 rounds of MDA. Effective MDA required planning and coordination at national, district and chieftain levels, trained and motivated HWs, informed and cooperative communities and their leaders, and monitoring, funding and drug supplies to treat the common side effects experienced by heavily parasitized individuals. Continued targeted MDA is required to achieve the national objective of schistosomiasis control. Maintenance of long term control will require surveillance, education and improved water and sanitation facilities.
DEVELOPING, MONITORING AND EVALUATING CAPACITY DEVELOPMENT OF CENTRE FOR NEGLECTED TROPICAL DISEASES (CNTD) SUPPORTED LABORATORIES AND STAFF MEMBERS IN ENDEMIC COUNTRIES

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Impact assessment of interventions against neglected tropical diseases in resource poor settings, requires good technical support through diagnostic laboratories manned by highly skilled technical staff. Many countries in Africa and Asia, endemic for NTDs (Neglected Tropical Diseases), lack the technical capacity and laboratory facilities that will facilitate good practices in impact assessment. To alleviate this problem, Centre for Neglected Tropical Diseases (CNTD) in 2009 embarked on an initiative to strengthen five regional laboratories to support national NTD intervention programmes and to reinforce the capacity of NTD endemic countries staff by training 10 members of staff through PhD studies on epidemiology and integrated control of lymphatic filariasis (LF) in countries of very low capacity, in Africa and Southeast Asia. The main goal of this capacity building effort is to ensure national programmes were able to demonstrate value for money for the funding provided by DFID to eliminate LF as a public health problem. To assist with this mission, CNTD has requested support from LSTM’s Capacity Development Impact Research (CDIR) unit to design, monitor and evaluate capacity development of the five laboratories (Ghana, Kenya, Malawi, Sierra Leone and Sri Lanka) and the research activities of the recruited PhD students in line with the activities and outputs specified in the project logframe. Each laboratory and in-country NTD staff will be at different stages of capacity development and they will progress at different rates so each must be monitored separately using both qualitative and quantitative indicators. Capacity development plans will incorporate activities at the levels of individuals, laboratories and national/international context and the plans and indicators will be developed and agreed with all key stakeholders. Although it is anticipated that there will be a need for ongoing inputs to capacity development of laboratories and training of NTD staff members in endemic at all levels, this project will cover the period 1st April 2012 - 31st March 2015.

IMPROVING NEGLECTED TROPICAL DISEASE (NTD) CONTROL OUTCOMES THROUGH NORTH-SOUTH GLOBAL HEALTH PARTNERSHIPS

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In Tanzania, over 45 million people are at risk of infection with 2 or more of the 5 Preventive Chemotherapy (PCT)-targeted Neglected Tropical Diseases (NTDs) which include Onchocerciasis, Lymphatic Filariasis, Soil Transmitted Helminthiasis, Trachoma and Schistosomiasis. Recognizing the need for increased cost-effectiveness in a resource limited environment, the Ministry of Health and Social Welfare has adopted an integrated approach to NTD control with support from various global organizations and NGOs. In 2009, the program targeted 26 out of 132 districts for integrated Mass Drug Administration (MDA) and in 2010-2011, with support from USAID through RTI/IMA, an additional 44 districts were targeted. In 2012, with increased commitment from multiple partners like USAID/RTI/IMA, DFID, SCI and CNTD, 23 additional districts will be targeted, taking the total coverage to 93 out of 132 districts.

With support from USAID, WHO, APOC, RTI and IMA, the number of treatments distributed has increased from 15.4 million in 2009 to 25 million in 2011. These valuable north-south partnerships provide support to local governments and communities to conduct MDA through training, advocacy and supportive supervision. In the 3 years that the integrated approach has been used, MDA trainings have been provided to 115,966 community drug distributors, 23,985 teachers and 11,259 frontline health workers. USAID support has allowed for the allocation of 3 IMA staff with specialized skills to the national NTD secretariat, thereby contributing to capacity building at the national level. To address the shortage of human resources at the national secretariat, IMA staff work jointly with Ministry staff to provide guidance and supervision at all levels of MDA. The various north-south partnerships that have been successfully established are helping to ensure that the NTD program is on the road to national coverage in Tanzania. Establishing similar partnerships in countries where they do not yet exist can further contribute to the control and elimination of NTDs globally.

DEVELOPMENT AND EVALUATION OF A RAPID DIAGNOSTIC TEST TO SUPPORT ONCHOCERCIASIS CONTROL AND ELIMINATION PROGRAMS

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Onchocerciasis, or river blindness, is a major cause of preventable blindness around the world. Caused by parasitic worms (Onchocerca volvulus) transmitted to humans through the bite of the blackfly, onchocerciasis typically affects poor, rural communities near fast-flowing streams and rivers. Mass administration campaigns of the microfilaricide drug ivermectin have significantly reduced the burden of the disease in many mesoendemic and hyperendemic regions to the extent that elimination has become a possibility. Assessment and monitoring of elimination requires rapid diagnostic tests that are sensitive and highly specific for exposure to O. volvulus. (Ov). We describe a new rapid diagnostic test (RDT) that detects IgG4 antibodies specific to a previously validated Ov antigen Ov16. To facilitate confirmatory reading of the results and thus quality control of epidemiological survey results, test results must be stable for a long period of time (>24 hours). We describe a novel approach to ensure test result stability on a lateral flow format. Performance data for this test based on panels of well-characterised Ov-infected sera and control sera (including other non-Ov filarial infections) demonstrate that the Ov16-based RDT performs equal to or better than Ov16-based ELISAs, can be used within whole blood, and has the design properties required (in terms of environmental temperature, humidity exposure profiles, and potential cost) by stakeholders in Ov-endemic regions of Africa and the Americas.

PITFALLS AND OPPORTUNITIES IN CONTROLLING CO-INFECTIONS

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There is increasing momentum in public health and the veterinary sciences towards a model of integration whereby multiple pathogens are targeted simultaneously. Little is known, however, about the epidemiology of co-infections and strategy for their control is nascent. Using gastro-intestinal nematodes as an example, we construct an epidemiological model that is used to simulate the between-parasite species interactions reported in published field studies and animal models. Many previous studies of nematode co-infections have attempted to infer species interactions based upon infection prevalence data and we demonstrate how this can give rise to spurious results. Uncovering the true nature of between-species interactions is critical in informing control and this is exemplified by the phenomena we describe as ‘slaving’ and ‘release’. ‘Slaving’ refers to the
tethering of synergistic pathogens co-circulating in a host population. In the situation whereby no effective chemotherapy is available for a particular pathogen, its control can still be achieved by targeting its co-circulating synergest. ‘Release’ refers to the inadvertent increase in a pathogen’s prevalence resulting from the control of a co-circulating antagonist. The success of integrated control programs, therefore, not only rests in the efficacy and spectrum of the available chemotherapeutics, but also in the interactions of the extant pathogen community.

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CONTROL OF NEGLECTED TROPICAL DISEASES IN POST-CONFLICT COUNTRIES IN AFRICA: CHALLENGES FOR LYMPHATIC Filarialis ELLIMINATION IN LIBERIA

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More than half of the 32 Lymphatic filariasis (LF) endemic countries in Africa are yet to implement mass drug administration (MDA) for the elimination of the disease, 11 years after the Global Programme to Elimination LF (GPELF) was launched in 2000. Majority of the countries that have not started MDA are post-conflict countries like Liberia which has a fragile health system in a resource poor setting recovering from the ravages of war. LF is endemic in 13 out of the 15 counties in Liberia with prevalence of infection ranging from 1-46%. Recent efforts to initiate MDA for the elimination of LF in the country have revealed enormous challenges. Planning for LF elimination on the platform for the integrated control of neglected tropical diseases (NTD) required inter-sectoral collaborations that did not exist. There has been strong resistance to the incorporation of vertical programmes for other NTDs, like onchocerciasis and soil transmitted helminths (STH), into a single integrated national NTD control programme. Liberia also faces logistical challenges for MDA implementation. The 14 year war destroyed technical capacity and physical infrastructure on a massive scale. Nevertheless significant progress has been made to ensure a successful launch of MDA implementation for LF elimination in 2012 using the CDI strategy. A massive social mobilization campaign is planned for hard to reach communities to sensitize them and encourage volunteers to serve as community drug distributors. In this presentation, the baseline data about the benefits of MDA and encourage volunteers to serve as

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HOW EFFECTIVE IS SCHOOL-BASED DEWORMING FOR THE COMMUNITY WIDE CONTROL OF SOIL TRANSMITTED HELMINTHS?

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The recent London Declaration on neglected tropical diseases was based in part on a new roadmap from WHO to “sustain, expand and extend drug access programmes to ensure the necessary supply of drugs and other interventions to help control by 2020”. Drug donations from the pharmaceutical industry form the backbone to this aim, especially for helmint infections. The increased availability of funds to control soil transmitted helminths (STHs) raises the question of how best to use these resources, given that treatment must be administered repeatedly in endemic areas in the absence of improvements in water and sanitation.

Deworming for STHs is often targeted at school children because they are at greatest risk of morbidity and because school-based deworming is remarkably cost-effective. However, the impact of school-based deworming on overall transmission in the wider community remains unclear. We examine the effect on transmission by estimating the proportion of parasites targeted by school-based deworming. We use methods derived from the description of the transmission dynamics of the worms, demography and school enrolment, and data from a small number of example settings where age-specific intensity of infection (either worms or eggs) has been measured for all ages. In these settings <30% of the population are 5-15 years old. Combining this demography with the age-intensity profile we estimate that in one setting school children output as little as 10% of hookworm eggs whereas in another setting they harbour up to 50% of ascaris worms (the highest proportion of parasites for our examples). In addition, it is estimated that from 40-70% of these children are enrolled at school. Thus, whilst school-based programmes have many important benefits, the proportion of infective stages targeted by school-based deworming may be limited, particularly where hookworm predominates. We discuss the consequences for transmission for a range of scenarios, including when infective stages deposited by children are more likely to contribute to transmission than those from adults.

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THE BENEFITS OF USING MOBILE PHONES IN MONITORING HEALTH INTERVENTIONS: THE PERSPECTIVE FROM THE NEGLECTED TROPICAL DISEASES CONTROL IN TANZANIA

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Tanzania’s health care system is overwhelmed with huge volumes of clients seeking care and served with a handful of qualified staff. This East African nation is ravaged by non-infections and infectious diseases including the Neglected tropical diseases like- lymphatic Filariasis, soil transmitted helminthiasis, Schistosomiasis Onchocerciasis and trachoma, most of which are nonexistent in the developed world. Inefficient service delivery mechanisms resulting from poor record keeping and reporting mechanisms further hamper proper planning and decision-making. The Tanzania Neglected Tropical Diseases (NTD) control program has successfully piloted Mass Drug Distribution (MDA) to over 9,000 at risk population using mobile phone technology synergized with web and desktop applications. Forty (40) community drug distributors (CDDs) were trained and equipped to use mobile phones to conduct house-to-house census, and later distributeivermectin and Albendazole to eligible population. The exercise run parallel with the existing/houting paper based census, drug distribution and reporting mechanism. The CDDs were able to quickly acclimatize to QWERTY mobile phone keyboards, learned the mobile application and conducted the census while uploading the data in real time -via internet– to the central server. With the data in time, the district, regional and national office could calculate drug need and allocate supplied accordingly. Mass drug administration was conducted with coverage report live updates in the central server and via the web. This allowed early intervention decision-making by relevant authorities. Mobile phones provide user-friendly, timely and efficient mechanisms to monitor and evaluate Neglected tropical diseases control activities-e.g. mass drug administration- at the village and sub-village level. In resource-limited setting, they provide a viable solution to data collection and reporting of health interventions programs. The Tanzania experience could be shared in the developing world!
PATENT EXTENSION: IMPROVING THE FDA’S NEGLECTED TROPICAL DISEASES REVIEW VOUCHER

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A Neglected Tropical Disease (NTD) is a condition that, despite its frequency is not necessarily low, has been for different reasons, especially for affecting the poorest people of the world, submitted to the ostracism of low investment to find better therapeutic options. The burden of disease coming from the NTDs is really high and is very close to other very prevalent conditions in terms of disability-adjusted life years (DALYs). But, none less important is the burden of annual losses on productivity within the low-income countries affected by NTDs. NTDs are not only a health problem, they are also an economic and social problem that is delaying the economies of these countries and why not, the whole world. Therefore, it is important to find the best way to stimulate the funding in the NTDs research arena. Unfortunately, it seems to be that not only good intentions are enough in order to obtain the funds to shorten the pipeline to find new compounds for these diseases. In 2006 a group of academics proposed what today we know as the FDA’s NTDs Voucher. The idea is quite simple; if a pharmaceutical company succeeds getting the approval from FDA of a compound for one of the NTDs, this company will obtain a Priority Review Voucher (PRV). This means that the time it takes FDA, within the Fast Track Program (FTP), to review a new drug application is reduced. The goal for completing a Priority Review is six months. This is supposed to be a tool that can be very useful to put new compounds for NTDs into the market but the outcomes so far are not like they were expected at the beginning. An improved version of the voucher to stimulate the development of drugs for NTDs is proposed. The idea is based on granting a Patent Extension Voucher (PEV) for the companies that achieve in marketing an NTD compound, but taking into account the possibility of second use compounds for NTDs, a demonstrated effectiveness, impact on the targeted NTD and the current advantages of the FDA’s PRV. Finally, a way to calculate the value of the proposed PEV is explained.

DYNAMICS OF HELMINTH INFECTIONS IN EASTERN INDONESIA DURING AND THREE YEARS FOLLOWING A MASS DRUG ADMINISTRATION CAMPAIGN TO ELIMINATE LYMPHATIC FILARIASIS

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The lymphatic filarial parasite Brugia timori occurs only in eastern Indonesia, where it causes high morbidity. We evaluated the effect of mass drug administration (MDA) with albendazole and diethylcarbamazine in a sentinel, B. timori endemic, village on Alor Island in annual surveys over a period of 10 years. Prior to the first round of MDA, the microfilaria (MF) prevalence was 26%, and 80% of the residents had filaria-specific IgG4 antibodies as determined by the Brugia Rapid test. In 2010, 34 months after the 6th and final round of MDA, the MF rate had dropped to 0.2%, and the antibody rate had decreased to 6.4%. The MDA campaign also had a beneficial effect on STH infections. Pre-MDA prevalence rates for Ascaris, hookworm and Trichuris were 34, 28, and 11%, respectively; these rates were the lowest after the 5th round of MDA with 27, 4, and 2%. Unfortunately, STH rates increased after cessation of MDA and were close to the pre-MDA rates 34 months after the 6th round of MDA. However, intensities of STH infections were still lower than baseline levels, and no heavy infections were detected. This study showed that MDA with DEC/albendazole has had a major impact on B. timori MF and IgG4 antibody rates, and it provides a proof of principle that elimination should be feasible. Our results also documented the value of annual DEC/albendazole as a mass de-worming intervention and emphasize the need for continued STH intervention after cessation of MDA for lymphatic filariasis.

DISCOVERY OF ANTIGENS FOR DIAGNOSIS OF SOIL-TRANSMITTED HELMINTH INFECTIONS

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The human soil-transmitted helminths whipworm (Trichuris trichiura), Ascarids (Ascaris lumbricoides), and hookworms (Necator and Ancylostoma) create a substantial burden for worldwide public health, with an estimated one-third of the world’s population infected with one or more of these nematode parasites. The current global strategy to control infections with intestinal nematodes involves mass drug administration of anthelmintic medicines without prior diagnosis. However, cure is often not complete, and the limited variety of available drugs has fueled concerns of parasite resistance. The most widely-used diagnostic method is the microscopic detection of parasite eggs, a labor-intensive technique with inadequate sensitivity and specificity. Therefore a rapid, sensitive, specific, and inexpensive method to detect parasitic worm infections without laboratory infrastructure or trained personnel would offer enormous advantages over current protocols. Using closely-related veterinary parasites, informatic and immunological research efforts have provided strong proof-of-concept that specific and sensitive detection of parasite antigens by ELISA and lateral flow assays is achievable. Controlled timecourse infections in canines show that pre-patent infections are detected and the antigens are quickly diminished following effective anthelmintic treatment. Building upon these data, studies are in progress to clone, express, and purify nematode targets from human parasites. Preliminary results demonstrate that antisera specific to A. lumbricoides antigens specifically recognize infected samples with a high level of sensitivity.

IMPLEMENTING PROGRAMS FOR LYMPHATIC FILARIASIS IN ONCHOCERCIASIS ENDEMIC COMMUNITIES: CHALLENGES TO BASELINE MEASUREMENTS IN ETHIOPIA

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Current (2011) WHO guidelines for launching a lymphatic filariasis (LF) program call first for mapping for the disease using the immunochromatographic card tests (ICT) in areas suspected to be endemic for W. bancrofti. In areas where ICT rates are >1%, sentinel sites are selected for monitoring impact of annual mass drug administration on prevalence of the disease. When microfilaremia in the sentinel sites are <1% (generally by year 5 of MDA), then a Treatment Assessment Survey (TAS) to determine if transmission has been interrupted and MDA can cease. In Ethiopia in 2007, mapping for LF using ICT found the disease to be co-endemic with onchocerciasis in three zones: Bench Maji, North Gonder, and Metekel. MDA with IVM has been ongoing in these Zones since 2004. In 2011, six sentinel sites (two each zone) were selected based on having populations of ≥500 and the highest ICT positivity (range 21%-65%) and baseline testing for MF was conducted. The total

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population in the six sites was 5,337 of which 2,748 were tested for Mf. Results found only 1 mf positive person in Bench Maji (0.11%), 3 in North Gondar (0.17%), and 2 in Metekel (0.12%), all of which are below the 1% threshold expected to be found after 5 years of MDA. Ivermectin is a known microfilaricidal and its historic use for onchocerciasis in these zones has likely caused the LF Mf to fall below the 1% threshold. LF prevalence, however, is a poor indicator for adult W. bancrofti worms and therefore cannot reliably be used to determine whether the adult worm population is still viable. For this reason, and because of the relatively low cost of adding ALB to the existing (VM MDA for onchocerciasis, we have decided to continue with MDA for LF (by adding albendazole to ivermectin) according to the WHO recommended strategy.

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**ATORVASTATIN AND ARTEMETHER COMBINATION THERAPY REDUCES INFLAMMATION AND IMPROVES RECOVERY OF MICE WITH LATE-STAGE EXPERIMENTAL CEREBRAL MALARIA**

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*Plasmodium falciparum* infection can cause a diffuse encephalopathy known as cerebral malaria (CM), a major contributor to malaria associated mortality. Despite appropriate anti-malaria treatment using quinine or artemisinin derivatives, CM mortalities may be as high as 30% while 25% of survivors experience neurological complications. Thus, adjunctive therapies are urgently needed to prevent or reduce such mortalities. A number of clinical trials involving potential adjunctive therapies for CM have not proven beneficial and some interventions have been deleterious stressing the need for better understanding of CM pathogenesis and development of effective therapies. Chemokines and cytokines have been implicated in the development of CM and CM associated mortalities. Interferon γ induced protein 10 (CXCL10) was recently found to be associated with fatal human CM in field studies in India and Ghana and linked to severity of other infectious diseases. Mice deficient in CXCL10 gene were partially protected against experimental cerebral malaria (ECM) mortality when infected with *P. berghei* ANKA indicating the importance of CXCL10 in the development of CM. We tested the hypothesis that utilizing synthetic products that reduce or neutralize the excessive production of CXCL10 during CM pathogenesis will increase survival and reduce mortality. Atorvastatin is a widely used synthetic drug that lowers cholesterol levels in blood by blocking the enzyme HMG-CoA reductase and has been shown to specifically reduce plasma CXCL10 levels. We determined the effects of atorvastatin/artemether combination therapy on CM outcome in ECM. We assessed immune determinants of severity of CM, survival, and parasitemia in mice receiving the combination therapy from day 6 to 9 post-infection in infected mice and compared the results with controls. The results showed that treatment with atorvastatin significantly reduced systemic inflammation (lower IL-1α, IL-6, IL-17, CXCL4, CXCL11, and IL-2), reduced potent anti-angiogenic factor CXCL10 and increased angiogenic factor VEGF production. Treatment of the late-stages of ECM in mice with a combination of atorvastatin and artemether improved survival (100%) over treatment with artemether monotherapy (70%), p<0.05. Thus, adjunctively reducing CXCL10 and enhancing VEGF levels by atorvastatin during anti-malarial therapy may represent a novel approach to treating CM patients in the future.

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**ANTIMALARIAL PRESCRIPTION PRACTICES IN THREE PUBLIC HOSPITALS LOCATED IN AREAS OF VARYING ENDEMICITY IN UGANDA**

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In Uganda antimalarials are often prescribed when malaria is unlikely, a problem that is becoming critical following the adaptation of effective but expensive ACTs as the 1st line treatment for uncomplicated malaria. Less known is the extent of irrational use of anti-malarials among hospitalized patients. We present data on anti-malarial prescription practices among hospitalized children with microscopy results. As part of an inpatient malaria surveillance program data was collected from three public hospitals in Uganda: Tororo (high transmission), Jinja (medium transmission) and Kambuga (low transmission). At each site, a standardized case record form was used to collect individual patient level data, including medicines prescribed during hospitalization. Between Jan to Dec, 15748 children were hospitalized in all three hospitals; Tororo (4949), Jinja (9202) and Kambuga (1597). Over 97% of patients had a thick blood smear performed. Proportion of hospitalized children with a positive blood smear was 60% in Tororo, 47% in Jinja and 34% in Kambuga. Of children with negative blood smear, 280 (14%) in Tororo, 3003 (63%) in Jinja and 683 (66%) were prescribed an antimalarial. Quinine was the most commonly prescribed anti-malarial among children with positive (94%) and negative (84%) test results. Among children prescribed an anti-malarial, the unadjusted odds of death was higher among those with negative results as compare to those with positive results (OR1.65 95%CI 1.25-2.17, P <0.001). When underlying diagnosis, severity of illness, age and antibiotic use were adjusted for in logistic regression, no significant difference in the odds of death was noted between the two groups. Much as there has been improvement in the proportion of children tested for malaria at the sites, prescription of anti-malarials to patients with negative malaria test results remains unacceptably high at two of the sites. With no clear benefit of this practice there is an urgent need to better understand reasons why clinicians continue to treat patients for malaria even when test results are negative.

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**NAMIBIAN MEDICINAL PLANT EXTRACTS AND THEIR MECHANISM OF ACTION AGAINST PLASMODIUM FALCIPARUM IN AN IN VITRO MODEL**

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New medicines for malaria are urgently needed, especially in developing countries where malaria is endemic. Malaria treatment depends strongly on traditional medicine as a source for inexpensive treatment of the disease in these countries. In Namibia, malaria is on the decline and the country is moving towards pre-elimination of the disease. However, some communities preferring traditional medicines and not accepting allopathic medicine may prevent elimination. Ethnomedicines need to be integrated into mainstream malaria case management to achieve malaria elimination by 2020. To do so, they need to be scientifically validated to allow for their safe and effective use. In this study, extracts from indigenous medicinal plants *Vahlia capensis*, *Nicolasia costata*, and *Dicerocarym*...
MALARIA CHEMOPREVENTION IN A HIGH TRANSMISSION SETTING: A RANDOMIZED CONTROLLED TRIAL OF MONTHLY DIHYDROARTESMISININ-PIPERAQUINE VERSUS MONTHLY SULFADOXINE-PYRIMETHAMINE VERSUS DAILY TRIMETHOPRIM-SULFAMETHOXAZOLE VERSUS NO THERAPY FOR THE PREVENTION OF MALARIA

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The burden of malaria remains high for infants in some parts of Africa despite the use of insecticide treated bednets (ITNs). Chemoprevention offers a potential means of reducing the malaria burden in infants, however, optimal drug and dosing strategies are unclear in areas were transmission occurs throughout the year and antifolate resistance is high. A cohort of infants aged 4-5 months were enrolled using convenience sampling in Tororo, Uganda, a rural area with perennial high transmission intensity. Infants received an ITN at enrollment and were followed for all their health care needs 7 days/week. At 6 months of age, infants were randomized using an open label study design to one of four treatment arms: no therapy, monthly sulfadoxine-pyrimethamine (SP), daily trimethoprim-sulfamethoxazole (TS), or monthly dihydroartemisinin-piperazine (DP). Study drugs were self-administered at home and continued until the infants reach 24 months of age. The primary end point was the incidence of malaria using passive surveillance between 6-24 months of age or early study termination. Malaria incidence was compared using a negative binomial regression model with measures of association expressed as the protective efficacy (PE=1-incidence rate ratio). Preliminary results are presented here. Of 400 infants enrolled, 393 were randomized to therapy of which; 38 were withdrawn before 24 months of age, 277 are actively being followed and 78 have reached 24 months of age. The incidence of malaria is 5.69 episodes per person year (PPY) among those randomized to no therapy; 5.47 episodes PPY among those randomized to monthly SP (PE=7%, 95% CI 17-26%); 4.32 episodes PPY among those randomized to daily TS (PE=26%, 95% CI 7-42%); and 2.32 episodes PPY among those randomized to monthly DP (PE=60%, 95% CI 48-68%). Preliminary results suggest that monthly SP is not effective at preventing malaria, daily TS is associated with only modest protective efficacy, and monthly DP is the most effective regimen. Final results will be available after Sept. 2012 when all infants reach 24 months of age.

CLINICAL EFFICACY AND SAFETY OF ARTESUNATE-AMODIAQUINE AND ARTEMETHER LUMEFANTRINE FOR THE TREATMENT OF UNCOMPlicated MALARIA AND PREVALENCE OF DRUG RESISTANCE MARKERS IN NGAOundere, North CAMEROON

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Cameron switched policy for treatment of uncomplicated malaria to amodiaquine-arteresunate (AS-AQ) and fixed dose artemether-lumefantrine (AL) respectively in 2004 against a backdrop of amodiaquine treatment failure. After four years of implementation of this drug policy, country-specific evidence-based data to support the continuous efficacy and safety profile of ACTs are still needed. This study was carried out in collaboration with the national malaria control program to generate data to support the continuous use of ACTs for malaria case management. A randomised open label trial was conducted between September and December 2007 at the Ngaoundere Protestant Hospital.
Ngaoundere, Cameroon. One hundred and fifty patients between six months to 14 years of age with uncomplicated malaria were randomized to receive standard doses either AS-AQ (73) or AL (77) and followed up for 28 days according to WHO 2003 protocol. Drug safety was evaluated using standard clinical and laboratory parameters and safety concerns classified according to the common toxicity criteria. Response was classified according to WHO and isolates were genotyped for the msp-2 gene to determine recrudescent parasites. Pre-treatment blood samples were used to determine the prevalence of resistant mutations in the pfCRT, pfmdr1, dhfr and dhps genes by sequencing. Ethical and administrative clearances were obtained from the National Ethics Committee and the Ministry of Public Health in Cameroon respectively. PCR-corrected cure rates were 100% for AL, and 96.4% for AS-AQ. The combinations were well tolerated clinically and biologically. By Day 14, the mean total bilirubin, creatinine and ALT values were slightly increased in subjects treated with AS-AQ. Changes in white cell counts and platelet count were significantly different (p<0.05) in the two drug groups, but were of no clinical significance. All side-effects were transient and therefore disappeared by the end of treatment. Both AS-AQ and AL are highly effective and well-tolerated for the treatment of uncomplicated malaria in Ngaoundere, Cameroon supporting their continuous use. High prevalence of mutant pfCRT and pfmdr1 alleles confirm long standing resistance to chloroquine among repeated infections. Our study suggests that North to South increase in high level CQ resistance and might compromise the use of AQ use in combination therapy. Long-term monitoring of safety and efficacy and molecular markers is however, highly solicited.

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SAFETY OF ARTEMISININS DURING EARLY PREGNANCY, ASSESSED IN 62 SUDANESE WOMEN

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Between June 2006 and October 2008, the safety of artemisinins during early human pregnancy was assessed in central-eastern Sudan. Pregnant women in the first or second trimester who were attending antenatal care clinics at the Wad Medani, Gadarif and New Halfa hospitals were interviewed. Each was asked if she had had malaria in the first trimester of the index pregnancy and, if so, what treatment they had received. The women who had received artemisinins were then followed-up until delivery and their babies were followed-up until they were 1-year-olds. Overall, 62 of the pregnant women reported receiving artemisinins - artesunate injections (48), artesunate plus sulfadoxine-pyrimethamine (11) or artemether plus lumefantrine (three) - during the first trimester. Medical records were available for 51 (82%) of these 62 women, and, in each case, these records showed the reported treatment and that malaria had been confirmed. Only nine (15%) of the 62 women given artemisinins had not known that they were pregnant when treated.

Two of the treated women (both given artesunate injections in the first trimester) had miscarriages, one at 20 weeks of gestation and the other at 22 weeks, each while receiving quinine infusions for a second attack of malaria. The other 60 women who had received artemisinins delivered apparently healthy babies at full term. No congenital malformations were detected, there was no preterm labor, no maternal deaths were recorded during the follow-up, and none of the babies died during their first year of life. It therefore appears that artemisinins may be safe to use during early pregnancy, although further study is clearly needed.

A MARKOV MODEL TO EVALUATE THE COST-EFFECTIVENESS OF DIHYDROARTESININ-PIPERAQUINE VS. ARTEMETHER-LUMEFANTRINE FOR FIRST-LINE TREATMENT OF UNCOMPROMICATED PLASMODIUM FALCIPARUM MALARIA IN AFRICAN CHILDREN

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Recent randomized multi-center trials showed that dihydroartesinin-piperaquine (DHAQ) is as efficacious as artemether-lumefantrine (AL) in treating uncomplicated malaria in African children in different endemicity settings, with comparable safety profiles. The study results also indicate that DHAQ has a longer post-treatment prophylaxis effect than AL, thus reducing the risk of re-infection following treatment and averting morbidity and mortality. The objective of our economic evaluation is to compare the health outcomes and costs of treatment with DHAQ or AL as first line therapy in children below six years of age with uncomplicated malaria, in view of the differing post-treatment prophylactic effect profiles of these two drugs. We developed a Markov model to simulate the effectiveness of the two treatment strategies in a hypothetical cohort of 1,000 children over a one-year period. Monte Carlo simulation is used to account for uncertainty in model parameters. The preliminary results of our model show that the estimated number of cases of acute malaria illness are 1545.9 (95%CI: 1543.3--1548.5) and 1716.4 (95%CI: 1713.4--1719.4) per 1,000 children over one year when treated with DHAQ or AL as first line therapy, respectively. The estimated number of severe malaria infections per 1,000 children are 25.3 (95%CI: 24.8--25.9) with DHAQ and 28.1 (95%CI 27.5--28.7) with AL treatment in a scenario.
where 90% of children with recurrent infections have access to early treatment for uncomplicated malaria. The number of deaths are estimated to decline from 17.6 (95% CI: 17.2–18.1) to 16.0 (95% CI: 15.6–16.4) per 1,000 children over a one-year period when AL is substituted with DHAPQ as first-line therapy. We conclude that even though the post-treatment prophylactic advantage of DHAPQ seems to be relatively small, changing the first-line therapy of uncomplicated falciparum malaria from AL to DHAPQ has the potential to significantly reduce malaria-associated morbidity and mortality and thus may provide substantial benefit to the population and appears to be cost-effective when the costs of the drugs are the same.

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ASSESSING THE EFFECT OF THE RECOMMENDED ARTEMETHER-LUMEFANTRINE DOSING REGIMEN ON THE RISK OF TREATMENT FAILURE IN PATIENTS DIAGNOSED WITH UNCOMPLICATED FALCIPARUM MALARIA

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Artemether-Lumefantrine (AL), the first line antimalarial treatment in 49 countries, is administered according to four weight bandings, patients at the margins of which deviate significantly from the optimal target dose. To assess the efficacy of administered lumefantrine through the total mg/kg spectrum, individual patient level data (N=8,927) from 43 clinical efficacy studies of uncomplicated Plasmodium falciparum treated with 6 doses of AL conducted between 1996 and 2011 (7,399 from Africa, 1,588 from Asia) were collated using standardised procedures. Factors associated with PCR adjusted efficacy were evaluated using Cox regression model with shared frailty to account for study effects. 24 studies ended follow-up at 28 days while 19 studies followed up for 42 days or longer. 192 recrudescent and 1,101 new infections were reported. The median total dose of lumefantrine administered was 65.5 mg/kg (IQR: 55.4-77.8 mg/kg), with children under 1 year receiving the greatest dose (median=90.0 mg/kg, IQR=80.0-102.9 mg/kg), compared to those aged 1-5 (median=65.5 mg/kg, IQR: 55.4-80.0 mg/kg), 5-12 (median=72 mg/kg, IQR: 65.5-84.7 mg/kg) and greater than 12 years (median=54.3 mg/kg, IQR: 48.0-62.6 mg/kg). The median mg/kg dose of lumefantrine in patients failing the treatment was 65.5 mg/kg (IQR: 57.5-79.3 mg/kg) which was similar to those who were cured (median=65.5, IQR: 55.4-77.9 mg/kg). In the multivariate model, the risk factors for recrudescence were lower age categories categories [age < 1 year (AHR= 5.11, 95% CI: 1.30-20.06, P=0.0190), age 1-5 years (AHR: 6.53, 95% CI: 1.76-24.14, P=0.0049)], logged baseline parasitaemia (AHR: 1.17 [1.02-1.34], P=0.0240) and total mg/kg drug dosage received (AHR: 0.96, 95% CI: 0.93-0.99, P=0.0160). Adjusting for confounding factors the most significant risk factor for recrudescence was the use of non fixed dose formulation (AHR: 3.07 [1.51-6.21], P=0.0021).

Patients treated with the FDC received a greater mg/kg dosage of AQ (median=29.56, IQR: 26.39-40.00) compared to those receiving a non FDC (median=25.00, IQR: 23.33-34.01). The fixed dose formulation provides better efficacy results than co-blister probably related to improved dosages. Prospective comparative studies of ASAQ formulations are warranted to confirm the benefits on efficacy and effectiveness of fixed dose formulations and a higher target dose of AQ.

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PROBABILISTIC RECORD LINKAGE FOR MONITORING THE SAFETY OF ARTEMISININ BASED COMBINATION THERAPY IN THE FIRST TRIMESTER OF PREGNANCY IN RURAL SENEGAL

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There are insufficient data on the safety in early pregnancy of the artemisinin-type antimalarials. Assessing the risk of teratogenicity requires large sample sizes. Limited pharmacovigilance infrastructure exists in malaria-endemic countries. Monitoring drug safety in the first trimester is especially challenging as it requires prospective follow-up to reduce recall and survival biases and accurate assessment of gestational age. Record linkage approaches for pregnancy pharmacovigilance using routinely generated health records could be an efficient approach, but it has not been evaluated in resource-poor settings. The aim of this pilot study was to assess the feasibility of record linkage using routinely collected health care data as pragmatic means of monitoring the safety of artemisinin-based combination therapy (ACT) in early pregnancy in Senegal. Data (2004-2008) were extracted from paper-based registers from outpatient clinics, antenatal (ANC) and the delivery unit from a dispensary in rural Senegal and entered into databases. Probabilistic record linkage was used to identify pregnancies exposed to ACT in the first trimester of pregnancy. Two record linkage software packages (Link-Plus and FRIL) were compared and output data were reviewed independently by two investigators. Information on 685 pregnancies was extracted, 536 of which were eligible for record linkage; 95.3% of them resulted in live-births, 2.3% in stillbirths and 2.5% in miscarriages. Major congenital malformations were identified in 1.6% of births. Seventy-one and 75 matches between pregnancy outcome and the outpatient treatment registers were identified by both software packages. All the 7 pregnancies exposed to ACTs in the first trimester identified resulted in normal live-births. Probabilistic record linkage is a potential cost-effective method to assess the safety of...
antimalarials in early pregnancy in resource-poor settings. It is suited to assess the risk of major birth defects and stillbirths in settings with good existing health records and well-defined target populations.

EMPOWERING VILLAGE HEALTH TEAMS, A VALUE ADDITION TO HEALTH SERVICES DELIVERY IN RESOURCE LIMITED SETTINGS; CASE STUDY OF KIBOBA AND KYANKWANZI DISTRICTS IN UGANDA

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The majority of the people in Uganda, especially children, can not easily access health care, because of distant health facilities and a critical shortage of health workers. This has resulted in high mortality rates especially due to malaria (23%), pneumonia (21%), and diarrhoea (17%). Emerging evidence supports the unique role of community health workers referred to in Uganda as Village Health Teams (VHTs) play in providing first level health care in their communities. This paper provides a case study on how VHTs have increase health care provision for children under 5years for malaria, diarrhoea and respiratory tract infections in central Uganda. In the districts of Kiboga and Kyankwanzi, records of 4 government health facilities and VHTs attached to them were reviewed. Out Patient Department (OPD) attendance records and VHT data registers for children under 5 years between January to October for the years 2009 and 2011 was done. Data on malaria, diarrhoea and respiratory tract infections was analysed under four variables of OPD attendance, access to treatment, timeliness of treatment, and patient referral. Two third (2/3) of children with the three diseases accessed treatment and were healed in the communities by VHTs. Sixty four percent (64%) of malaria, 78% of diarrhoea and 65% of acute respiratory infections have been seen by VHTs. Only 12% of the children were referred to health facilities for further management. Furthermore 44% of all the children treated by VHTs, received their treatment within 24 hours of onset of illness. VHTs reduce attendance in OPD. This implies that there is reduced workload for the already constrained human resource at facility level. Timely treatment of diseases at community level is likely to reduce children that may slide into complications.

A PILOT STUDY ON ANTI-MALARIAL INTERVENTIONS FOR MALARIA IN PREGNANCY IN EDO STATE, NIGERIA

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Malaria in pregnancy (MiP) is a major public health problem in Nigeria, despite available interventions. This abstract describes the collaborative efforts between international organizations, local communities, and stakeholders in the fight against MiP in Nigeria. In 2009, a study was undertaken in some communities in Edo State, Nigeria, assessing knowledge, attitude and practice (KAP) of MiP among women of reproductive age (15 - 49 yrs), and primary healthcare providers. Thereafter, interventions, including peer education on KAP of MiP were provided for health workers, and the women, through workshops, rallies, and door-to-door campaign. In addition, women advocacy groups were inaugurated and supported to continue the dissemination of appropriate KAP of MiP to all stakeholders in the communities. Finally, post-intervention survey was conducted to assess the impact of the intervention among the women. Furthermore, some factors responsible for low utilization of anti-malarial intervention during pregnancy were noted, including non-availability of insecticide treated bed nets (ITNs) and anti-malarial medications. A total of 1955 women of reproductive age (mean age ±sd, 27.88 ± 9.98) was surveyed. In all, 109 primary care providers (medical officers, nurses, community health extension workers, etc), and 37 women peer educators were trained. The flag-off awareness campaign attracted a large audience, via in-person and local television station. The door-to-door campaign and rallies reached about 3,000 persons within the study communities. Overall, peer education was effective in improving knowledge on malaria prevention among women of productive age, as knowledge increased significantly between pre- and post- intervention studies. Following the study, the National Malaria Control Program of Nigeria’s Federal Ministry of Health, in 2011, through a local NGO, CHRADiP, freely distributed over 1000 long lasting ITNs to pregnant women and young children across the various communities in the state. The educational intervention improved KAP of MiP among the stakeholders in the communities. In addition, the primary care workers, and the entire community were mobilized and empowered on appropriate KAP of malaria. The provided free ITNs were appreciated. A national scale-up of a similar intervention is recommended.

Efficacy of artesunate and artesunate-azithromycin for the treatment of uncomplicated Plasmodium falciparum malaria in Vietnam

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Reports of reduced susceptibility of artesunate in the treatment of uncomplicated Plasmodium falciparum malaria in western Cambodia highlights the urgent need to contain and reduce the spread of artesunate resistant strains. As part of this effort it is important to monitor the spread artesunate resistance and to evaluate new artemisinin based combination therapies (ACT). The objective of the present study was to determine the efficacy of artesunate alone and artesunate-azithromycin for the treatment of uncomplicated P. falciparum malaria in south-central Vietnam. The latter ACT was assessed because of azithromycin’s favourable pharmacokinetic properties and safety record in young children and pregnant women. In an open labelled study carried out in 2010, 36 patients (children aged 6-14 years, n=10, adults aged 15-60 years, n=26) were allocated a 7-day course of artesunate (~4 mg/kg on D0, 2 mg/kg daily for D1 to D6) with a follow-up period of 28 days and 38 patients (children: n=14, adults: n=24) received a 3-day course of artesunate (4 mg/kg daily) plus azithromycin (~20 mg/kg daily) with a follow-up period of 42 days. The treatments were well tolerated, with no obvious drug associated adverse events. The PCR genotype corrected cure rate was 91% for both treatment groups. This study showed that the malaria strains at the study site were still highly susceptible to artesunate alone. Artesunate-azithromycin was also efficacious in the treatment of P. falciparum malaria and may provide an alternative option for the treatment of young children and pregnant women.

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A SURVEILLANCE SYSTEM TO MONITOR THE QUALITY AND AUTHENTICITY OF ARTEMISININ COMBINATION TREATMENTS IN AFRICA AND SOUTHEAST ASIA

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Poor quality Artemisinin Combination Therapies (ACTs) in malaria-endemic countries pose an enormous threat to malaria patients. The lack of reliable estimates of the prevalence of poor quality ACTs and their impact on public health makes it difficult for the national regulatory authorities (NRAs) to determine the need and scale of interventions to put in place. Our aim is to provide robust estimates of the frequency of substandard, counterfeit and degraded artemisinin containing drugs, and to develop standardised methodologies for sample collection. As part of the overall project we have explored the use of different sampling strategies to collect drugs from public and private healthcare providers in Rwanda, Cambodia, Ghana and Tanzania, with sampling in other locations underway. Once collected all samples are logged onto a database, the packages scanned and, tablets weighed and measured. Qualitative (mass spectrometry, near infrared and Raman spectroscopy) and quantitative (high performance liquid chromatography and high performance liquid chromatography-mass spectrometry) content analyses are then conducted. Thus far over 3,500 ACTs have been analysed. Preliminary content analyses indicate that a number of samples fall below the internationally recommended thresholds (90-110 %) for their stated active pharmaceutical ingredient with variations found to occur both between and within batches of the same brand. To assist in classifying whether the ACTs are degraded, due to environmental impact rather than manufacturing practices, we are investigating the ageing of a set of patented ACTs in field and in laboratory based studies, with quantitative analysis carried out on these samples at regular intervals over a period of four years. Following cross verification between the three collaborating laboratories, the results will be shared with the country specific NRA and stored on the “Counterfeit Drug Forensic Network - CODFIN” database.

LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP): A NOVEL TOOL TO INVESTIGATE MIXED MALARIA INFECTIONS IN MALARIA ELIMINATION SETTINGS

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Improved diagnostics for malaria will be required if elimination is to be achieved. We aimed to modify a novel nucleic acid amplification system, loop-mediated isothermal amplification (LAMP), to serve as a high-throughput, sensitive and specific diagnostic technique for the identification of sub-patent malaria infection. We developed a high-throughput 96-well plate LAMP (htLAMP) assay using a colorimetric agent that produces a visually detectable colour change. The htLAMP assay was applied to filter paper (FP) control parasitemia samples ranging from 0.0005- 2% to determine analytical sensitivity. htLAMP was also applied to whole blood and filter paper samples. Further optimization of the htLAMP assay was required to achieve the desired analytical sensitivity of <10 parasites/µL. The high-throughput, colorimetric htLAMP assay shows promise as a diagnostic tool for rapid detection of low parasitemias encountered in elimination settings.

DIAGNOSING MALARIA IN PREGNANCY: COMPARING IMMUNOFLUORESCENT MICROSCOPY TO OPTICAL MICROSCOPY AFTER GIESMA STAINING

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Malaria in pregnancy remains a significant threat in sub-Saharan Africa as it is associated with sub-optimal pregnancy outcomes. The current standard of diagnosis, optical microscopy after staining with Giemsa, requires well trained microscopists and may require as long as two hours before results are obtained. Alternate rapid diagnostics tests thus need to be evaluated, particularly in pregnancy when changes in the immune response could potentially affect the performance of rapid tests based on antibody detection. We assessed the diagnostic performance of an alternate method, fluorescent microscopy, compared to optical microscopy after Giemsa staining. As part of two baseline studies of mother-to-child transmission of HIV in the Buea Health District, 407 consenting pregnant women were enrolled. Venous blood samples were collected and tested by optical microscopy after Giemsa staining (OM) and by fluorescent microscopy (FM) using the Partec-Cycope (Partec GmbH, Münster, Germany). All participants were asymptomatic at the time of enrollment. Both slides were read by experienced microscopists and evaluated qualitatively as being positive or negative for plasmodia. Of the 407 samples tested by OM, 255 (62.5%) were plasmodium-positive. Of these 255, 207 were also plasmodium-positive by FM, thus a sensitivity of 81.1% (95%CI: 75.8, 85.8%). Of the 152 samples negative by OM, 75 were also negative by FM, thus a specificity of 49.3% (95%CI: 41.1, 57.6%). The positive and negative predictive values of FM were respectively 72.9% (95%CI: 67.3, 78.0%) and 61.0% (95%CI: 51.8, 69.6%). The percentage agreement between both methods was 69.3% (Kappa=0.32, p-value<0.01). There was moderate agreement between FM and OM. The low specificity and negative predictive value of FM suggest a high likelihood of false negative results if FM is used in place of OM.

THERMAL CONTRAST SIGNIFICANTLY IMPROVES THE SENSITIVITY OF LATERAL FLOW ASSAYS FOR MALARIA DIAGNOSIS

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Malaria rapid diagnostic tests (RDTs) using lateral flow immunoassays (LFAs) are one of the few low-cost assays that can diagnose malaria in a point of care setting without laboratory infrastructure. With LFAs, a positive detection occurs when the test region of the assay membrane strip appears visibly red if the target analyte from a patient’s sample is captured by an antibody bound to the membrane and an antibody bound...
to the surface of gold nanoparticles. This sandwich capture occurs as antigen-bound gold nanoparticles migrate across the membrane to form the test line. When compared to microscopy, the best of the current LFAs have >95% sensitivity and specificity for Plasmodium falciparum infections in which parasitaemia is ≥200 parasites/µL, but have significantly decreased sensitivity for infections with <200 parasites/µL and for P. vivax, P. ovale and P. malariae. Malaria elimination campaigns will require RDTs with a sensitivity that exceeds that of microscopy. We recently demonstrated that the sensitivity of RDTs can be dramatically enhanced by laser heating of the gold nanoparticles resulting in quantifiable release of heat. An infrared camera can then measure the heat released, which is directly proportional to the number of gold nanoparticles, and this technique can quantitatively measure the antigen burden in the sample. This new technology is termed thermal contrast and was recently demonstrated for Cryptococcal meningitis, as reported previously. Herein we show that this technology can also be used to enhance the sensitivity of RDTs for malaria, showing an 8-fold increase in sensitivity as compared to standard RDT testing during serial dilutions of clinically positive P. falciparum blood samples. Thus, while current RDTs have a limit of detection at 200 parasites/µL, thermal contrast can enable detection at the level of ~25 parasites/µL, significantly improving the sensitivity of RDTs to malaria in those with low-level parasitaemia. Further improvement on the order of 100-fold in sensitivity is possible with redesign of LFAs to reduce nonspecific background laser absorption and enhance specific gold nanoparticle absorption. Finally, inexpensive existing technologies are being evaluated to design a robust, battery operated point-of-care RDT thermal contrast reader (<$100) for resource limited settings.

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PERSPECTIVES ON MALARIA RAPID DIAGNOSTIC TESTS AFTER NATIONAL ROLLOUT IN TANZANIA’S PUBLIC SECTOR - PROVIDER AND CONSUMER VIEWS FROM MBeya REGION

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As part of its national strategy to improve malaria case management, Tanzania has been gradually rolling out malaria rapid diagnostic tests (mRDT) in the public sector since 2008. A multidisciplinary evaluation to assess the effectiveness of this strategy is currently underway. We present results of qualitative research conducted in two districts in Mbeya Region where mRDTs were introduced in February 2011. Qualitative interviews were conducted with health authorities, providers and community members about their experiences with malaria diagnosis and treatment post mRDT implementation. A total of 28 interview transcripts were reviewed for content analysis. Several conflicting views and practices with respect to mRDTs emerged. Whereas laboratory and pharmacy officers were more likely to express confidence in the accuracy of mRDTs, other health authorities were less convinced of their usefulness as a diagnostic tool; some suggested more studies to assess the quality of mRDTs were needed. Others expressed concerns that clinicians were ignoring negative mRDTs in favor of artemether-lumefantrine (ALu) treatment. Our interviews with providers and community members confirm their suspicions. While some providers acknowledged ignoring negative mRDTs results for patients with malaria symptoms, they also noted that such patients often improved after ALu treatment. Other providers adopted a “wait and see” approach, advising their patients to return in 2-3 days if symptoms persisted. Although the extent of such practices is not known, the use of ALu for negative mRDTs was cited as one of the malaria challenges for the region. Stock outs of mRDTs were mentioned as another. Regional authorities noted that within the first 8 months of implementation, 4 out of 8 districts had experienced a stock out. Although mRDT stock outs were less disruptive for facilities that also practiced microscopy, facilities without microscopes reported reverting to clinical diagnosis. These challenges will need to be addressed early on if improvements in malaria case management are to be achieved. Strategies to consider include identifying effective measures to improve provider adherence to mRDTs, as well as addressing bottlenecks in the mRDT supply chain.

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STUDY OF HOSPITAL BASED MALARIA CASES IN THE PEDIATRIC DEPARTMENT OF KORLE BU TEACHING HOSPITAL, GHANA

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Malaria kills about one million children, under five years of age, each year worldwide, with nine out of 10 deaths occurring in sub-Saharan Africa. This study was carried out to determine the incidence of malaria in the pediatric department of Korle Bu Teaching Hospital from January 2011 to October 2011, and to compare available diagnostic tests for malaria. 978 suspected cases of malaria (507 males and 471 females, aged 1 day - 12 years), attending the OPD and admitted as inpatients in the ER of the Pediatric Department were included in this study. 1.0 mls of blood sample was collected into EDTA bottle. Thick and thin smears were prepared, stained and examined. Subsequently, the blood samples were subjected to antigen detection using the First Response Malaria pLDH/ HRP 2 Combo Test according to the manufacturer’s instructions. The results were tabulated and analyzed statistically. 51 cases out of 978 suspected cases were positive for malaria, with an incidence of 5.2%. Out of these 40 (78.4%) were positive for Plasmodium falciparum, 5 (9.8%) were positive for P. malariae, 2 (3.9%) were positive for P. ovale, and 4 (7.8%) were positive for both P. falciparum and P. malariae. The First Response Malaria pLDH/ HRP 2 Combo Test detected 51 positive cases compared with the blood smear study, which detected 41 cases. 36 cases were detected both by the First Response Malaria pLDH/ HRP 2 Combo Test and blood smear study. 15 cases were positive by the First Response Malaria pLDH/ HRP 2 Combo Test, but not by the blood smear study. 5 cases detected to be positive by the blood smear study were found to be negative by the First Response Malaria pLDH/ HRP 2 Combo Test. 937 cases were negative both by the First Response Malaria pLDH/ HRP 2 Combo Test and the blood smear study. Among 51 positive cases, 35 were males with a percentage of 68.6% as compared to females (31.4%). The sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic efficiency of the First Response Malaria pLDH/ HRP 2 Combo Test when compared to microscopy, were 87.5, 96.8, 90, 98.9, and 96%, respectively. In conclusion, the incidence of malaria in this present study was 5.2%. The sensitivity of First Response Malaria pLDH/ HRP 2 Combo Test is very close to microscopy. It is a simple, sensitive, and effective diagnostic test for P. falciparum, P. malariae, P. vivax and P. ovale malaria.
We followed up 131 children with microscopically confirmed malaria at 7 standard in 308 episodes of fever in a cohort of children under five years. For diagnosis of malaria was compared using expert microscopy as a gold treatment monitoring and diagnosis of recurrent episodes of malaria in become negative more quickly. We assessed the accuracy of HRP2 and Plasmodium lactate dehydrogenase (pLDH) based RDTs treatment.

A HIGH TRANSMISSION SETTING IN UGANDA

Rapid diagnostic tests (RDTs) for malaria diagnosis have attracted interest to improve fever management in malaria endemic areas where microscopy is simple to perform and may be used to monitor treatment and diagnose new episodes of malaria.

THE DIAGNOSTIC VALUES OF RAPID DIAGNOSTIC TEST BASED ON HISTINE RICH PROTEIN II OF CARESTARTTM MALARIA IN DETECTING MALARIA INFECTION IN PREGNANT WOMEN SAN AND KITA

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We conducted from July 2009 to February 2010 in San and Kita (Mali), a study on the diagnostic values of the Histine Rich Protein II (HRP-II) CareStartTM Malaria compared to the thick smear for the detection of malaria infection during pregnancy as part of a pilot study for monitoring the therapeutic efficacy of Sulfadoxine-Pyrimethamine during in vivo testing of 42 days. Result showed both the sensitivity and specificity of HRP-II CareStartTM was higher than that observed in the thick film. CareStartTM Malaria positivity is proportional to parasite density detected by the thick film. HRP-II of CareStartTM Malaria is simple to use and fast. The HRP-II TDR can be used in the detection of malaria infection among pregnant women.

ACCURACY OF TWO RAPID DIAGNOSTIC TESTS FOR DIAGNOSIS AND MONITORING TREATMENT OF MALARIA IN A HIGH TRANSMISSION SETTING IN UGANDA

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Rapid diagnostic tests (RDTs) for malaria are simple to perform and may improve fever management in malaria endemic areas where microscopy is not readily available. Most RDTs detect Histidine-rich protein 2 (HRP2) which persists in the blood stream for variable lengths of time after treatment. Plasmodium lactate dehydrogenase (pLDH) based RDTs become negative more quickly. We assessed the accuracy of HRP2 and pLDH based malaria RDTs for initial diagnosis of uncomplicated malaria, treatment monitoring and diagnosis of recurrent episodes of malaria in young children in a hyperendemic area in Uganda. Accuracy of the RDTs for diagnosis of malaria was compared using expert microscopy as a gold standard in 308 episodes of fever in a cohort of children under five years. We followed up 131 children with microscopically confirmed malaria at 7 day intervals for 28 days to determine clearance time of HRP2 and pLDH antigenemia. Both RDTs were done for children with recurrent episodes of fever after treatment of malaria. Of the 308 children tested, sensitivity was 98% for HRP2 and 87.1% for pLDH; specificity was 54.7% for HRP2 and 95.3% for pLDH. Positive predictive value (PPV) was 80.5% for HRP2 and 97.2% for pLDH; negative predictive value (NPV) was 93.5% for HRP2 and 79.5% for pLDH. Persistent antigenemia from recent malaria episodes contributed to the low specificity of HRP2 while the lower sensitivity of pLDH was due to poor antigen detection at low parasite densities. The mean duration of antigenemia was 21 days for HRP2 and 2 days for pLDH.

Pre-treatment parasite density predicted the duration of antigenemia of HRP2. We documented 61 episodes of fever after antimalarial treatment. For these, HRP2 had sensitivity of 100%, specificity of 34.5%, PPV of 62.7% and NPV of 100%. pLDH had sensitivity of 90.6%, specificity of 100%, PPV of 100% and NPV of 90.6%. The HRP2 based RDT though accurate for initial diagnosis of malaria, was limited by low specificity due to persistent antigenemia.The pLDH based RDT showed rapid clearance of antigenemia and was accurate for diagnosis of malaria in children with recurrent fever after treatment. In patients who had malaria in the previous three weeks and present with fever, pLDH based RDTs should be used to monitor treatment and diagnose new episodes of malaria.

EVALUATION OF PRE- AND POST-TRAINING KNOWLEDGE AND PRACTICES OF HEALTH WORKERS IN THE USE OF RAPID DIAGNOSTIC TEST FOR PARASITOLOGICAL DIAGNOSIS OF UNCOMPlicated MALARIA IN CAMEROON

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Rapid diagnostic tests (RDTs) for malaria diagnosis have attracted interest in recent years because of their high specificity and sensitivity and are suitable for resource-constrained settings as they require minimal infrastructure. However, many health workers who are the main players for the effective use of this new technique do not yet master it. The Cameroon National Malaria Control Programme (NMCP) only recently
introduced RDTs in 50 pilot districts in 2011. Health workers (HWs) from mission and public health facilities in Yaounde and Bamenda cluster randomized in a research to evaluate the provision of appropriate treatment to malaria patients were invited to attend a 1 day and 3 days workshop on “Ensuring appropriate treatment for uncomplicated malaria” and “Improving quality of care for management of suspected malaria” respectively. All workshop attendees completed a pre training questionnaire which covered aspects such as clinical manifestation and methods of malaria diagnosis, the role of an RDT, who should conduct an RDT, the practical steps, time to read and interpretation of the results, treatment according to test results. During the training, HWs received lectures and practical exercises on all the above mentioned aspects including practical steps with assistance of a 16- step WHO RDT job aid and treatment guidelines from NMCP. Participants were also individually supervised during the performance of an RDT and graded using a checklist. The same questionnaire was used for post training evaluation. Of the 54 HWs from Yaounde, 62.5% were nurses, 20.8% medical doctors and 16.7% laboratory technicians. The knowledge increase on clinical manifestation and diagnostic methods for malaria for pre and post training was 10.42% while knowledge on RDT use had an increase of 52.3%. Knowledge on treatment based on test results had an increase of 28.2% while practical skills improved from 0% to 80%. Of the 40 HWs from Bamenda, 62.5% were nurses, 25% medical doctors and 12.5% laboratory technicians. The knowledge increase on clinical manifestation and diagnostic methods for pre and post training was 8.9% while knowledge on the RDT had a 35.4% increase. The knowledge on treatment based on test results had an increase of 17.6% while practical skills improved from 0% to 86%. If HWs are given appropriate training, clear instructions with appropriate job aids, they can use RDTs appropriately irrespective of their cadre and setting.

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MALARIA PARASITE DENSITY ESTIMATED FROM ACTUAL WBC COUNT OF PATIENTS CORRELATES WITH ESTABLISHED WBC REFERENCE VALUE IN CENTRAL GHANA

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White Blood Cells count (WBCc) is a bed-rock in the estimation of malaria parasite density in malaria field trials, interventions and patient management. WBCs are indirectly and relatively used in microscopy to estimate the density of malaria parasite infections. Due to frequent lack of facilities, in some malaria endemic countries, to quantify WBCc of patients, an assumed WBCc of 8.0 × 10⁹/L has been set by the WHO to help in estimating malaria parasite densities. The comparative analysis study, in Central Ghana, compiled laboratory data of 5902 Plasmodium falciparum (Pf) malaria parasite positive samples. Samples were obtained from consented participants of age-group less than 5 years. Full Blood Counts (FBC) of participants’ samples were analysed using the ABX Micros 60 Haematology Analyzer. Blood slides were read by two competent microscopists to produce concordant results. All internal and external quality control measures were carried out appropriately. Parasite densities were calculated using participants’ absolute WBCc and assumed WBCc of 5,000 to 10,000 per microlitre of blood. From the 5902 Pf malaria positive samples, the mean (SD) WBCc and geometric mean parasite density were 10.4 (4.6) × 10⁹/L and 7557/µL (95% CI 7144/µL to 7994/µL) respectively. The difference in the geometric mean parasite densities calculated using actual WBCs and compared to densities with assumed WBCcs counts were significantly lower for 5.0 × 10⁹/L; 3937/µL, 6.0 × 10⁹/L; 4725/µL and 8.0 × 10⁹/L; 6300/µL. However, the difference in geometric mean parasite density, 7874/µL (95% CI, 7445/µL to 8328/µL), with assumed WBCc of 10.0 × 10⁹/L was not significant. In conclusion, using the assumed WBCc to estimate malaria parasite densities in Pf infected children less than 5 years could result in significant errors in the estimation of parasite burden in a malaria endemic region. Assumed WBCc of 10.0 × 10⁹/L at 95% CI of geometric mean of parasite density statistically agreed with the parasite densities produce by the absolute WBCc of participants. This correlates with the established reference value for WBC in Central Ghana. We therefore suggest where available, the use of the WBC reference value to estimate malaria parasite density when obtaining absolute WBCc is not possible especially in drug efficacy and vaccine trials.

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PRODUCTION OF VERTICAL FLOW RAPID MALARIA TEST KIT TO DETECT PLASMODIUM FALCIPARUM AND PLASMODIUM VIVAX

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An endeavour to produce in-house rapid diagnostic test in Thailand to supplement the use of various commercial test kits available the market. The test aimed to produce a vertical flow immunochromatographic test to detect either Plasmodium falciparum or Pan malaria parasites and hoped to be the alternative tool for field users. The test were produced by using the in-house monoclonal antibodies produced against either Plasmodium lactate dehydrogenase (pLDH) or Plasmodium glycolaldehydes-3-phosphate dehydrogenase (pGAPDH). Preliminary study was done against 38 wild type malaria and 39 negative control samples and found that the test kit gave sensitivity and specificity to P. falciparum 89.5 % and 98.3 % and to P. vivax 82.4 % and 98.8 % respectively. Even the test gave somewhat high diagnostic values but its sensitivity positive correlated with parasitism levels. However, at least, this study got an alternative RDT prototype to be validated its feasibility for using in field onward.

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INTRODUCING RAPID DIAGNOSTIC TESTS INTO COMMUNITY-BASED MANAGEMENT OF MALARIA: EVIDENCE FROM A CLUSTER-RANDOMIZED TRIAL IN TWO AREAS OF HIGH AND LOW TRANSMISSION IN UGANDA

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Universal access to diagnostic testing for malaria is now recommended by WHO, to encompass all levels of health care, including community-based treatment programmes. Rapid diagnostic tests (RDTs) provide a simple means of confirming malaria diagnosis in locations lacking electricity and qualified health staff. Some countries have begun to introduce RDTs at community level, and data on the impact of diagnostic testing on treatment and referral practices by community health workers is still limited. A cluster-randomised trial to evaluate the impact and cost-effectiveness of RDTs when used by community medicine distributors (CMDs), compared with presumptive treatment, has been conducted in two areas with contrasting malaria transmission in Rukungiri District, Uganda since June 2010. The trial aims to evaluate the impact of diagnostic testing on the proportion of children who receive appropriate ACT treatment and referral under low and high transmission, as defined by malaria microscopy on a research slide collected at the same time as the RDT. The study will also provide evidence on the operational challenges and community acceptability of RDTs. A total of 120 communities (379 CMDs) were randomised to training either in use of RDTs or presumptive
INTRODUCING RAPID DIAGNOSTIC TESTING FOR MALARIA INTO THE PRIVATE SECTOR: EVIDENCE FROM A CLUSTER-RANDOMIZED TRIAL IN REGISTERED DRUG SHOPS IN UGANDA

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Universal access to diagnostic testing for malaria is now recommended by WHO, to encompass all treatment providers. Many malaria cases are treated outside the formal health sector, with drug shops often being the first, and only, source of treatment. Rapid diagnostic tests (RDTs) provide a simple means of confirming malaria diagnosis in drug shops, and improved diagnosis may also help to ensure that the drugs sold are appropriate. As yet, there is little evidence of the impact of diagnostic testing on antimalarial drug sales and referral practices by drug shops, particularly in Africa. A cluster-randomised trial to evaluate the impact and cost-effectiveness of using RDTs in registered drug shops, compared with presumptive treatment, has been conducted in Mukono District, Uganda since October 2010. The trial aims to evaluate the impact of diagnostic testing on the proportion of drug shop clients who receive appropriate ACT treatment, in line with parasitological status as defined by malaria microscopy on a research slide collected at the same time as the RDT. The study will also provide evidence on the feasibility, operational challenges and acceptability of this approach. A total of 60 drug shops were randomised to receive training either in the use of RDTs or presumptive diagnosis of malaria. All drug shop vendors (DSVs) were trained on the national malaria treatment guidelines, use of rectal artesunate pre-referral treatment, and when to refer. Supporting interventions included activities to raise community awareness, and close support supervision to DSVs for the first 3 months of implementation. Since January 2011, supervision has been scaled back to mimic levels typically seen in health systems in rural Africa. Nonetheless, adherence to RDT results by DSVs has remained high, with over 95% of ACT treatments sold being consistent with RDT test results. We will describe the design of the intervention in drug shops, and present data on adherence to RDT result and treatment guidelines by DSVs; referral practices; and changes over the first 15 months of the trial.

SCALING-UP RAPID DIAGNOSTIC TESTS FOR MALARIA: BARRIERS AND OPPORTUNITIES IN NIGERIA’S PRIVATE SECTOR

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Throughout Nigeria, about 80% of treatment for fevers occurs in private shops, pharmacies and clinics. Many private practitioners presumptively diagnose childhood fevers and a multitude of other symptoms as malaria without conducting blood tests, for a variety of reasons. With the increased availability of artemisinin combination therapy (ACTs) to effectively treat malaria, there is growing recognition that accurate diagnosis prior to treatment is needed. Effective diagnosis not only saves on health expenses and reduces the risk of drug resistance, but also reduces the risk of misdiagnosis and delayed treatment of non-malaria illness. The Global Health Group at UCSF conducted qualitative research in early 2012 in collaboration with the University of Ibadan, Nigeria, to determine the availability and acceptability of using Rapid Diagnostic Tests (RDTs) as an easy and inexpensive tool for diagnosis of malaria in the absence of microscopy. Results from qualitative analysis indicate very little knowledge or use of RDTs and that significant structural barriers exist in deploying RDTs within the private sector. These barriers include legal and technical constraints among different types of providers; regulatory limitations; widespread beliefs that diagnostic tests are not needed and that results are unreliable; and inadequate incentive structures for balancing profit motives with priorities for providing quality care. This research suggests that private sector providers may respond to patient requests for diagnosis prior to treatment if awareness of the value of diagnosis is increased and greater demand for RDTs can be fostered. A second phase of research is underway which will test malaria prevalence as well as acceptability and adherence to RDT results among purchasers of anti-malaria medication at private outlets in Nigeria.

HIGH FREQUENCY MALARIA PARASITE DETECTION BY BUFFY-COAT SMEAR AMONG PATIENTS WITH NEGATIVE THICK BLOOD FILM TESTS

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Malaria remains one of the world’s most serious global health concerns. Prompt and accurate diagnosis is critical to effective management of malaria. Although microscopy by thin- and thick-blood smears is the current standard for malaria diagnosis, there is a risk of false negative results when the patient has recently been treated with antimalarial medications. To explore how frequently *falciparum* malaria parasites are detected, by quantitative buffy-coat capillary tube test, among patients with prior negative results by thick-film blood-smeare tests. We studied 36 patients with uncomplicated *falciparum* malaria, confirmed by conventional thick- and thin-film microscopy diagnostic methods. The patients were admitted and treated at the Bangkok Hospital for Tropical Diseases. Fingerpricks for conventional blood smear and concurrent buffy coat blood smears were conducted every 6 hours until the patients exhibited negative parasitemia, and then finger-prick tests were performed daily until each patient was discharged from hospital. A negative result by conventional thick-film test was compared with the concurrent buffy-coat thick-film test. By study method, we found that of 36 patients with negative thick films, buffy-coat smears detected 10 patients (27.8%) with asexual form *Plasmodium falciparum*. We concluded that quantitative buffy coat capillary tube tests can accurately and efficiently detect malaria parasites, even when conventional thick films show negative parasitemia.
COMPARATIVE EFFICACY OF UNCONTROLLED AND CONTROLLED INTERRMEDIATE PREVENTIVE TREATMENT DURING PREGNANCY (IPTp) WITH COMBINED USE OF LLNS IN HIGH RESISTANCE AREA TO SULFADOXINE-PYRIMETHAMINE IN CÔTE D’IVOIRE

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In recent years, Intermittent Preventive Treatment for pregnancy (IPTp) with SP has become policy in much of sub-Saharan Africa. But resistance to SP has been spreading across sub-Saharan Africa and thus the effectiveness of SP-IPT has been questioned. The present study, therefore, sought to assess incidence of placental malaria, LBW and anemia of two approaches IPTp-SP (DOT scheme versus no DOT) in Anonkoua-kouté and Sama where the reported prevalence of dfr single mutant 108 was respectively 62% and 52.2%. The study was a longitudinal design involving pregnant women and was conducted in Anonkoua-kouté (Côte d’Ivoire), a suburban area, and Sama, a rural area, from January 2008 through March 2009. Women of a pregnancy less than 28 weeks duration were randomized to receive SP (1500 mg of sulfadoxine and 75 mg of pyrimethamine) in a single intake twice and were followed up monthly until delivery. Doses were administered under supervision in the controlled IPTp group, while in the uncontrolled IPTp group, drug was given free to women and it was recommended to take it at home. The primary end point was the proportion of low birth-weight (LBW) infants (body weight <2500 g) and the secondary, the rate of severe anemia and placental malaria detected at delivery. A total of 420 pregnant women were enrolled (212 and 208 respectively in controlled and uncontrolled groups). Delivery outcome was available for 378 women. In the modified intention to treat (ITT) analysis, LBW infants were born from 15.5% women of the uncontrolled IPTp group and from 11.9% of women on controlled IPTp group (p= 0.31). The per-protocol population (PP) analysis showed consistent results. The proportions of women with placental malaria infection, moderate anemia (Hb<11 g/dL), and severe anemia (Hb <8 g/dL) at delivery were similar between the two groups (p>0.05). In conclusion, the study showed that the two approaches were equivalent suggesting the use of unsupervised IPTp with SP free of charge in areas where implementation of DOT scheme suffer from many constraints.

EFFICACY OF ARTEMETHER/LUMEFANTRINE SINCE ADOPTED AS A FIRST LINE TREATMENT FOR UNCOMPLICATED PLASMODIUM FALCIPARUM MALARIA IN ETHIOPIA IN 2004

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In Ethiopia, unacceptably high level of resistance to sulphadoxine/pyrimethamine prompted the change to a combination of artemether and lumefantrine (AL) as a national first line treatment for uncomplicated Plasmodium falciparum malaria in 2004. Regular monitoring of the efficacy of the recommended regimen for falciparum malaria is essential to suggest whether the required high level of efficacy is maintained or to detect any early indication of resistance. These studies were conducted to assess the current level of AL efficacy in the country and provide credible information to national malaria control program managers for evidence based decision making. The studies were conducted between 2007-2011 malaria peak transmission seasons in seven sentinel sites using the revised WHO protocol. A minimum of ninety patients with uncomplicated P. falciparum malaria aged six months and above were enrolled in each study site. Each patient was treated with a standard six dose regimen of AL given twice daily for three days under partial supervision. The clinical and parasitological responses were assessed during a twenty eight days follow up period. Outcome of treatment were defined according to the standard WHO classification. Recurrent parasitaemia were genotyped to distinguish between recrudescence and new infection. PCR corrected adequate clinical and parasitological response (ACRP) at Day twenty eight in the per protocol analysis was greater than 95% in all sites except in Shele where the ACRP was 92.5%. There was no early treatment failure and most of the recurrent infections were due to late parasitological failure. Parasite and fever clearance rates were rapid and all patients were cleared of their gametocytes by day 14. Mean hemoglobin value had also improved on day twenty eight compared with the baseline. No serious adverse events were reported. However, mouth ulcer was recorded in some children after treatment and resolved spontaneously. A regimen of AL is highly effective in the study localities, after six years of use as first line treatment in the country. The high cure rate of AL reported in this study is encouraging and support the continued use as first line treatment for uncomplicated falciparum malaria in the country. However the 7.5% recrudescence infections observed in Shele highlights the need for regular monitoring the efficacy in Shele and other part of the country.

ASSESSMENT OF THE MOLECULAR MARKER OF PLASMODIUM FALCIPARUM CHLOROQUINE RESISTANCE (PFCRT) IN SENEGAL AFTER SEVERAL YEARS OF CHLOROQUINE WITHDRAWAL

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Since 2006, the artemisinin- combination therapies (ACTs); artemether–lumefantrine (AL) and artesunate plus amodiaquine (AS/AQ) were adopted for uncomplicated malaria treatment. After several years of CQ withdrawal the current study wished to determine the level of CQ resistance at the molecular level in selected sites in Senegal since interest in using CQ again has been raised by the scientific community. Finger prick blood samples were collected from Plasmodium falciparum positive children below the age of 10 (n = 474) during cross sectional surveys conducted in two study sites in Senegal with different malaria transmission level. All samples were analyzed for single nucleotide polymorphisms (SNPs) in the P. falciparum chloroquine resistance transporter gene (PfCRT – codons 72-76) using PCR-SSOP ELISA and Real Time-PCR methods. In total, 449 blood samples (94.7%) were PCR positive, 285 and 164 from Central and Southern sites in Senegal, respectively. In both study areas the prevalence of the PfCRT wild type single CVMNK haplotype was very high; Central study at 70.5% in 2009 and 74.8% in 2010 and in Southern study site at 65.4% in 2010 and 71.0% in 2011. Comparing data with older studies in Senegal, a sharp decline of the mutant type PfCRT prevalence is evident. From 65%, 64% and 59.5% in samples collected from various sites in 2000, 2001 and 2004, to approximately 30% in our study. A similar decrease in mutant type prevalence is noted in other neighboring countries. With the continued development of increased CQ susceptibility in many African countries it may be possible to re-introduce CQ again in the near future, in a drug combination, possibly given to non-vulnerable groups and demanding a close monitoring of possible reemergence of CQ resistance development.
MONITORING ANTIMALARIAL DRUG RESPONSE IN PLASMODIUM FALCIPARUM FIELD ISOLATES USING AN EX VIVO DAPI ASSAY

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Malaria treatment efforts are hindered by the rapid emergence and spread of drug resistant parasites. Simple assays to monitor parasite drug response in clinical samples are important, as they can detect drug resistance before it becomes clinically apparent as well as inform changes in treatment policy to help prevent the spread of resistant parasites. We surveyed malaria cases in a clinic in Thies, Senegal from 2008-2011 and employed a DAPI-based ex vivo drug assay to test parasite response to amodiaquine, chloroquine and mefloquine in approx. 400 clinical isolates. We genotyped known drug resistance-associated mutations and culture adapted a subset of parasites in order to compare their in vitro and ex vivo drug responses. The DAPI ex vivo drug assay is comparable or superior to SYBR-based assays using clinical samples, with a median signal to noise ratio of 4:1 and excellent agreement between technical replicates. Mutations in pfcrt and pfmdr1 were associated with changes in drug response, and we observed strong concordance between the in vitro and in vivo IC50s of culture adapted parasites. Overall, the DAPI ex vivo assay is robust and can be used to monitor parasite response to antimalarial drugs in field settings.

SNPs ON ABC TRANSPORTERS AND IN VIVO MALARIA PARASITE NON CLEARANCE AFTER CHLOROQUINE TREATMENT IN MALIAN CHILDREN

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Plasmodium falciparum malaria remains one of the major causes of morbidity and mortality in sub-Saharan Africa. PFCRT K76T mutation was demonstrated to play a central role in the P. falciparum resistance to chloroquine. Previous study have shown that SNPs on several ABC transporters genes are associated with in vitro chloroquine resistance. We aimed to find any association between SNPs on ABC transporter and the in vivo parasite non clearance after chloroquine treatment in Mali. We carried out a chloroquine efficacy study in the rural village of Kollé, Mali. P. falciparum DNA was extracted from filter paper and SNPs on Pfcrt, Pfmdr1, PFG30 and PFG47 were analyzed by nested and MS PCR. The study protocol and informed consent document were reviewed and approved by the National ethical committee. The data were statistically analysed by Epi Info® and STATA. 196 children suffering from uncomplicated malaria were included and 54 (27.5%) of them failed to the treatment at D14. The mutant alleles PfCRT 76T and Pfmdr1 86Y were associated with parasite non clearance with p=0.00001 and 0.03 respectively. However, the association of SNPs on PFG30 and PFG47 genes with parasite non clearance was not statistically significant, p =0.43 and 0.57 respectively. The logistical regression analysis showed that the mutant allele Pfmdr186Y contributed positively to the Pfcrt 76T parasites non clearance (p=0.02). This gene has been already described as a modulator of chloroquine resistance in several in vitro and in vivo studies from different settings. However, the SNPs on PFG30 and PFG47 genes did not contribute to the parasite non clearance. In conclusion, our findings have shown a lack of association between SNPs on the new putative transporters genes and parasite non clearance in children in Mali. But Pfcrt76T and Pfmdr186Y alleles were associated with the in vivo parasite non clearance in these settings.

PREVALENCE OF MUTATION OF PFCRT AFTER THE USE OF AMODIAQUINE IN INTERMITTENT PREVENTIVE TREATMENT IN CHILDREN (IPTC) IN SENEGAL

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Chloroquine was until 2002 the most commonly used drug against uncomplicated malaria in Africa in general and Senegal in particular. After several years of the withdrawal of chloroquine due to an high level of resistance of Plasmodium falciparum. Studies were conducted in Africa and showed a decline in the prevalence of resistance marker pfcrt-76T, molecular marker of P. falciparum resistance to chloroquine and amodiaquine. In Senegal the IPTC is a strategy for preventing malaria in children using sulfadoxine-pyrimethamine and amodiaquine. So the aim of this study is to evaluate the prevalence of pfcrt mutation in Senegal after three years of implementation of IPTC. This study was conducted in three health districts in Senegal (Mbour, Fatick and Bamby) with 54 health posts. The genotype of the pfcrt gene for polymorphisms C72S and K76T was determined in at least two multiplex real-time PCR runs with full agreements using the Rotorgene 3000 platform representing CVIET, CVMNK and SVMNT haplotypes. 3D7, Dd2 and 7G8 DNA obtained from the Malaria Research Reagent Resource (MR4) was used to provide sequence-specific positive control and nuclease free water was included as a negative control. Analysis was done with 47 isolates in 2008, all 2009 samples (n= 42) and 116 of the 125 isolates in 2010, were PCR positive. 10 (21%), 21 (48%) and 48 (41%) carried the CVIET haplotypes in 2008, 2009 and 2010 respectively. The SVMNT haplotypes were found in 32 (68%), 18 (42%) and 58 (50%) isolates in 2008, 2009 and 2010 respectively. Mixed haplotypes infections (CVMNK/CVIET) were found in 5 (10%), 3 (6%) and 10 (8%) of the 2008, 2009 and 2010 samples respectively. The SVMNT haplotypes was not found in any of the isolates. The prevalence of pfcrt mutation between 2004 and 2006 was approximately 60% in Senegal and Kenya, as reported previously. In our study, we found a decrease in the prevalence of pfcrt gene resistance compared to previous level in Senegal and in Africa. However this level remains high with percentages of 48% observed in 2009.

PREVALENCE OF MOLECULAR MARKERS OF SULPHADOXINE-PYRIMETHAMINE RESISTANCE IN AN AREA OF INTENSE, YEAR-ROUND MALARIA TRANSMISSION IN RURAL MALAWI

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Malaria infection in pregnancy is associated with severe maternal morbidity and increased perinatal mortality. Although sulphadoxine-pyrimethamine (SP) is no longer recommended as treatment for uncomplicated malaria due to resistance, SP is still recommended for intermittent preventive treatment in pregnancy (IPTp). Increasing resistance threatens the use of SP for IPTp. In 2010, we conducted cross sectional studies of the prevalence of molecular markers of resistance to SP among parasitemic patients presenting to the outpatient department (OPD) and delivery ward of Machinga District Hospital. In addition, pregnant women between 16 and 32 weeks of gestation with asymptomatic parasitemia were enrolled from antenatal clinic (ANC). Polymerase chain reaction was performed to examine molecular markers for SP resistance. Not all specimens could be amplified at all loci, therefore, percentages are given out of those that were amplified. We enrolled 196 OPD attendees and 291
pregnant women: 245 from ANC, and 46 at delivery. Primigravidae made up 44% of those from ANC and 59% of those at delivery. The overall prevalence of double (Gly-437/Glu-540 dhps), triple (Asn-108/Ile-51/Arg-59 dhfr) and quintuple mutants (double plus triple) was high (98%, 93%, and 92%, respectively), with no statistical difference among the groups. The prevalence of dhfr 164 was low (2%). The prevalence of dhps 613 was higher in OPD attendees than pregnant women (19% vs 5.4%, p-value<0.0001). In this study characterizing molecular markers of Plasmodium falciparum resistance to SP in Malawi, the prevalence of the quintuple mutant was high, while the prevalence of dhfr 164 remained uniformly low. The prevalence of dhps 581 is significantly higher among pregnant women at delivery, suggesting that IPTp with SP during pregnancy is selecting for this mutation. Given the high levels of molecular resistance to SP, we need to develop new tools for preventing malaria in pregnancy.

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**HIGH PREVALENCE OF PFCRT, PFDHPS AND PF DHFR DRUG RESISTANT HAPLOTYPES IN THE SOUTH BUT NOT IN THE NORTH OF CÔTE-D'IVOIRE**


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The national malaria treatment policy changed twice in 2003 from Chloroquine to Amodiaquine, then in 2005 (adoption of ACT). However, the gap between the time of decision and the effective implementation of switch to ACT favored an abusive use of CQ and SP. The nationwide coverage of recommended ACTs is not quite effective due in part to disparities in the distribution of healthcare infrastructure infrastructures which are more concentrated in the south and mainly in Abidjan the economical capital of Côte-d’Ivoire. The level of resistance to CQ and SP in the country may greatly varies according to local usage of these two drugs. A prospective study was undertaken in Côte-d’Ivoire in 2008–2009 to assess by means of the pyrosequencing technology, the distribution of allelic frequencies of molecular markers associated with resistance to CQ and SP between two sites from the south and one site from the north, in 2008, after the changes of treatment policy for acute Plasmodium falciparum malaria in Côte-d’Ivoire. A total of 123, and 86 samples were collected from two southern sites, Ayamé and Anonkoua-kouté respectively, and 121 samples were collected from, Dabakala up north. Out of the samples collected from each of the three sites, 98 samples from the district of Ayamé were successfully amplified, 80 from Anonkoua-kouté, and 117 from Dabakala. As major finding this work points out that in 2008, the prevalence of the three key resistance-conferring haplotypes, the triple mutants PfCRT IET and PfDHFR IRN, and the simple mutant PfDHPS SGK, were higher in Ayamé and Anonkoua-kouté in the south while the sensitive haplotype PfCRT MNK significantly predominated in Dabakala. The triple mutant IRN was rare at North. While ACTs are strongly recommended in the country to treat malaria, our work indicates a variable CQ and SP pressure nationwide. Mainly, these two drugs are still in used in the south where pressure is higher than up north. There could be a variable level of compliance to malaria treatment recommendations from the malaria control program.

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**INVESTIGATING THE ROLE OF CANDIDATE MOLECULAR MARKERS OF LUMEFANTRINE AND AMODIAQUINE RESISTANCE IN CLINICAL OUTCOMES OF ARTEMISININ COMBINATION THERAPIES (ACT) OF PLASMODIUM FALCIPARUM MALARIA**

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The initial reduction in peripheral Plasmodium falciparum parasitemia following artemisinin-based combination therapy (ACT) is driven predominantly by the peripheral parasite population. The rapid action of the artemisinin component, but overall efficacy requires sustained therapeutic concentrations of the longer-acting partner drug. Candidate molecular markers associated with resistance in the pfcrt and pfmdr1 genes of *P. falciparum* have been reported to be involved in decreased sensitivity to amodiaquine and lumefantrine. However, the utility of these markers for predicting therapeutic responses to artesunate-amodiaquine and artemether-lumefantrine remains unclear. Correlation studies are confounded by the high PCR-corrected parasitological cure rates associated with these ACTs and by regional variation in immunity and parasite genetic background. Seventeen research groups have pooled their data on treatment outcomes and candidate resistance markers from efficacy studies conducted in different parts of the world. A total of 27 studies with 5,300 patients in 15 countries were included in the analysis. Our objectives were to investigate whether known polymorphisms in *P. falciparum* can predict treatment outcomes following artemether-lumefantrine and artesunate-amodiaquine therapy, and to determine whether resistance-associated genotypes are selected in recurrent infections. We have investigated associations between polymorphisms in pfcrt and pfmdr1 at the time of treatment and parasite clearance, recurrence, and PCR-determined recrudescence and reinfection. We have also assessed early and post-treatment selection of resistance-mediating genotypes. The results of these pooled analyses will clarify the roles of molecular markers for partner-drug resistance in monitoring ACT efficacy and will help to guide the selection of informative genetic markers in future studies.

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**ASSOCIATION OF PFMDR1 AND PFCRT POLYMORPHISMS WITH SLOW CLEARANCE OF PLASMODIUM FALCIPARUM AFTER ARTEMISININ COMBINATION THERAPY IN WESTERN KENYA**

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Artemisinin Combination Therapies (ACTs) are now considered the best treatment for Plasmodium falciparum malaria and have been widely deployed. A decline in the efficacy of artemisinin monotherapy in western Cambodia, characterized by slow parasite clearance, has recently been reported. The molecular mechanism of this reduced response to artemisinins has not been established. Artemisinins are thought to act within the parasite digestive vacuole and proteins found on the vacuole membrane may play a role in modulating drug sensitivity. Genes encoding two such proteins, pfcrt and pfmdr1, have been analyzed for sequence polymorphisms in samples collected during a clinical trial of artemether-lumefantrine (AL) and dihydroartemisinin-piperaquine (DHA-PIP) in Kenya in 2009. Genotypic analysis of the results showed significant associations between slow parasite clearance and CQ-sensitive haplotypes of pfmdr1 and pfcrt as measured by real-time quantitative PCR. The amino acid NFD mutant was high, while the prevalence of dhfr 164 remained uniformly low. The prevalence of dhps 581 is significantly higher among pregnant women at delivery (37% vs 2.6% at ANC and 1.3% in OPD, p-value<0.0001). This study characterizing molecular markers of Plasmodium falciparum resistance to SP in Malawi, the prevalence of the quintuple mutant was high, while the prevalence of dhfr 164 remained uniformly low. The prevalence of dhps 581 is significantly higher among pregnant women at delivery, suggesting that IPTp with SP during pregnancy is selecting for this mutation. Given the high levels of molecular resistance to SP, we need to develop new tools for preventing malaria in pregnancy.
Findings from this evaluation are being used to inform planning of future national program to come up with better indicators in the near future. In addition to the findings, the tools developed, will potentially help the and barriers to effective implementation of DOTs at community-levels. on improving knowledge of VMWs in order to address misconceptions and transportation. The national malaria program should aim to focus the second and third doses), reportedly caused by stock-outs, distance in Cambodia's national strategy to reduce malaria morbidity and and treatment using Rapid Diagnostic Tests and Artemisinin-based Combination Therapies in hard-to-reach villages (>5km from closest health facility). VMWs play a key role in control and prevention, diagnosis and treatment of malaria as well as in delivering behavioral change communication (BCC) interventions to this target population. To evaluate the implementation of these activities performed by VMW/MMWs, a quantitative and qualitative assessment was conducted in 5 provinces of western Cambodia in order to: (i) understand job satisfaction of VMWs and MMWs vis-a-vis their roles and responsibilities; (ii) assess their performance according to their job descriptions; and (iii) gain insights into the challenges faced in delivery of diagnosis, treatment and health education activities to their communities. A total of 196 VMWs/MMWs were surveyed in October 2011 using a combination of quantitative and qualitative methods. Triangulation of quantitative and qualitative data helped to gain a deeper understanding of the success factors of this intervention and the challenges faced in implementation. Overall, levels of VMW performance were in line with the expected performance (80%); however, some performance gaps were identified in the areas of knowledge of malaria symptoms, treatment regimens, and key messages. In particular, there were low levels of practice of the recommended direct observed treatments (DOTs) approach for malaria treatment (especially for the second and third doses), reportedly caused by stock-outs, distance and transportation. The national malaria program should aim to focus on improving knowledge of VMWs in order to address misconceptions and barriers to effective implementation of DOTs at community-levels. In addition to the findings, the tools developed, will potentially help the national program to come up with better indicators in the near future. Findings from this evaluation are being used to inform planning of future activities and interventions such as DOT in a context where artemisinin drug resistance is a significant public health issue.

EVALUATION OF COMMUNITY MALARIA WORKER PERFORMANCE IN WESTERN CAMBODIA: A QUANTITATIVE AND QUALITATIVE ASSESSMENT

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Village/ Mobile Malaria Workers (VMWs/MMWs) are a critical component in Cambodia’s national strategy to reduce malaria morbidity and mortality. Since 2004, VMWs have been providing free malaria diagnosis and treatment using Rapid Diagnostic Tests and Artemisinin-based Combination Therapies in hard-to-reach villages (>5km from closest health facility). VMWs play a key role in control and prevention, diagnosis and treatment of malaria as well as in delivering behavioral change communication (BCC) interventions to this target population. To evaluate the implementation of these activities performed by VMW/MMWs, a quantitative and qualitative assessment was conducted in 5 provinces of western Cambodia in order to: (i) understand job satisfaction of VMWs and MMWs vis-a-vis their roles and responsibilities; (ii) assess their performance according to their job descriptions; and (iii) gain insights into the challenges faced in delivery of diagnosis, treatment and health education activities to their communities. A total of 196 VMWs/MMWs were surveyed in October 2011 using a combination of quantitative and qualitative methods. Triangulation of quantitative and qualitative data helped to gain a deeper understanding of the success factors of this intervention and the challenges faced in implementation. Overall, levels of VMW performance were in line with the expected performance (80%); however, some performance gaps were identified in the areas of knowledge of malaria symptoms, treatment regimens, and key messages. In particular, there were low levels of practice of the recommended direct observed treatments (DOTs) approach for malaria treatment (especially for the second and third doses), reportedly caused by stock-outs, distance and transportation. The national malaria program should aim to focus on improving knowledge of VMWs in order to address misconceptions and barriers to effective implementation of DOTs at community-levels. In addition to the findings, the tools developed, will potentially help the national program to come up with better indicators in the near future. Findings from this evaluation are being used to inform planning of future activities and interventions such as DOT in a context where artemisinin drug resistance is a significant public health issue.

MOLECULAR MARKERS OF ANTIMALARIAL DRUG RESISTANCE IN SOUTH-CENTRAL VIETNAM

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Malaria control programs introduced in Vietnam since 1990s have resulted in significant reduction in the number of malaria cases and deaths from the disease. Introduction of artemisinin derivatives and later artemisinin-based combination therapies (ACTs) for malaria treatment have contributed greatly to the overall success of this program. However, recent reports by WHO of reduced susceptibility of artesunate in Binh Phuoc Province in south Vietnam is of immense concern, which may hamper future efforts to control malaria in the country. Since 2006, we have conducted four drug efficacy trials (i.e. artemisinin-piperquine, dihydroartemisinin-piperazine and two formulations of artesunate-amodiaquine) in Phuoc Chien Commune, Ninh Thuan Province in south-central Vietnam for the treatment of uncomplicated Plasmodium falciparum malaria. The ACTs were found to be highly effective with PCR-corrected cure rates >98%. In the present study we have analysed the in vitro susceptibility of the field isolates collected in these trials to the commonly used antimalarials, as well as the polymorphism in
the major drug resistant markers: pfcr, pfdrf-ts and pfmdr1. Data on
the prevalence of polymorphisms in these markers, as well as the drug
susceptibility profiles of the clinical isolates will be discussed in the context
of the ACT efficacy trials results. We also compared our findings at Ninh
Thuan Province with published data from Binh Phuoc Province.

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CONTRASTING PATTERNS OF PLASMODIUM
VIVAX MULTIDRUG RESISTANCE GENE 1 (PVMDR1)
POLYMORPHISMS IN THAILAND AND CAMBODIA

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Plasmodium vivax remains a major source of malaria-related morbidity
in Thailand and Cambodia. Data on drug resistance polymorphisms in
vivax malaria populations from this region remains sparse. Studies have
linked polymorphisms in the P. vivax multidrug resistance (pvmdr1) gene
to chloroquine resistance and increase in pvmdr1 copy number to reduced
susceptibility to mefloquine and other ACT partner drugs. In this study,
we compared pvmdr1 resistance patterns between clinical isolates from
northwestern Thailand and southern Cambodia collected between 2006
and 2009. Pvmrd1 copy number was quantified by a novel multiplex
Taqman® real time PCR assay in 109 Cambodian and 49 Thai samples.
Copy number was considered increased if the calculated value was greater
than 1.7. Isolates were also sequenced to characterize the prevalence of
two pvmdr1 mutant SNPs (Y976F and F1076L). In total, a greater
proportion of Thai isolates displayed increased Pvmrd1 copy number (20%
vs. 0.9%, p<0.001). Prevalence of double mutants was higher among
Cambodian isolates than Thai isolates (95% vs. 7.7%, p<0.001).
The 1076L single mutant was dominant among Thai samples; while both
mutations occurred together in the vast majority of Cambodian samples.
Our data highlight contrasting patterns of pvmdr1 polymorphisms in
Thailand versus Cambodia. Selection for different Pvmrd1 haplotypes in
these two areas has likely been shaped by different drug policies in the
countries. Further studies looking at the distribution of drug resistance
alleles using microsatellites will help us gain a better understanding of the
evolution of drug resistant P.vivax malaria.

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COMPARING CHANGES IN EFFICACY OF ARTESUNATE-
MEFLOQUINE COMBINATIONS FOR THE TREATMENT OF
UNCOMPLICATED FALCIPARUM MALARIA IN THAILAND
DURING FIVE YEARS OF CLOSED DRUG MONITORING (2006-
2010)

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The treatment of uncomplicated Plasmodium falciparum malaria in
Thailand has been modified several times during the past 30 years to
counter the rapid emergence and spread of drug resistance. This study
was to compare the changes in efficacy of two-day and three-day
combination of artesunate and mefloquine (AS2M and AS3M). The study
was conducted during 2006-2010 in 7 international bordered provinces
to Burma, Cambodia and Malaysia. A total of 1,034 Uncomplicated
falciparum malaria patients were enrolled in two phases, during 2006 and
2007, received ASM2 while those recruited during 2008-2010 received
ASM3. All were followed for 42 days. This study found that the efficacy of
artesunate-mefloquine combination was not only based on the drugs, but
also the treatment regimen and variation of parasite genetics in different
locations. Continuation of the monitoring of antimalarial drugs efficacies
are necessary to cope up with the changing in efficacies.

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A RANDOMIZED TRIAL OF TEXT MESSAGE REMINDERS TO
INCREASE ADHERENCE TO MALARIA TREATMENT

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Despite the massive international efforts made over the past decades,
malaria continues to be one of the primary causes of under-5 mortality
worldwide. Several recent studies document low adherence to artemisinin-
based combination therapies (ACTs). Low adherence undermines the
chances of patients fully recovering from acute malaria and increases
the likelihood of the emergence of resistant strains of the parasite. We
conducted a randomized controlled trial to investigate the impact of
text message reminders on adherence to ACTs in Tamale, Ghana. One
thousand one hundred forty participants were recruited from drug
shops, licensed chemical sellers, public and private hospitals, and other
ACT vendors when purchasing malaria medicine. Participants were
randomized by automated system to the treatment group or the control
group. Patients in the treatment group received six reminders, one for
each dose of malaria treatment, sent out in 12 hour intervals. The primary
outcome was adherence based on completion of treatment regimen.
Adherence was assessed through observation of pill-packets and through
self-reports at unannounced home follow-ups timed to coincide with
the completion of treatment. The follow-up rate was 99.6%. Receiving
text message reminders increased the odds of adherence by 34% (95%
CI [0.992-1.810], p-value 0.056) when a short message was used, and
by 0% (95% CI [0.747-1.316], p-value 0.954) when a long message was
used. Text message reminders appeared to work best when sent to the
caretaker of child patients, with short messages increasing the odds of
adherence by 125% (95% CI [1.299-3.890], p-value 0.004).

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THE INFLUENCE OF ENVIRONMENTAL RISK FACTORS AND
INDIVIDUAL BEHAVIORS ON MALARIA OCCURRENCE IN
LAHAD DATU DISTRICT OF SABAH, MALAYSIA: A CASE
CONTROL STUDY

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Malaria is a parasitic disease and continue to be a major public health
problem worldwide, it is estimated that 300 million people were infected
by malaria with more than a million deaths throughout the world.
In Malaysia, malaria incidence has decreased from 23.4 per 10 000
population in 1991 to 2.8 cases per 10 000 population in 2003. The
incidence of malaria in the state of Sabah is the highest in Malaysia. In
2004, the incidence was 9.56 cases per 10 000 population which was
the highest among the states. In the same year, Lahad Datu district
recorded incidence of 24 cases per 10 000 population. Thus, this study
was conducted to identify the factors which influence malaria infection in
Lahad Datu district of Sabah. Malaria cases which were notified to district

www.astmh.org
and controls which were matched by age, sex and mukim were analyzed. McNemar Odds Ratio (OR,) calculated using McNemar Calculator for bivariate analysis while Conditional Logistic Regression was used for multivariate analysis. Data were coded and analyzed by using SPSS version 12.0. From the results, most of the malaria patients (95%) that included in the study were below 50 years old. 80.7% were male and only 19.3% were female. Based on OR, it was found that socioeconomic status, risk perception, movement, preventive behaviours, house structure and design, and presence of potential mosquito-breeding place were risk factors for malaria infection. Multivariate analysis showed cases were more likely to have a high risk occupation (OR= 4.14, 95% CI= 1.04-16.57), low risk perception towards danger of malaria (OR= 6.01, 95% CI= 1.22-29.63), no residual spray done at home (OR=3.70, 95% CI=1.02-13.39) and have history of movement in the last two weeks (OR=5.25, 95% CI= 1.03-26.71). As a conclusion, some individual behaviours and preventive factors are strong risk factors for the occurrences of malaria cases in Lahad Datu district. With the identification of the potentially modifiable risk factors, a proper public health intervention can be implemented.

ELEVATED INCIDENCE OF NON-FALCIPARUM MALARIA DURING THE RAINY SEASON 2011 IN NIGER

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Non-falciparum malaria is not benign disease states, but little attention is devoted to them in sub-Saharan Africa due to the prominence of falciparum malaria and its attendant complications. Most studies in sub-Saharan western Africa describe the frequency of non-falciparum malaria as 10% or less. Following implementation of a 2 step protocol utilizing both light microscopy (LM) and the addition of a malaria rapid diagnostic test (RDT) (Standard Diagnostics, Malaria Antigen, 05FK60) a retrospective review of laboratory records for 282 patients during a single four month rainy season was performed. Results indicated a higher than anticipated frequency of 24.6% positive tests for non-falciparum malaria species and 79.4% falciparum-positive tests. When LM alone was used for diagnosis, the rate of positive smears was 79%. When LM was combined with an RDT, the rate of positive tests decreased to 32%. With a population suffering from high prevalence of malnutrition and disadvantaged economic status, the Nigerian population is at significant risk from non-falciparum malaria. Potential reasons for an elevated incidence of non-falciparum malaria in this population are discussed.

THE EVALUATION OF EASY ACCESS GROUPS AS A TOOL FOR MONITORING TEMPORAL CHANGES IN MALARIA TRANSMISSION AND COVERAGE OF CONTROL INTERVENTIONS IN MALAWI: THE EVALMAL STUDY

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Currently recommended tools for measuring progress of malaria control involve large, logistically and financially demanding population-based household surveys that provide national and provincial level estimates at intervals of 2 - 5 years. Since malaria transmission intensity and disease incidence can vary widely within a country though, programmatic decisions are often made at district level. With the change of focus from control of burden to reduction of transmission and the recent progress made, malaria trends in older (and asymptomatic) age groups become more important. Timely, valid, low-cost district and local level estimates of short- and medium-term control progress are urgently needed to support the move towards the control and elimination of malaria. Opportunistic sampling in planned or spontaneous aggregations of sub-groups of the population, the so-called Easy Access Groups (EAGs), offers the prospect of a less resource intensive method of deriving estimates of control progress. Children >4 months presenting at the EPI vaccination clinic at Chikwawa District Hospital, any accompanying older sibling(s) aged <15 years, and their parents/guardians were surveyed monthly since April 2011. A modified version of the RBM MERG MIS questionnaire will be administered to the parent/guardian. A finger blood sample was collected for a blood film, a malaria rapid diagnostic test, haemoglobin assessment and a filter-paper blood spot for serology. The estimates of burden of disease and uptake of control interventions were compared to that of a rolling Malaria Indicator Survey (mMIS) in the same population. The data presented is from the first year of the study (April 2011 - March 2012). The results will focus on the comparison of estimates derived from the EPI EAG and the mMIS. In conclusion, we determined if valid population level estimates of malaria intervention coverage and burden indicators and their short-term temporal trends can be obtained from opportunistic sampling in EAGs.

NON-RANDOM ASSOCIATIONS OF T-CELL EPITOPES IN PLASMODIUM FALCIPARUM CIRCUMSPOROZOITE PROTEIN, LILONGWE, MALAWI

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Cellular immunity to Plasmodium falciparum circumsporozoite protein (CS) is mediated by two immunodominant T cell epitopes (TH2 and TH3). Recent studies have found non-random associations between these epitopes in natural parasite populations, suggesting constraints on permissible TH2/TH3 combination haplotypes. Using sequence data from parasites from Lilongwe, Malawi (235 isolates) and The Gambia (44 isolates), we evaluated the extent of non-random association between the epitopes. In both populations, T-cell epitopes did not assort randomly. In fact, some combinations of TH2 and TH3 epitopes occurred more frequently than expected by random chance in both populations. The mechanism driving this deviation from random assortment is unclear; however it appears similar in parasite populations from East and West Africa. Potential explanations would include selection of certain combinations (by human immunity or within the mosquito host) or functional constraints on protein secondary/tertiary structure. Interestingly, among the Malawian isolates some of the most over represented combinations were highly similar to the T-cell epitopes contained in the RTS,S vaccine. In total, we found 118 (50.2%) isolates having at least a TH2 or TH3 epitope within one amino acid of the RTS,S vaccine type, while 230 (97.9%) have a TH2 or TH3 epitope within two amino acids of the RTS,S. Further characterization of this phenomenon is ongoing and is likely to be important in the design of next generation CS based vaccines.
DIFFERING SIGNATURES OF SELECTION ON TWO PLASMODIUM VIVAX CANDIDATE VACCINE ANTIGENS

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To date, candidate vaccine antigens for Plasmodium vivax have primarily been selected based on their orthology to P. falciparum vaccine candidates. In several studies, falciparum antigens have shown genomic signatures of immune selection. However, few studies have evaluated if candidate vivax antigens show similar signatures of selection. We explored the genetic diversity of two P. vivax vaccine candidates, the circumsporozoite protein (pvcsp) and the merozoite surface protein 1 (pvmsp1), in a panmictic Cambodian vivax population, using scalable next-generation sequencing. We assessed these loci for evidence of selection and compared the results to similar analyses of other, geographically distinct P. vivax populations. In geographically diverse P. vivax populations, the 42-kd region of pvmsp1 consistently displayed a signature of strong balancing selection. Moreover, interspecies comparisons of orthologous antigens revealed that a subregion within the 42-kd block of pvmsp1 is under strong selection for non-synonymous nucleotide changes. In contrast to pvmsp1, the N-terminal and C-terminal conserved regions of pvcsp showed minimal evidence of balancing or directional selection. However, pvcsp sequences were highly heterogeneous, due to the central repeat region which appears to be under immune selection. As evidence, VK210 repeat arrays display a significantly higher proportion of non-synonymous nucleotide polymorphisms compared to VK247 arrays. In addition, repeat length polymorphisms appear to have occurred by a rapid and recent expansion as determined by mismatch distributions of the repeat arrays. These results demonstrate that immune selection on these antigens likely results in two different adaptive patterns, each increasing the genetic diversity of these candidate vaccine antigens in a different way. Similar to falciparum malaria, genomic approaches to detect alleles under immune selection may identify novel targets of immunity.

THE EFFECT OF MALARIA AT DELIVERY ON FETAL ANEMIA AND THE ROLE OF INTERMITTENT PREVENTIVE TREATMENT DURING PREGNANCY IN MALAWI

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Fetal anemia is common in malaria areas and is a risk factor for infant morbidity and mortality. Malaria during pregnancy may decrease cord hemoglobin (Hb) and cause fetal anemia among newborns. Intermittent preventive treatment in pregnancy (IPTp) with sulfadoxine-pyrimethamine (SP) is protective against malaria, but has also been hypothesized to contribute to fetal anemia by affecting hematopoiesis. Peripheral, placental, and cord blood were examined for malaria parasitemia and hemoglobin concentration in a cross-section of 3,848 mothers and infants delivered at Queen Elizabeth Central Hospital in Blantyre, Malawi between 1997 and 2006. Unconditional linear and logistic regressions were performed with multiple imputation for missing covariates to assess the associations between malaria, IPTp with SP, and fetal anemia (cord Hb <12.5 g/dL). The overall prevalence of fetal anemia was 7.9% (n=304).

Malaria parasitemia at delivery was associated with an increase in cord Hb of 0.24 g/dL (95% confidence interval (CI): 0.05, 0.42), adjusting for SP use, gravidity, year, and season of delivery. The adjusted prevalence odds ratio (POR) for the effect of malaria on fetal anemia was 1.41 (95% CI: 1.05, 1.90). Primigravidae who did not take IPTp had infants at highest risk for fetal anemia (adjusted POR: 3.37, 95% CI: 1.68, 6.78), and density of parasitemia was correlated with a decrease in cord Hb of 0.33 g/dL (95% CI: 0.14, 0.53) and 0.35 g/dL (95% CI: 0.13, 0.57) per log increase in placental and peripheral parasitemia respectively. There was no significant association between SP use and cord Hb or fetal anemia (adjusted POR: 0.98, 95% CI: 0.69, 1.39). Malaria during pregnancy, but not IPTp, decreases cord Hb and is a risk factor for fetal anemia in Malawi. IPTp with SP may continue to be safe and effective in preventing malaria during pregnancy and fetal anemia despite development of SP resistance.

MODELLING THE DEVELOPMENT OF ACQUIRED IMMUNITY TO PLASMODIUM FALCIPARUM MALARIA

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Development of immunity to malaria is a significant epidemiological process. It influences the transmission dynamics of the infection and disease, dictates how severe an infection will be and influences the evolutionary dynamics of the parasite due to host immune pressure. Despite the importance of immunity, it is unclear what mechanisms are involved in its development. The primary goal of this project is to describe the process of acquisition of immunity in a population with repeated exposure using a mathematical model of malaria transmission. A plethora of malaria control strategies have been implemented and others such as a malaria vaccine offer future prospects. The impact of these programmes on malaria transmission and subsequently the effect they have on the rate of development of immunity will also be evaluated in this study.

GENOTYPING OF PLASMODIUM FALCIPARUM USING ANTIGENIC POLYMORPHIC MARKERS AND TO STUDY ANTI-MALARIAL DRUG RESISTANCE MARKERS IN MALARIA ENDEMIC AREAS OF BANGLADESH

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In the past many regions of Bangladesh were hyperendemic for malaria. Malaria control in the 1960s to 1970 eliminated malaria from the plains but the Chittagong Hill Tracts remained as a difficult to control reservoir. Northeastern Bangladesh with many tea plantations has malaria rates below 1% with approximately 10-20% Plasmodium vivax. The Chittagong Hill Tracts have areas with between 1 and 10% annual malaria rates predominately 90-95% P. falciparum. In southeast Asia multiplicity of infection for hypendemic regions has been approximately 1.5. Few studies on the population dynamics of P. falciparum have been performed in Bangladesh. Here we report polymorphic and drug resistant genotype analysis on 33 paired recrudescent infections after drug treatment in the period 2004-2008 in the Chittagong Hill Tracts which is just prior to countrywide provision of artemisinin combination therapy. Overall the multiplicity of infection for MSP-1 was 2.7 with a slightly smaller parasite diversity post-treatment. The 13 monochlonal infections by both GLURP and MSP-1 were evenly divided between pre- and post-treatment. The MSP-1 MAD block was most frequent in .66 of the samples. The prevalence of the K76T PfCRT chloroquine resistant allele was approximately 82% of the samples, while the resistant Pfmdr1 N86Y was present in 33% of the samples. Interestingly the post-treatment samples had a small but
significantly higher amount of the sensitive PFCRT alleles by RT-PCR. In conclusion, the parasite population retains a high population diversity despite hypoendemic transmission with retention but decrease in the chloroquine resistant allele and Pfmdr1 resistant alleles.

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POPULATION STRUCTURE AND SPATIAL DISTRIBUTION OF PLASMODIUM FALCIPARUM CIRCUMSPOROZOITE PROTEIN NANP REPEATES IN LILONGWE, MALAWI

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Humoral immunity to Plasmodium falciparum circumsporozoite protein (CS) is mediated by a central region of the protein containing a repetitive tetra-amino-acid repeat termed the “NANP repeat.” Genetic analysis suggests that variants with different repeat lengths have spread recently in the population by a rapid mechanism such as slip-strand mispairing. It has been suggested that this is an adaptive mechanism of the parasite to evade immune recognition by the host. In some studies of RTS,S vaccine efficacy, levels of antibodies to this region of CS have been the most highly correlated marker of protective immunity. To date there have been no descriptions of the population structure of P. falciparum based upon differences in these repeats. Using filter paper blood spots from 100 participants in a study in Lilongwe, Malawi, we used capillary electrophoresis to determine the size of the NANP repeat region of parasite variants. Preliminary results confirm that infection by multiple genetically distinct variants of the parasite is common and that genetic diversity of P. falciparum infections is similar in adults and children. As each participant is geolocalized, this allows us to assess the spatial distribution of parasite variants, spatial variation in parasite diversity and the impact of environmental factors (such as proximity to water) in a multivariable spatial model of this diversity using our ArcGIS database.

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GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY PREVALENCE IN THE GAMBIA

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Current malaria treatment guidelines recommend the use of primaquine as gametocytocidal treatment for falciparum malaria in settings targeting elimination. However, fears on the primaquine’s potential hemolytic effect in individuals with glucose-6-phosphate dehydrogenase deficiency (G6PDd) have precluded its implementation, particularly in sub-Saharan African countries where the prevalence of G6PDd is either unknown or outdated. In this study, we present genotype and phenotype data in The Gambia and describe phenotype profiles for mutations with reported high prevalence in the Senegambia region. Filter paper blot spots from 3,100 healthy children aged 6-14 years collected during a school survey were analysed. Enzyme activity was determined quantitatively with a commercial test kit (Atlas Medical®) and results adjusted for individual haemoglobin level. The frequencies for the A (A376G) and A- variant mutations, G202A, T968C and A542T were determined using Taqman® assays. The correlations between genotype and enzyme activity was also studied. Fifty-two percent of children were male and the mean haemoglobin was 12.4 (SD 1.3) g/dL. Preliminary analysis showed median activity of 6.5 (range 0-22) U/gHb in the analysed subset. The prevalence the A (A376G) and A- (A202G) mutations was 37.8% and 4.0%, respectively. The wide range of enzyme activity observed together with a low prevalence of the A- (A202G) genotype suggests that phenotype-based assessment may be needed before wide scale use of primaquine.

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RESPONDENT-DRIVEN SAMPLING ON THE THAILAND-CAMBODIA BORDER. I. CAN MALARIA CASES BE CONTAINED IN MOBILE MIGRANT WORKERS?

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Reliable information on mobility patterns of migrants is a crucial part of the strategy to contain the spread of artemisinin-resistant malaria parasites in Southeast Asia, and may also be helpful to efforts to address other public health problems for migrants and members of host communities. In order to limit the spread of malarial drug resistance, the malaria prevention and control programme will need to devise strategies to reach cross-border and mobile migrant populations. The Respondent-Driven Sampling (RDS) method was used to survey migrant workers from Cambodia and Myanmar, both registered and undocumented, in three Thai provinces on the Thailand-Cambodia border in close proximity to areas with documented artemisinin-resistant malaria parasites. 1,719 participants (828 Cambodian and 891 Myanmar migrants) were recruited. Subpopulations of migrant workers were analysed using the Thailand Ministry of Health classification based on length of residence in Thailand of greater than six months (long-term, or M1) or less than six months (short-term, or M2). Key information collected on the structured questionnaire included patterns of mobility and migration, demographic characteristics, treatment-seeking behaviours, and knowledge, perceptions, and practices about malaria. Workers from Cambodia came from provinces across Cambodia, and 22% of Cambodian M1 and 72% of Cambodian M2 migrants had been in Cambodia in the last three months. Less than 6% returned with a frequency of greater than once per month. Of migrants from Cambodia, 32% of M1 and 68% of M2 were planning to return, and named provinces across Cambodia as their likely next destinations. Most workers from Myanmar came from Mon state (86%), had never returned to Myanmar (85%), and only 4% stated plans to return. In conclusion, information on migratory patterns of migrants from Myanmar and Cambodia along the malaria endemic Thailand-Cambodian border within the artemisinin resistance containment zone will help target health interventions, including treatment follow-up and surveillance.

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MALARIA AND GRAVIDITY INTERACT TO MODIFY MATERNAL HAEMOGLOBIN CONCENTRATIONS DURING PREGNANCY

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Since the implementation of intermittent preventive treatment (IPTp) in sub-Saharan Africa, the effect of malaria-focused preventive measures on anaemia in relation to gravidity has been seldom investigated. We analysed data from 3 studies carried out in nearby areas in south Benin between 2005 and 2012. At inclusion (ANV1) women’s age, area of residence, schooling, parity, gestational age, weight and height were recorded. Thick blood smears were performed on ANV1, second visit (ANV2) and at delivery. Women’s serum ferritin and CRP concentrations...
were also assessed. The impact of gravidity on maternal haemoglobin (Hb) was analysed using a logistic or linear regression depending on the outcome. The statistical significance was set to $P < 0.05$.

The study was approved by the Ethics Committee of the Faculty of Medicine of Cotonou in Benin. In total, data from 3591 pregnant women were analysed. Both univariate and multivariate analyses showed a constant association between Hb concentrations and gravidity in the 3 periods of Hb assessment (ANV1, ANV2 and delivery).

Mean Hb concentration was significantly lower in primigravidae than in multigravidae at ANV1 (mean difference = -2.4 g/L, $P < 0.001$). Afterwards, it increased importantly in primigravidae only, with a tendency to reversal between primigravidae and multigravidae which was confirmed at delivery (mean difference = 2.8 g/L, $P < 0.001$). The prevalence of malaria was halved between ANV1 and delivery in primigravidae while it decreased only by 38% among multigravidae, who were less prone to be infected (malaria prevalence at ANV1, 20% and 10% respectively). Iron deficiency was more common in multigravidae, and it decreased slightly in this group between ANV1 and delivery. In a context of IPTp, primigravidae were shown to improve progressively haemoglobin concentration throughout pregnancy. In multigravidae, the improvement was less perceptible as anaemia was mainly due to iron deficiency. There is a need to reinforce malaria prevention strategies in both groups.

### ASSESSING THE ASSOCIATION BETWEEN MALARIA CHEMOPREVENTION AND THE NUTRITIONAL STATUS OF A COHORT OF YOUNG AFRICAN CHILDREN

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Malaria and malnutrition are common causes of morbidity and mortality in African infants. Data are limited as to whether antimalarial chemoprevention improves nutritional status. We compared the nutritional status of 393 infants living in Tororo, Uganda and randomized to 4 antimalarial chemoprevention arms at 6 months of age; no therapy, monthly sulfadoxine-pyrimethamine (SP), daily trimethoprim-sulfamethoxazole (TS) or monthly dihydroartemisinin-piperine (DP).

Anthropomorphic measures were made monthly and the primary outcomes of interest were a drop of $>1$ standard deviation (SD) in the height-for-age (HAZ) and weight-for-age (WAZ) z-scores from 6 to 18 months of age. Covariates of interest included breastfeeding status, maternal age, household wealth and chemoprevention arm. Associations between worsening nutritional status and covariates of interest were estimated using multivariate logistic regression. Mean baseline HAZ and WAZ scores were -0.98 and -0.42, respectively. From 6 to 18 months of age, 45% and 23% of infants had a drop of $>1$ SD in their HAZ and WAZ scores, respectively. Continued breastfeeding at 18 months was protective against a $>1$ SD drop in the HAZ ($OR=0.53$, $p=0.04$) and WAZ ($OR=0.18$, $p<0.001$) score. Compared to a maternal age of over 25 years, a maternal age of 18 years or younger was protective against a $>1$ SD drop in the HAZ ($OR=0.30$, $p=0.001$) and WAZ ($OR=0.36$, $p=0.03$) score. There were no significant associations between household wealth or chemoprevention and worsening nutritional status and with the exception of a trend towards a lower odds of a $>1$ SD drop in the WAZ score ($OR=0.46$, $p=0.06$) among infants randomized to monthly SP compared to those randomized to no therapy. In this cohort of infants living in a rural area of Uganda with high malaria transmission intensity, chemoprevention did not clearly improve nutritional status but sustained breastfeeding and younger maternal age were protective against worsening nutritional status from 6 to 18 months of age. Results will be updated through September 2012 when all infants have reached 24 months of age.

### MATERNAL ANEMIA IN PREGNANCY: ASSESSING THE IMPACT OF PREVENTIVE MEASURES IN A MALARIA ENDEMIC AREA

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Although widely implemented, the effectiveness of anaemia preventive measures (intermittent preventive treatment in pregnancy (IPTp), anti-helminthic and haematinics) on maternal anaemia at different time points of gestation in sub-Saharan Africa still need to be documented. 1005 pregnant women participating in a clinical trial of IPTp were followed from early pregnancy until delivery between 2010 and 2012 in southern Benin, where malaria transmission is perennial. On inclusion (ANV1), baseline characteristics of the women were recorded. At ANV1, the second antenatal visit (ANV2) and delivery, gestational age was assessed and anthropometric measurements were made. The first and second intakes of IPTp were given on ANV1 and ANV2 under supervision. A treatment dose of albendazole and haematinics were given at ANV1 to be taken home. At all time points, haemoglobin (Hb) levels, malaria and helminth infections were determined. Serum iron, folate, vitamin B12,
A PILOT SCHOOL SURVEY TO ESTIMATE THE MALARIA BURDEN IN A SETTING WITH DECLINING ENDEMICITY

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In a setting with declining malaria endemicity such as The Gambia, identification of malaria infected individuals is increasingly harder. School surveys may represent an easy and inexpensive method to identify foci of malaria transmission. The aim of this study was to evaluate the use of school surveys to estimate the malaria burden and identify foci of transmission, in the population. We carried out a school survey in the Upper River Region, The Gambia, in May-June 2011, before the start of the malaria transmission season. Thirty two primary schools were selected with probability proportional to size and in each of them 100 pupils were randomly enrolled. Data on socio-demographic variables and was collected through a questionnaire. A blood sample was collected for 1) detection of antimalarial antibodies against merozoite surface protein (MSP) 1, MSP2, and apical membrane antigen-1 (AMA-1) by ELISA, 2) microscopy (sexual forms and gametocytes), 3) PCR detection of malaria infection, and 4) haemoglobin by Hemocue. Three thousand two hundred seventy seven children (48% girls) were included in the survey. The mean age was 10 years (range: 4-21). Bed net use was 73%. About 17% had a history of fever in the past 48 hours while 3% had fever (axillary temperature ≥37.5°C) at the time of the survey; none was positive by rapid diagnostic test. About 11% of the children had anaemia (haemoglobin<11g/dL). The parasite prevalence was 10% (309/2681) for Plasmodium genus, and 9% (277/2871) for P. falciparum species. There was evidence of heterogeneity in the parasite prevalence across schools. In addition there was heterogeneity in age, reported use of bed nets, and anaemia across the schools. School survey data can be used to determine the malaria burden and identifying foci of malaria transmission in regions of declining endemicity. These foci will be sites for other studies to determine the cause of heterogeneity and interventions that can contribute to malaria elimination efforts.

THE MALARIA HOUSEHOLD COST OF CHILDREN UNDER FIVE YEARS OLD IN BURKINA FASO (WEST AFRICA)

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An estimated 700,000-2.7 million persons die of malaria each year, 75% of them African children. This disease is the major cause of health facilities use. This paper estimates the direct and indirect cost of simple and severe malaria for under 5 years children for household in order to provide a better understanding of the burden of malaria to household. Data have been collected from 506 households of 24 villages of Nanor’s demographic surveillance site in Burkina Faso. A random sampling of household was done. We included households with children under five years old and we exclude children enrolled on the malaria vaccine trial. The obtained informations has been used to estimate the direct and indirect costs of malaria treatment. The direct cost of malaria treatment includes all cash expenditures on seeking malaria care by patients and their caretakers. The indirect costs included all cash expenditures on transportation and non medical supplies. Households were interviewed about their expenditure on malaria treatment for children under 5 years old. Simple random sampling was used to select villages and households with at least one child under 5 years old. Malaria was ascertained not by parasitological tests but through fever using a recall period of one month. The survey was conducted during the high transmission season in 2010. Durinh household survey, the average cost of treating an episode of the disease including direct and indirect costs for household were 7.83USD. During exit interview this cost was evaluated 8.5 USD at OPD and 7.19 USD respectively at Outpatient OPD and Inpatient IPD. The average total cost for rich households was higher than the poorest one. The productivity cost was 6.01 USD at household survey, 5.72 USD at OPD and 37.71 USD at IPD exit interview. In conclusion, there is an equity access of malaria care for children under 5 years old. Productivity cost are the most important for household.Indirect cost reduction will contribute to individuals and family well being.

DRUG THERAPY OF SUSPECTED MALARIA CASES BEFORE THEIR ADMISSION IN A DISTRICT HOSPITAL IN BURKINA FASO DURING THE DRY SEASON

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Misuse of antimalarial drugs has led to the emergence of resistant Plasmodium falciparum strains. Malaria treatment protocols were reviewed at the beginning of 2000 in African countries and artemisinine based combination therapy (ACT) was introduced. To describe the treatment itinerary of suspected cases of malaria before their admission to the district hospital of Do, seven years after the introduction of ACT in Burkina Faso. From December 2010 to May 2011, we conducted a cross-sectional survey of suspected malaria cases admitted at the district Hospital during the dry season (malaria low incidence season). We included all patients aged 6 months or above, recorded as suspected malaria according to the criteria of national malaria control program, excluding those with chronic defects. 476 suspected cases, out of which 422 (88.7%) uncomplicated and 54 (11.3%) complicated, were recruited, representing 7.9% of the admissions. The number of cases decreased from December (207 cases) to May (14 cases) with a monthly average of 79 cases. The average age was 14.4 years, ranging from 6 months to 76 years. Cases under 5 years were 168 (35.3%). Treatment itineraries were mainly: initial consultation in a health facility of first resort (public or private clinic), 20 cases (4.2%); direct consultation to the district hospital, 104 cases (21.8%); initial consultation with a traditional healer, 3 cases (0.6%); initial self-medication, 346 cases (72.7%); out of the latter, 331 cases (95.6%) then consulted directly at the district hospital. The practice of self-medication did not differ between those aged less than 5 years and those above 5 years and over (OR = 0.6, 95% IC = 0.4 - 1.0), or by gender (OR = 1.2, 95% IC = 0.8 - 1.9). Self-medication drugs involved were mainly antipyretics (n = 327) and antimalarials (n = 58). Out of the latter, ACT was used in 39.6% of cases, quinine in 19.0% and non-recommended antimalarials, such as sulfadoxine-pyrimethamine, amodiaquine and chloroquine, in 41.4%. A total of 112 cases (23.5%) had positive thick blood smear, including 18
cases (16.1%) who had taken an antimalarial. During the dry season, the treatment itinerary of suspected malaria cases is marked by a short circuit at health care level and use of non recommended antimalarials by self-medication. Complementary analysis of the itinerary during the epidemic season may help to define more appropriate strategies to sensitize the population.

REGIME SHIFTS, HETEROGENEOUS TRENDS AND INDIAN OCEAN DIPOLE INDUCED SYNCHRONY IN MALARIA TIME SERIES FROM KENYAN HIGHLANDS

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Large malaria epidemics in the East African highlands during the mid and late 1990s kindled a stream of research on the role that global warming might have on malaria transmission. Most of the inferences using temporal information have been derived from a malaria incidence time series from Kericho. Here we examined whether observed patterns in that time series were common across other localities in the lake Victoria basin of Western Kenya. We found that temporal trends were decreasing yet heterogeneous. Time series from localities above 1600 m showed regime shifts that coincided with the 1998 Indian Ocean Dipole,IOD. We found all the time series to more closely follow the interannual patterns of Variability of the IOD than El Niño Southern Oscillation, and we found the time series had a synchronous pattern that resembled a Moran effect, i.e., their patterns of concerted fluctuation were higher than the observed environmental correlation. The heterogeneity in malaria trends probably reflects the multitude of factors that can drive trends of malaria transmission and highlights the need for both spatially and temporally fine-grained data to make sound inferences about the impacts of climate change on secular changes in malaria transmission. Nevertheless, synchronous malaria epidemics call for the integration of knowledge on the forcing of malaria transmission by environmental variability to develop robust malaria control and elimination programs.

ASYMPTOMATIC PLASMODIUM SP. INFECTION AND COGNITION AMONG PRIMARY SCHOOLCHILDREN AGED 6-14 YEARS IN A HIGH MALARIA TRANSMISSION SETTING IN UGANDA

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In areas of high malaria transmission, asymptomatic Plasmodium infection is commonplace among school children, yet little is known about its impact on children’s cognitive function. We investigated the association between asymptomatic Plasmodium infection and measures of sustained attention and abstract reasoning among primary school children in Tororo district, Uganda, a high malaria transmission area. In randomized placebo controlled trial assessing the impact of intermittent preventive treatment for malaria on morbidity and cognitive function, 740 children were enrolled. A detailed history and physical examination was conducted. Stool samples were examined for helmith infections and blood smears for malaria parasites. Two tests of cognition were administered to children: Raven’s matrices for abstract reasoning and code transmission tests for sustained attention. Differences in mean test scores were analysed using t-tests and multivariable linear regression models. Of the 740 children at baseline, the mean (SD) age was 9.9 (4.3) years and 53.3% were females. The prevalence of Plasmodium spp. infection was 30.1%, with the majority of infections due to P. falciparum. Ninety percent of children reported coming from a household with at least one mosquito net but net use the previous night was only 36%. Children with Plasmodium infection had significantly lower mean scores in tests of abstract reasoning (37.6 versus 42.7 p<0.0001) and sustained attention (42.2 versus 49.5 p<0.0001) compared to uninfected children. Other factors significantly associated with poor scores in the cognitive function tests included sex, age and weight. In conclusion, despite high household coverage of mosquito nets, net use is unacceptably low among schoolchildren and Plasmodium infection remains highly prevalent and is strongly associated with poor cognition in schoolchildren. Such results underline the need for targeted approaches to malaria prevention and control in schoolchildren.

PREVALENCE AND CORRELATES OF MALARIA PARASITEMIA IN PEOPLE LIVING WITH HIV/AIDS ATTENDING THE LAQUINTINIE HOSPITAL IN DOUALA, CAMEROON

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A substantial number of people living with HIV/AIDS (PLWHA) inhabit areas of high malaria transmission. To provide data to improve the prevention and care of malaria in such patients, we assessed the prevalence and socio-demographic and clinical correlates of malaria parasitaemia in PLWHA. Between April-June 2010, a cross-sectional study of adult PLWHA attending the Douala Laquintinie Hospital was conducted. After obtaining consent, socio-demographic and clinical data were obtained via a standardized questionnaire. Malaria parasitaemia was determined by blood smear microscopy. To determine correlates, means were compared using t-tests while proportions were compared using chi-square tests. The 238 PLWHA enrolled had a mean age of 40.8±10.5 years. Most (67.6%) were females, 48.3% were on antiretroviral therapy and 41.4% had CD4+ counts<200 cells/ml. The proportion of participants who reported using bed nets and insecticides were 36.1% and 37.4% respectively. Overall, the malaria prevalence was 24.8%. Malaria prevalence was not significantly lower in patients using bed nets (23.3%), using insecticides (23.6%), nor in those on antiretroviral therapy (24.4%). Although malaria prevalence was higher in patients with CD4+ counts <200 cells/ml (30.6%) compared to those with CD4+ counts ≥200 cells/ml (20.1%) this did not achieve statistical significance (P=0.07). Malaria parasitaemia was prevalent in this population of PLWHA. Very few patients reported using preventive methods and even then, the self-reported measures taken to prevent malaria did not seem to be effective. Because of the potential for worse HIV outcomes in the presence of malaria (even when asymptomatic), malaria prevention and treatment (if indicated) needs to be reinforced.

MALARIA GAMETOCYTE PREVALENCE IN NORTHERN KWAZULU-NATAL, SOUTH AFRICA

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South Africa has embarked on the ambitious goal of halting malaria transmission within its borders by 2018. If this goal is to be attained, all malaria cases must be detected and effectively treated. It has been suggested that primaquine become standard first line treatment together with artemether-lumefantrine to prevent onwards transmission. This suggestion has raised some health concerns as primaquine has been associated with haemolysis in glucose-6-phosphate dehydrogenase (G6PD) deficient individuals. To address this issue a pilot study to assess gametocyte carriage in northern KwaZulu-Natal was conducted during 2011/2012 malaria season. The Umkhanyakude Municipality of KwaZulu-Natal, South Africa was selected for the study due to its relative high malaria prevalence. Filter paper finger prick blood spots were collected during a community based survey. Parasite mRNA extracted from the
filter paper samples was subjected to a gametocyte specific reverse transcriptase PCR. Results from this study will be used to inform a policy on primaquine usage in KwaZulu-Natal.

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AN EVALUATION OF CHART ABSTRACTION TO ASSESS THE QUALITY OF CASE MANAGEMENT FOR INPATIENTS WITH SEVERE MALARIA - BENIN, 2010

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Ensuring high quality care for inpatients with malaria is critical. Chart abstraction offers a potentially efficient method to assess quality of care. However, as the availability and quality of inpatient charts in Benin was unknown, we conducted a study of the feasibility of sampling and abstracting charts, the validity of abstracted data, and the extent of missing data. Chart abstraction was conducted in July 2010 from a probability sample of inpatients (any age) in five Beninese hospitals (method 1). We also compared abstraction to interviews with health workers (HWs) and their patients admitted 12-48 hours earlier (N=11) (method 2), and interviews with HWs regarding patients they discharged within 72 hours (N=10) (method 3). Analysis of all methods focused on 11 signs of suspected malaria and severe disease, test results, and treatment. For method 1, we sampled 4% (60/1383) of inpatients admitted in June 2010. Of 60 patients sampled, 45 (75%) charts were retrieved and abstracted; 43 suspected malaria cases were identified. Of 473 signs, 179 (37%) were documented in charts. In 74% (32/43) of charts, at least one sign was present to identify severe disease. Antimalarial treatment was documented in 81% of charts of patients with suspected malaria (35/43). Interviews of HWs and admitted patients (method 2) showed that 96% (45/47) of documented signs were valid. HW interviews regarding discharged patients (method 3) showed that 35% (19/55) of non-documented signs were not assessed by HWs. Malaria test results were documented in 65% of charts (41/63) (methods 1, 2, 3). Abstraction from inpatient charts was feasible, and documented data were valid. Despite poor documentation, data were sufficient to identify severe illness for three-quarters of patients. Charts contained moderate levels of testing and high levels of treatment information. This study was limited by small sample size and possible recall bias. We recommend chart abstraction for an inpatient survey and introducing a standard admission form as an intervention to improve documentation and quality of care.

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GENETIC DIVERSITY OF PLASMODIUM FALCIPARUM IN THE THREE MAJOR ECOLOGICAL ZONES OF GHANA

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Parasite genetic diversity information is useful for malaria control activities such as drug and vaccine failure rates. Parasite diversity varies with transmission intensity in varying geographical settings. Our objective was to describe the level of Plasmodium falciparum genetic diversity in the three ecological zones Ghana. A random selection of 379 baseline (Day 0) filter paper blood blots from children under five years, recruited between 2005-2008 during the monitoring of the efficacy of antimalarial drugs for the treatment of uncomplicated malaria in Ghana were genotyped. DNA was extracted; allele frequency and genetic diversity were investigated by nested PCR of the block three of the MSP2. The samples were drawn from towns in the three major ecological settings of Ghana: Navrongo (Sudan Savanna), Begoro and Bekwai (forest zone) and Cape Coast (Coastal Savanna). Differences in clonal diversity were observed that can be explained by geographical location. In general FC27 was the dominant allele in the Ghanaian parasite isolates in comparison with the 3D7 (Wilcoxon sign rank z, 2.953: p=0.0031). There were significant differences in clonal diversity in the different ecological zones when the carriage of a single clone was compared with carrying two clones (Wilcoxon sign rank z, 2.062:p=0.039), single clone vs. three clones (Wilcoxon sign rank z, 2.717 p=0.007) and two clones vs. three clones (Wilcoxon sign rank z, 2.953: p=0.003). This clonal diversity can be further explained by the differences in ecological zones, with the Sudan Savanna showing less diversity than the forest zone and the coastal savanna zone. Also, the forest zone showed more clonal diversity than the coastal Savanna and the two forest based towns did not show significant differences in clonal diversity. Greater genetic diversity was observed in the FC27 alleles than the 3D7 alleles which may translate into more diversity in the different ecological zones and population differentiations of the MSP2 alleles probably due to differences in ecological zones resulting in varying transmission intensity and out-crossing of parasite isolates during sexual reproduction.

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ADOLESCENT PREGNANCY AND THE RISK OF PLASMODIUM FALCIPARUM MALARIA AND ANEMIA - A PILOT STUDY FROM SEKONDI-TAKORADI METROPOLIS, GHANA

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The problem of malaria in adolescence has been surpassed by the immense burden of malaria in children, most especially less than 5. A substantial amount of work done on malaria in pregnancy in endemic regions has not properly considered the adolescence. The present study therefore aimed at evaluating the prevalence of Plasmodium falciparum and anaemia infection in adolescent pregnant girls in the Sekondi-Takoradi metropolis, Ghana. The study was carried out at four hospitals in the Sekondi-Takoradi metropolis of the western region of Ghana from January 2010 to October 2010. Structured questionnaires were administered to the consenting pregnant women during their antenatal care visits. Information on education, age, gravidity, occupation and socio-demographic characteristics were recorded. Venous bloods were screened for malaria using RAPID response antibody kit and Geimsa staining while haemoglobin estimations were done by cyanmethemoglobin method. The results revealed that adolescent pregnant girls were more likely to have malaria infection than the adult pregnant women (34.6% verse 21.3%, adjusted OR 1.65, 95% CI, 1.03-2.65, P = 0.039). In addition, adolescent pregnant girls had higher odds of anaemia than their adult pregnant women equivalent (43.9% versus 33.2%; adjusted OR 1.63, 95% CI, 1.01-2.62, P = 0.046). Taken together, these data suggest that adolescent pregnant girls were more likely to have malaria and anaemia compared to their adult pregnant counterpart. Results from this study shows that proactive adolescent friendly policies and control programmes for malaria and anaemia are needed in this region in order to protect this vulnerable group of pregnant women.

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INTERMITTENT PREVENTIVE TREATMENT OF MALARIA IN PREGNANCY (IPTP): EMPHASIS ON ADEQUATE DOSAGE AND TRIMESTER UPTAKE

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Intermittent preventive treatment in pregnancy (IPTp) with sulphadoxine-pyrimethamine (SP) has been adopted as policy by all countries in sub-Saharan Africa. However, studies on the post-implementation effectiveness and coverage of the therapy are being evaluated upon possible resistance that has been reported. This study assessed the effectiveness and uptake of the therapy by pregnant women attending antenatal care with some emphasis on trimesters and supposed doses taken. This cross-sectional study compared malaria and anaemia prevalence among 754 pregnant women using IPTp-SP with non-IPTp-SP users. The results showed that 57.8% (436/754) used IPTp-SP while 42.2% (318/754) did not. In general, 81.4% of the IPTp-SP users were anaemia negative, while 18.6% were malaria positive and those who received ≥2 doses had significantly reduced prevalence of malaria. Furthermore, of those that received IPTp-SP, 20.4% were in their 3rd trimester while 71.3% were in their 2nd trimester. However, only 3% of the pregnant women completed 3 doses while 30% completed the full ≥2 doses of IPTp-SP. In multivariate analysis, malaria infection in 3rd trimester was associated with increased odds of anaemia (adjusted OR 6.81, 95% CI, 1.19-38.94). IPTp-SP usage among pregnant women reduces malaria and its efficacy must be strengthened by proper dosage completion.

DEVELOPING A SCHOOL-BASED MALARIA EPIDEMIC DETECTION SYSTEM IN ETHIOPIA

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Epidemics of malaria occur periodically in Ethiopia, often resulting in substantial morbidity and mortality. Effective surveillance and response systems are required to allow early identification of epidemics and minimise the impact on health of the population. The current strategy for epidemic detection in Ethiopia relies upon charting weekly malaria cases against a ‘normal’ calculated using the third quartile method from the previous five years of data. However, there are limitations to the quality and frequency of reporting and analysis of these data. The current study explores the use of simple, easy to measure indicators of malaria morbidity and infection at school level, including school absenteeism, with a view to identifying an indicator which can be used for an epidemic early warning system. It is expected that this approach will allow the community to notice unusual increases in malaria morbidity, prompting an in-depth investigation by district health officials. In locations with limited accessibility to district towns, this may overcome some of the challenges of the current health facility based epidemic detection system. A series of eight repeated cross-sectional school surveys were conducted at six sites in southern Ethiopia over the short malaria transmission season (April to June 2012), randomly selecting 110 children for each survey. Indicators collected were multi-species rapid diagnostic test result, microscopy examination of blood film, reported fever on survey day and in prior two weeks and measured axillary temperature. Participants were asked their normal frequency of school attendance and number of absences from school in the previous two weeks. School records of pupil absenteeism were collected on completion of the study, as well as routine data on clinical and confirmed malaria from health facilities serving the study population. Survey indicators and school absenteeism rates were compared to health facility data, in order to identify indicators which correlate with and offer a lead time over the standard malaria epidemic detection system.
A NOVEL METHOD TO ASSESS THE SAFETY OF SULFADOXINE-PYRIMETHAMINE FOR INTERMITTENT PREVENTIVE TREATMENT IN INFANTS USING ROUTINE HEALTH FACILITY DATA FROM SOUTHERN TANZANIA

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Intermittent preventive treatment with sulfadoxine-pyrimethamine is recommended for malaria prevention in infants (IPTi-SP) in areas of moderate to high malaria transmission and where parasite resistance to SP is not high. Serious adverse events, including Stevens-Johnson syndrome (SJS), have been reported following SP exposure, but few infant-specific data exist. Within the context of a cluster randomized controlled trial of IPTi-SP in southern Tanzania, we captured routine health facility data on infant outpatient attendance from all health centres. Data included diagnosis, allowing classification of attendance for a non-scabies skin condition. We investigated the association of IPTi-SP with attendance for skin conditions using a number of methods, including the self-controlled case series method. This novel methodology allowed estimation of the relative incidence of attendance among infants presenting with a skin condition who had received SP for IPTi-SP or for malaria treatment.

Among these infants, the rates of attendance were compared between ‘unexposed’ and ‘exposed’ periods of time, using infants as their own comparison - thus adjusting for all measured and unmeasured time-fixed confounders. Based on previous studies and the half-life of SP ingredients, we defined an ‘exposed’ period as the 42 days following an SP dose, and compared whether rates during this period differed to those during an infant’s ‘unexposed’ time. Data were available for 9880 infants over 12 months, with >8000 doses of SP received. No diagnoses of SJS were recorded. The incidence of attendance with a skin condition was 0.062/year among all infants, and 0.071/year among the 3983 infants who had received >=1 dose of SP for IPTi-SP or malaria treatment. In total 239 infants attended for a skin condition and received at least one dose of SP, and these were included in the self-controlled cases series analysis. In comparison to the rate during ‘unexposed’ periods, the age-adjusted rate of attendance among these 239 infants during the 42 days after exposure was almost 50% lower suggesting no detectable increased rate of attendance for skin condition during the six weeks following an SP dose. These results provide reassurance about the safety of SP in infants from this setting, and provide a worked example of how the self-controlled case series method may be used to assess safety of interventions in developing countries using routine health facility data.

A PROSPECTIVE ANALYSIS OF PLASMODIUM FALCIPARUM INFECTION RISK FROM INFANCY TO ADULTHOOD IN AN AREA OF INTENSE, SEASONAL TRANSMISSION IN MALI

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Time to first malaria episode is often used as a measure of clinical immunity to Plasmodium falciparum. In contrast, the risk of P. falciparum infection irrespective of clinical disease can be measured by the time required to detect parasites in the blood by sensitive methods such as polymerase chain reaction (PCR). Pre-erythrocytic malaria vaccines aim to elicit a protective immune response against the sporozoite or liver stage of P. falciparum to prevent infection, but little is known about naturally acquired pre-erythrocytic immunity in endemic areas. Prospective studies that have assessed the risk of P. falciparum infection in endemic settings have failed to show correlations between antibodies to pre-erythrocytic antigens and infection, and evidence for age-dependent acquisition of protective immunity to infection is limited. To evaluate for evidence of naturally acquired pre-erythrocytic immunity, we conducted a prospective analysis of P. falciparum infection during a single malaria season from May to December 2011 in Kalifabougou, Mali. Of the 695 subjects enrolled in this study, 372 were uninfected by PCR at enrollment before the malaria season. Of these, we determined time to P. falciparum PCR positivity using dried blood spots collected at two-week intervals over the entire study period. We found no evidence that pre-erythrocytic immunity is acquired with increasing age/exposure. Unexpectedly, younger children (<4 years old) showed decreased risk of infection compared to older children (4-17 years old) when adjusted for sex, hematocrit, Schistosoma co-infection, sickle cell trait, and spleen size (hazard ratio 0.44; 95% CI, 0.32-0.60; p<0.0001). Based on these data, antibody responses to Anopheles gambiae salivary gland extract, pre-erythrocytic antigens, and blood-stage antigens are being measured before and after the malaria season in an attempt to determine if the observed decreased risk in younger children is due to differences in vector/parasite exposure versus other factors.

MALARIA IN THE KINGDOM OF SAUDI ARABIA - IS ELIMINATION A REALISTIC GOAL?

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In 1998, after two exceptional years of heavy rain, the Kingdom of Saudi Arabia (KSA) suffered its worst epidemic of malaria, with total cases reaching 40,796. Almost 90% of these were locally acquired, and incidence reached 11/1000 in the main malarious areas of Asser and Jazan, in Southern KSA. Since then, KSA has scaled up vector control with IRS, ITNs and larviciding and improved on case management. Today’s data tells a very different story: the number of autochthonous cases since 2008 has been less than 100 per year, just 4% of total cases and an incidence rate of <0.05/1000, far lower than the rate of 5/1000 that WHO
developed. Population genetics analysis can allow us the opportunity to explore the genetic relatedness of parasites found in soldiers and DRC. The results from the study support the epidemiologic findings that the parasites from soldiers were closely related to parasites found in the DRC (Nei’s GD=0.28). Clustering of isolates from soldiers and DRC was confirmed by principal coordinate analysis. The parasites from the Guatemalan soldiers were characterized and genetic relatedness was calculated using Nei’s genetic distance. Seven neutral microsatellite markers were used to characterize the parasites. The study reveals that malaria still remains a major public health concern in both the DRC and Guatemala. The parasites from the two regions were found to be closely related, suggesting that there is a high degree of genetic similarity between the two populations. This highlights the importance of implementing effective malaria control strategies in both regions to prevent the spread of malaria.

LANDSCAPE GENETICS OF FALCIPARUM MALARIA IN GEOGRAPHICALLY DISPERSED CONGOLESE SITES AND IN UN PEACEKEEPING SOLDIERS RETURNING TO GUATEMALA

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GENDER DIFFERENCES IN INSECTICIDE TREATED NETS (ITN) USE AFTER A UNIVERSAL FREE DISTRIBUTION CAMPAIGN IN KANO STATE, NIGERIA

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The shift from targeted groups to universal coverage of Insecticide Treated Net (ITN) raises issues of gender equity and equality in access and use. There is a need for gender-based analysis to assess the effects of gender on the uptake of this key intervention for malaria control. The recent post-campaign survey in Northern Nigeria offers an opportunity to look at gender differences in ITN use. The post-campaign survey was conducted October 19-November 4, 2009 and included a random sample of 4,638 individuals in Kano State. The survey was carried out using a questionnaire adapted from the Malaria Indicator Survey. Using binary logistic regression and controlling for several covariates, we assessed the effect of gender on ITN use among all the individuals living in households with at least one ITN. ITN ownership increased more than tenfold, from 6% to 71% before and after the campaigns. There was no significant difference between the proportion of females and males living in a household with at least one ITN. However, a higher percentage of females used ITN compared to males (56% vs. 46%). After controlling for several covariates, females remained more likely to use ITNs compared to males (OR: 1.5, 95%CI: 1.3-1.7). In conclusion, this study reveals gender inequality in ITN use with men less likely to use ITN. Notably, the uptake of the intervention among the most-at-risk group (females) is higher. However, there is a need to also ensure that males equally use ITN to achieve universal coverage.
TRANSMISSION BLOCKING EFFICACY OF ANTI-MALARIAL PLANT EXTRACTS ON PLASMODIUM FALCIPARUM GAMETOCYTES FIELD ISOLATES

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Targeting gametocytes, gametes and/or oocinete, i.e. the stages of the malaria parasite responsible for its transmission from the human host to the Anopheles vectors, is key for pharmacological malaria control strategies. Research efforts to identify such compounds have significantly increased over the last years. However, at present, only two drugs are available, namely primaquine and artesunate, that are acting on late stage gametocytes (stage IV-V). In this study, we assessed the antiplasmodial effects of 5 extracts from 2 plants against gametocyte to oocinete stages of Plasmodium falciparum field isolates in an ex vivo assay; Anopheles gambiae females were membrane fed on gametocyteic human blood, treated with the plant extracts and transmission blocking activity evaluated on day 7 by assessing oocyst prevalence and density. Two of the 5 tested extracts showed significant transmission blocking activity: the commercial neem (Azadiracta indica) extract NeemAzai®; completely blocked oocyst development at 500, 250 and 70 µg/ml. A 90% inhibition was still found at a dosage of 50µg/ml of this seed kernel extract. Transmission blocking activity was also found with an ethyl acetate leave extract from the same plant species, inhibiting oocyst development completely at 500 µg/ml and by 80% at 250 µg/ml. The results of this study highlight the potential of anti-malarial plants for the discovery of novel transmission blocking molecules, but open also the challenging perspective of using standardized, transmission blocking herbal formulations as a complement to artemisinin combination therapy in the management of malaria and the control of the parasite’s transmission.

REDUCTION OF MALARIA PREVALENCE BY INDOOR RESIDUAL SPRAYING: A META-REGRESSION ANALYSIS

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Indoor residual spraying (IRS) has become an increasingly popular method of insecticide use for malaria control and many recent studies have reported on its effectiveness in reducing malaria burden in a single community or region. There is a need for systematic review and integration of the published literature on IRS and the contextual determining factors of its success in controlling malaria. This study reports the findings of a meta-regression analysis based on 13 published studies which were chosen from over 400 articles through a systematic search and selection process. The summary relative risk for reducing malaria prevalence was 0.38 (95% CI = 0.31-0.46) meaning a risk reduction of 62%; however, an excessive degree of heterogeneity was found between the studies. The meta-regression analysis indicates that IRS is more effective with high initial prevalence, multiple rounds of spraying, use of DDT, and in regions with a combination of Plasmodium falciparum and P. vivax.

A NEW DEVICE FOR SURVEILLANCE AND CONTROL OF OUTDOOR BITING MOSQUITOES: ITS DESIGN, FIELD TESTING AND APPLICATIONS FOR PREVENTION OF MOSQUITO BORNE INFECTIONS IN AFRICA

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Mosquitoes that seek blood outdoors continue to contribute significantly to transmission of diseases such as malaria, filariasis and viral infections. To achieve the goals of eliminating mosquito-borne diseases, new tools that can be used outdoors are required to complement existing indoor interventions, such as insecticide-treated bed nets. We developed an odor-baited device called the Mosquito Landing Box (MLB), which can be used to control and monitor mosquitoes biting outdoors. The MLBs were baited with smelly socks and carbon dioxide gas. Field experiments were conducted in rural Tanzania to assess: a) the number of wild host-seeking mosquitoes visiting the MLBs, b) whether the mosquitoes stayed long or left shortly after arrival, c) the time of night when the mosquitoes were most active and d) whether the visiting mosquitoes could be contaminated and killed. There were significantly more mosquito vectors, Anopheles arabiensis (df=1, P<0.001), An. funestus (df=1, P<0.001), Culex species (P<0.028) and Mansonia species (P<0.001) visiting baited MLB than unbaited controls. Increasing sampling frequency from 2-hourly to either 1-hourly or half-hourly was associated with an increase in number of mosquitoes caught (df=2, P<0.002), suggesting that many mosquitoes visited the device but left shortly afterwards. Outdoor host-seeking activity was highest from 2000hours to 2200hours and from 0400hrs to 0600hrs. Adding a partially open screen-cage around the MLB did not affect catches of An. arabiensis (df=1, P=0.986), An. funestus (df=1, P=0.776) or the culicines (df=1, P>0.681). Nearly half (47.1%) of the An. funestus caught visiting an insecticide-treated MLBs died compared to 1.2% in controls. Further studies are underway to identify more effective and long-lasting mosquito killing agents to apply on the MLBs. These findings indicate that the MLB might be useful for sampling and possibly controlling outdoor-biting mosquitoes; by attracting, contaminating and ultimately killing the mosquitoes, hence potentially reducing disease transmission.

VIRTUAL SCREENING FOR POTENTIAL LIGANDS OF G-PROTEIN COUPLED RECEPTORS (GPCRs): GATEWAY TO IDENTIFICATION OF NOVEL SCAFFOLDS FOR POTENTIAL TREATMENT OF NEGLECTED DISEASES

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Helminthic diseases caused by parasitic nematodes affect about 550-750 million people global. Also, malaria, caused by Plasmodium falciparum, was responsible for about 200 million cases and 650 000 deaths in 2010. Yet, little work has been done to discover new drugs that curb helminthic diseases. Also, the mosquito which transmits the malaria parasite has become resistant towards currently used insecticides. So, there is need to constantly search for new anti-parasitic drugs. Nematode FMRFamide-like peptides (FLPs) and the mosquito adipokinetic hormones (AKHs) alongside their G-protein coupled receptors (GPCRs) have been identified. GPCRs are known drug targets; hence intervention of the signalling pathway of FLPS and AKHS would lead to a reduction in nematode infections and the spread of malaria. Here, structure-based virtual screening of the ZINC database was performed using a previously modelled structure of a mosquito GPCR, AKHR to identify potential GPCR ligands. Docking calculations to study binding modes and affinities of a mosquito AKH (AKH-1) were done. ZINC compounds were then docked to AKHR and those with higher binding affinities for the receptor were selected for insecticidal evaluations and tested for activity towards nematode GPCRs. Docking results showed that AKH-1 had binding affinities of ΔGb =
and long-lasting insecticidal nets (LLINs) may be needed to achieve
Samson S. Kiware
INSECTICIDES BY MOSQUITOES
STOCHASTIC MODELS FOR THE AUTODISSEMINATION OF
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OF ACCESS TO MALARIA PREVENTION AND TREATMENT
RESOURCES AMONG BURMESE MIGRANTS IN TAK
FINDINGS FROM A RAPID QUALITATIVE ASSESSMENT
THAILAND
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Recent evidence of the declining efficacy of Thailand's first line artemisinin-
based combination therapy (ACT), mefloquine-arthensuate, in western
Thailand has raised concerns that artemisinin resistance is already present
on the Thai-Burma border. In Thailand, malaria occurs mainly in the
border provinces, with the highest incidence occurring among Burmese
migrants in Tak Province. Strategies to ensure migrants' timely access
to effective malaria treatment and prevention resources are therefore
of utmost importance. Although malaria case management and vector
control interventions in Tak Province should be targeted to Thai nationals,
refugees, and migrant workers alike, little is known about how Burmese
migrants who live or work in non-refugee camp settings actually access
and use malaria resources. This dearth of information on migrant malaria
care-seeking behaviors has hampered Thailand's ability to assess how
well malaria interventions are reaching these populations. To address
this gap, in February 2012 we conducted a rapid qualitative assessment
of community- and provider- level factors that affect migrants' use of
malaria prevention and treatment resources in Tak Province, Thailand.
Qualitative data were collected in 4 villages and 2 commercial farm
settings in two districts and included 8 focus group discussions, 11 key
informant interviews, 10 health provider- and 31 community- in-depth
interviews, and 7 seasonal calendars. Interviews were conducted in Karen,
Burmese, and Thai, digitally recorded, and transcribed in the language
of interview. Interview transcripts were translated into English and content
analysis conducted. Preliminary results indicate that several community
and health facility factors interact to limit migrants' access to effective
malaria prevention and treatment resources, particularly among non-
registered migrants. These include misunderstandings about the causes
of malaria, the limited availability of insecticide-treated nets for migrants,
delays in reaching health facilities due to financial and legal barriers, ACT
procurement and distribution delays, and language barriers between
providers and their migrant patients. To minimize malaria transmission and
the spread of artemisinin resistance, additional measures should be put in
place to help achieve better access to malaria resources among displaced
populations living in Thailand's border areas.

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FINDINGS FROM A RAPID QUALITATIVE ASSESSMENT
OF ACCESS TO MALARIA PREVENTION AND TREATMENT
RESOURCES AMONG BURMESE MIGRANTS IN TAK
THAILAND
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Mosquito borne diseases are major causes of mortality and morbidity
in the tropics. Over 20 Anophelines are of medical importance but in
Uganda, Anopheles gambiae and An. funestus are the major malaria
vectors. Globally, Malaria accounts for closely 1 million annual deaths,
over 90% of which is from Africa. In Uganda 12.3 million cases occur
with over 100,000 annual deaths and an estimated 384 child deaths per
day. The comprehensive approach for malaria control includes indoor
residual sprays (IRS), insecticide treated bed net distribution and malaria
treatment with ACTs as well as fansidar prophylaxis in pregnant women.
Despite these interventions, the malaria scourge prevails mainly due
to resistance in both the parasite and the vector. There are promising
results for a malaria vaccine but extensive trials are still needed before it's
operationalised. Proper vector management emphasizes a multifaceted
bio-rational approach where bacterial agents have been handy. Bacillus
thuringiensis and B. sphaericus are the most widely studied and advanced
biological agents but the spreading wave of resistance is their major
setback. Their virulence has been noted to vary according to species
variant and the region the agent is isolated. Therefore, there is a need
to search for alternatives but none of such studies have been done in
Uganda. Tree holes and water pond soil samples were pasteurized and
isolates were identified microscopically for Gram and endospore reactions.
They were exposed to 3rd instars of Aedes aegypti and mortality was
observed from 3 hrs to 72 hrs. 39 isolates (9.4%) were larvacidal against
Ae. aegypti, 80% of which were from tree holes. 1 unique isolate had
terminal endospores with a drumstick appearance and produced 60% mortality
within 3 hrs while the other 38 were within 24 hrs. Biochemical,
molecular characterization, optimal lethal doses and Anopheles
comparative assays are to follow. In conclusion, 39 isolates have potential
of being incorporated in biological control and thus recommending their
characterization and sampling from tree holes.

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STOCHASTIC MODELS FOR THE AUTODISSEMINATION
OF INSECTICIDES BY MOSQUITOES
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Vector control techniques that complement indoor residual spraying
(IRS) and long-lasting insecticidal nets (LLINs) may be needed to achieve
malaria elimination. Semi field system (SFS) experiments were conducted
to evaluate the potential for the autodissemination of pyriproxyfen
(PPF) by adult mosquitoes into their breeding sites and its impact
on adult mosquito emergence. We present stochastic mathematical
models parameterized using SFS data to help design field trials for the
autodissemination of PPF by adult mosquitoes. We incorporate stochastic
characteristics of the autodissemination technique into mosquito life
cycle: (1) To show how the fit between simulated and experimental data
may be used to design field trials. (2) To run the model using parameters
derived from mosquitoes innate life cycle characteristics such as blood-
feeding, resting, flight range, and oviposition behaviour. (3) To guide field
trial strategies that achieve effective insecticide coverage of breeding
sites using optimal dissemination stations. Our models developed using
experimental data may help design field trials for the autodissemination
technique by exploiting different parameters of mosquitoes' life cycle. The
model outputs suggest the design of dissemination stations, the distance
between them, PPF treatment proportions and intervals necessary to
achieve high coverage of breeding sites that may reduce adult mosquito
density up to 40%. Mathematical models parameterized using semi-field
experimental data are used to design field trials for the autodissemination
of insecticides by mosquitoes.

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ISOLATION AND CHARACTERIZATION OF GRAM POSITIVE
ENDOSPORE FORMING BACTERIA WITH BIO-LARVACIDAL
EFFECT AGAINST MAJOR MALARIA VECTORS IN UGANDA

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MALARIA PREVENTION MEASURES EXPENDITURES IN BURKINA FASO: HOW MUCH DO THEY COST TO HOUSEHOLDS?

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The efficacy of insecticide-treated nets (ITNs) has demonstrated in the protection from mosquito bites. The provision of insecticide-treated nets (ITNs) is widely accepted in Burkina Faso. However, other methods of prevention are used by the households. In the perspective of the introduction of the vaccine, it is important to know more about the household expenditure on the non-net products and practices. This paper aims to estimate the amounts spent on different malaria prevention measures in Burkina Faso. A cross sectional survey was conducted during the high transmission season in 2010. Simple random sampling was used to select villages and households. Data collection was carried out among 506 households in Nanoro district in Burkina Faso. Households were asked about expenditures on other forms of malaria prevention over the previous month including expenditure on coils, indoor spraying, aerosols, repellents, herbs, cleaning surrounding, environment and clearing vegetation; and any other forms of prevention. More than 50% of households owned at least one bed net. Bed nets were used by 94% of household head, 88% of under five children, 85% of husband or wife and 83% of children up to 5 years. 32% of households use smoke, 20% clean outside environment, 16% cultivate specific plants, 8% use coils, and 3% indoor spraying. The majority of households did not spend money on malaria prevention measures. Households spent an average of 2450F CFA on bed net, but only 6% of bed net were bought. Households spent an average of 910 F CFA on coils and 1768 F CFA on indoor spraying in the previous month. Household wealth was associated with coils (p=0.008), indoor spraying (p=0,04) and cleaning surrounding (p=0,009). Most of households received bed nets with the implementation of the new program of distribution of bed nets. They used more than one prevention measure. The introduction of the vaccine will affect the households’ practices and it is important that it doesn’t affect the utilization of the other malaria prevention practices.

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EFFECTS OF INSECTICIDE RESISTANCE AND CREPUSCULAR BITING BEHAVIOR ON THE COST EFFECTIVENESS OF A MASS, LONG-LASTING, INSECTICIDAL NET DISTRIBUTION: A MODELING STUDY

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The effectiveness of insecticide treated nets (ITNs) in preventing malaria is threatened by developing insecticide resistance and changing biting behavior. Data from experimental hut studies on the effects of a third generation long lasting ITN on eight anopheline mosquito populations with varying levels of insecticide resistance (from less than 10.6 % mortality in 0.05% deltamethrin WHO cylinder tests to fully susceptible) were used to parameterize malaria models. The effectiveness of a mass distribution of ITNs against malaria, in terms of episodes averted during the effective lifetime of the batch, and in terms of net health benefits expressed in disability adjusted life years (DALYs) averted, depending on resistance, biting behavior, and pre-intervention transmission level, was studied using an ensemble of 14 model variants in OpenMalaria. With the most resistant mosquito population, at the transmission level where ITNs were most effective (4 infectious bites per adult per annum (ibpapa) pre-intervention), the ITN mass distribution averted up to about 35% less episodes and DALYs than with susceptible populations. This was similar to the loss of effectiveness if 40% instead of 10% of the mosquitoes always bit during times when people are not under an ITN. Over the ranges studied, effectiveness of ITNs was more sensitive to the pre-intervention transmission level than to the level of insecticide and behavioral resistance. ITNs had positive net health benefits in most scenarios. Only at pre-intervention transmission levels above 128 ibpapa, a minority of variants of the model ensemble showed (slightly) negative net health benefits. ITNs are likely to be cost effective against malaria even in areas with strong pyrethroid resistance and where a large proportion of host-mosquito contact occurs during times when ITN users are not under their nets.

THE ASSESSMENT OF IEC/BCC MATERIALS AND DELIVERY CHANNELS IMPLEMENTED ALONG THE THAI-CAMBODIAN BORDER

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The assessment of information, education and communication (IEC) products and communication channels was carried out in Chanthaburi and Si Sa Ket Provinces, eastern Thailand in February, 2010. The assessment was conducted to determine the acceptance, cultural appropriateness, and effectiveness of IEC materials produced to promote the behavior changes in the project “A strategy for the containment of artemisinin resistant malaria parasites in South-East Asia”. The main objective of the assessment was to understand the interim effects of IEC materials on the target audience and to find out the most effective communication channels or media to reach out to the targeted mobile and migrant population in the project. The assessment used both qualitative and quantitative methods including interviews and focus group discussions. The qualitative component helped triangulate the quantitative findings. A total of 81 respondents were interviewed in the survey. Two focus group discussions were conducted with the target audience in both provinces. The results were encouraging and showed that the coverage of IEC materials was high (80.2-83.9%) in both areas. Overall, the pamphlets were highly received among the respondents in all aspects, i.e. contents, colours, sizes, and illustrations. It was found that most of the respondents could comply with key messages appeared on the pamphlets. The findings of FGD showed similar outcomes as compared with the quantitative survey. Majority of the respondents highly accepted the posters as shown by survey. The results showed that the IEC materials were culturally appropriate and very well received by the target population. More importantly, we found that the interpersonal communication was the highly preferred communication channel for health education. These findings should be utilized as an evidence for enhancing the capacity of our field staff and volunteers to effectively deliver the health education program to the target population, as the aim set to change people’s behaviors and finally to save people’s health from malaria.

FIELD EFFICACY AND PERSISTENCE OF LONG LASTING INSECTICIDE TREATED MOSQUITO NETS (LLINS) IN COMPARISON WITH CONVENTIONAL INSECTICIDE TREATED MOSQUITO NETS (ITN) AGAINST MALARIA VECTOR IN THAILAND

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In Thailand, the conventional Insecticide Treated mosquito Nets or ITN have been used over the years by the villagers. These mosquito nets are treated with permethrin 10%w/w EC manually as under the guidance of the health workers. These treated nets have efficacy for 6 months and need re-treated again. Long Lasting Insecticide treated mosquito Nets or LLINs, which can retain persistence for at least 3 years, are being considered to replace the conventional ITN. This study is intended to monitor the bioefficacy of two products of LLINs under field conditions in Thailand. These nets are PermaNet® and OlysetNet®. The study was carried out in a malaria endemic area of Kanchanaburi province. PermeNet®, OlysetNet® and conventional ITN were distributed to the households were allowed to use the bed nets. The nets were washed at every 6 months intervals and only conventional ITN were re-treated after washings. WHO standard procedures for cone bioassay tests were conducted with the bed net samples collected from the households that were using the nets and laboratory reared Anopheles minimus were used in cone bioassay tests to access the efficiency of mosquitoes nets. Results of the study showed that both LLINs (PermaNet® and OlysetNet®) offered > 80% mortality on Anopheles minimus over the entire 3 years period of field evaluation. The conventional ITN performed similar to LLINs except the fact that ITN were re-treated at 6 months intervals. Interestingly the ITN offered only 15% mortality after 6 months use and were washed without re-treatment.

ISOLATION OF ANTIPOLIOVIRUS AGENTS FROM ZEPHYRANTHES CANDIDA LINDL AND CASSIA SIAMEA LAM

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Polio eradication by vaccination of children in Nigeria has been largely unsuccessful due to the characteristic problems of accessibility, limited supervision, cultural hindrances and occasional vaccine-associated paralytic poliomylitis. The need to consider alternative ways of managing the infection becomes imperative. This led to the ethnobotanical study of plants used for control of viral infection in South-Western Nigeria. The objective of this study was to screen for efficacy, isolate and characterize antiviral agents from these plants. Fourteen medicinal plant samples were extracted by maceration into absolute methanol at room temperature and subjected to antiviral assay. Ability of extracts to inhibit viral-induced cell death in tissue culture was evaluated three days post-infection by
PROPERTIES OF C. SIAMEA, Z. CANDIDA, AND B. VIRGINICA

Five compounds were isolated from Z. candida and C. siamea with IC₅₀ values of 1.2 x 10⁻³ µg/mL and 2.3 x 10⁻¹ µg/mL, respectively. Hexane fraction retained in the chloroform fractions of C. siamea and C. siamea with IC₅₀ of 1.2 x 10⁻³ µg/mL and 2.3 x 10⁻¹ µg/mL, respectively. Activities were determined using the MTT colorimetric assay. Linear regression was used to determine IC₅₀ and CC₅₀ from which Selectivity Index (SI) was calculated. Bioassay-guided fractionation of extracts, repeated column and preparative thin layer chromatography of active fractions led to isolation of active compounds. Chemical structures of compounds were elucidated using spectroscopic techniques. The crude extracts of whole plant Zephyranthes candida and stem bark Cassia siamea were the most active of the extracts with IC₅₀ of 1.85 x 10⁻³ µg/mL and 2.1 x 10⁻¹ µg/mL, respectively. Activities were retained in the chloroform fractions of Z. candida and C. siamea with IC₅₀ of 1.2 x 10⁻³ µg/mL and 2.3 x 10⁻¹ µg/mL, respectively. Hexane fraction of C. siamea was also comparatively active with IC₅₀ of 5.1 x 10⁻¹ µg/mL. Five compounds were isolated from Z. candida, namely; 7-hydroxy-3”,4”-methylenedioxyflavan, lycorine, trisphaeridine, β-sitosterol glucoside and stigmasterol. Lupeol, lupenone, betulinic acid, emodin, chrysophanol, psycosin and β-sitosterol glucoside were obtained from C. siamea. Lupeol was the most active compound from C. siamea with IC₅₀ value of 1.4 x 10⁻² µg/mL. Lycorine was the most active compound from Z. candida with IC₅₀ value of 5.8 x 10⁻² µg/mL. Two compounds; 7-hydroxy-3”, 4”-methylenedioxyflavan and trisphaeridine are reported from Z. candida and their anti-poliovirus properties are established. This study provides chemical entities that may be lead for development of antiviral agents.

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STUDY OF ARBOVIRUSES IN ARCHIVED SPECIMENS FROM ACUTE FEBRILE ILLNESS STUDIES IN BANDUNG, INDONESIA

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Numerous recent arboviral outbreaks have demonstrated that arthropod-borne pathogens continue to be significant public health threats. These outbreaks have not been limited to tropical or developing countries as people and goods can be moved anywhere in the world within days. Indonesia might be a very important country for emerging viruses having several endemic arboviruses including dengue, chikungunya, and Japanese encephalitis viruses. Outbreaks due to these viruses have occurred several times in Indonesia but to date studies on the existence of other arboviruses in Indonesia are scarce. Therefore, a study to detect evidence of arboviral pathogens in archived samples is currently in progress. The main purpose of this project is to identify the presence of emerging arboviruses of pandemic risk in Indonesia. We have identified archived samples from prior acute febrile infection studies that enrolled patients in two hospitals in Bandung, West Java, Indonesia from 2004-2005. The original study enrolled a total of 406 hospitalized suspect-dengue cases; the majority (311) had evidence of recent dengue infection. However, infecting etiologies on the remaining samples had not been determined. The current study includes testing for other specific endemic arboviruses as well as unknown arboviruses in the dengue negative samples. Initially, samples are tested against panels of several arboviruses including flaviviruses, alphaviruses, and bunyaviruses employing RT-PCR and IgM detection assays. Suspect positive samples are further tested with virus specific RT-PCR, viral isolation, and DNA sequencing targeting several viruses including Japanese encephalitis virus, West Nile virus, Zika virus, Chikungunya virus, Ross River virus, and hantaviruses. Our study is the first systematic survey on emerging viruses in Indonesia with gold standard molecular, serological, and virus isolation assays to estimate the magnitude of circulation of arboviruses.

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HBSAG PREVALENCE AMONG PRE-VACCINE AND VACCINE ERA CHILDREN IN BANGLADESH: PRELIMINARY RESULTS

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Hepatitis B virus infection is a leading cause of morbidity and mortality due to hepatocellular carcinoma and liver cirrhosis worldwide. Some previous small scale studies in Bangladesh observed hepatitis B surface antigen (HBSAg) prevalence as 3%-7%. During 2003-2005 Bangladesh introduced hepatitis B (HepB) vaccine into the routine childhood vaccination schedule provided at 6, 10 and 14 weeks of age. While a birth dose of HepB vaccine is widely recommended, the country’s high prevalence of home births (85%) presents logistical difficulties to timely administration of a birth dose. This study evaluated the impact of HepB vaccine introduction in Bangladesh in the absence of a birth dose by comparing HBSAg prevalence among children born before and children born after HepB introduction. Using probability proportional to size cluster sampling, we selected a nationally representative sample of 2,100 pre-vaccine era children born from April 1, 2001 to March 31, 2002 and 2,100 vaccine-era children born from November 1, 2005 to October 31, 2006 from 105 clusters. In each cluster, starting from the center to a randomly chosen direction, we visited all households until we found 20 pre-vaccine era and 20 vaccine-era children. After taking written consent from their guardians, we collected a blood sample from each child along with vaccination and demographic information. We performed a rapid test of HBSAg in the field (Abbott Determine; sensitivity: 95%-100% and specificity: 96%-100%). Confirmatory testing will use standard serologic assays. To enroll 2,100 children from each group, we approached 2,203 pre-vaccine era children (refusal rate 5%) and 2,270 vaccine-era children (refusal rate 8%). Among the enrollees, 97% of vaccine-era children received HepB from national childhood vaccination program and 2.7% of pre-vaccine era children received HepB from private sources. None of 2,100 vaccine-era children were HBSAg-positive; by comparison, 24 (1.1%; 95% CI=0.7-1.7) of 2,100 pre-vaccine era children were HBSAg positive. Preliminary results suggest that even without a birth dose, the HepB vaccine program was highly effective in Bangladesh. Considering the long term efficacy of HepB, childhood HepB vaccination will continue to reduce the national hepatitis B burden. These findings support continued investment in HepB in other countries who introduced HepB vaccine into childhood immunization programs but have not yet evaluated impact.

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SEROPREVALENCE OF CYTOMEGALOVIRUS INFECTION IN A COHORT OF CHILDREN EXPERIENCING DIFFERENTIAL MALARIAN TRANSMISSION DYNAMICS IN WESTERN KENYA

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Cytomegalovirus infection is a serious cause of congenital disease in western countries where it causes sensorineural hearing loss and neurodevelopmental disorders and is one of the main viruses associated with transfusion related infections. The virus can be reactivated and causes fatal infection in immunosuppressed individuals. The prevalence of CMV infection in American adults is approximately 54% while approximately 85% of Gambian children acquire CMV infection by their first birthday. However, little is known about the prevalence of CMV in Kenyan children.
Our aim was to determine the CMV seroprevalence in a cohort of children from western Kenya. Infants were enrolled from two rural sites in Western Kenya: Kismu District where malaria transmission is holoendemic and Ndii District where malaria transmission is sporadic. Blood samples from infants born to HIV-seronegative mothers were taken from 1 month through 24 months of age to measure CMV viral load in peripheral blood and CMV antibodies. CMV-specific IgG was assessed using a luminex bead based array assay and viral loads were measured in DNA extracted from whole blood using quantitative PCR. Preliminary results show that CMV seroprevalence increases with age with a seroprevalence of 60% versus 22% at three months, 80% versus 52% at 6 months, 91% versus 83% at 12 months and 100% versus 92% at 24 months in malaria holoendemic and sporadic areas respectively. Studies are ongoing to analyze CMV IgM to confirm whether there is primary infection by 3 or 6 months of age. These preliminary results show higher seroprevalence of CMV in infants living in a malaria endemic area within the first year of life compared to infants from a region with sporadic malaria transmission. Further studies are needed to understand why seroprevalence of CMV in a malaria endemic region is so high early in infancy.

A SURVEY OF ARBOVIRUSES CIRCULATING IN KENYA 2007-2010

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Arbovirus surveillance in mosquitoes during the inter-epidemic period of 2007-2010 in six sites in Kenya indicated that several viruses were in circulation. The sites Garissa, Magadi, Turkan, Tanadel, Budalangi and Naivasha are well spread across the country. A total of 25 isolates were made comprising of 4 viruses from three arbovirus families. 11 of the isolates have not yet been fully characterized. The mosquitoes were collected using CO2-baited CDC light traps from December 2009 to June 2010 in the selected sites during wet seasons. Mosquitoes were identified to species and pooled (≤ 25 mosquitoes per pool). Consequently, the mosquitoes were homogenized in 1.5ml eppendorf tubes containing one 4.5mm copper bead and 750μl of minimum essential medium supplemented with 15% FBS, 2% L-Glutamine and 2% Antibiotics mixture. The homogenates were centrifuged at 10,000 rpm at 4°C for 15min and the resultant supernatants inoculated in monolayers of VERO cells in 24 well plates. The cultures were incubated at 37°C and monitored for cytopathogenic effects (CPE) daily up to 14 days. Cultures showing CPE were harvested and viruses identified by RT-PCR and sequencing. Bunyavirus isolates were obtained from pools of Aedes mcintoshi (4), Anopholes funestus (3) and Ae. tricholabis (1). Five of these mosquitoes were collected from Garissa, two from Magadi and one from Tanadel. Alphaviruses were isolated from pools of Cx. univittatus (3), Culex sp (1), Ae. lirudis pools (2), Ae. tricholabis (2) and Aedes spp (1). Six of these isolates were collected Garissa, two from Naivasha, one from Budalangi and three from Tanadel. All Flavivirus isolates were isolated from Cx.univittatus mosquitoes; five of which were from Garissa and one from Turkan. Eight Bunyaviruses (4 Bunynamvera, 1 Pongola), 11 Alphaviruses (4 Ndumu, 3 Babanki) and 6 Flaviviruses (6 West Nile viruses) were isolated. Four of the Alphaviruses and three of the Bunyaviruses have not yet been fully characterized. This results indicate that continued arbovirus surveillance in this region is important to avert future arbovirus outbreaks.

SEROLOGICAL EVIDENCE OF NIPAH LIKE VIRAL INFECTION IN PIGS IN BANGLADESH

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Nipah virus (NiV) causes fatal encephalitis in humans; Pteropus bats are their natural reservoir. Pigs may act as a potential amplifier of NiV and transmit the disease to humans as in Malaysia 1998-99. We looked for serological evidence of NiV infection in pigs in three North-western Districts in Bangladesh where human NiV infections were repeatedly identified. From May to September 2009 we collected blood samples from 312 pigs (Sus scrofa) (Rajshahi, n=100; Nawabganj, n=102; and Naogaon, n=110) from backyard and nomadic herds over six months of age. We tested the serum samples for antibody binding to a NiV recombinant G glycoprotein using a Luminex assay. Samples having median fluorescence intensity over 1000 were considered positive. Samples tested positive for antibody were further tested by serum neutralization test against the NiV N protein. To understand the difference between exposure (age, sex, pig raising pattern) and outcome (NiV antibody) we performed Wilcoxon rank-sum test and chi-squared. Of the 312 pigs, 60 had antibody against NiV G protein by luminex testing [19%, 95% Confidence Interval (CI): 14 - 24]. However, none of the serum samples demonstrated serum neutralization. The prevalence of NiV G protein antibody in pigs was higher in Rajshahi 26% [95% CI 17 - 38] and Nawabganj 23% [95% CI 16 - 33] than in Naogaon district 9% [95% CI 4 - 16]. Compared with the pigs that lacked NiV G antibody, pigs with NiV G antibody did not differ by age (median 23 months vs. 22.8 months, P=0.9); sex (21% female vs. 18% male, P=0.5); and raising pattern (pigs with NiV antibody: 20% raised in backyard vs. 15% raised in nomadic herds, P=0.4). In conclusion, this study identified serological evidence of Nipah or perhaps a closely related virus infecting pigs in Bangladesh. The difference in prevalence by geography suggests that the positive antibody tests did not result from nonspecific binding with porcine antigens. Actively screening pigs to identify henipavirus infections, may identify viruses of public health importance.

SEROLOGICAL AND MOLECULAR CHARACTERIZATION OF SUSPECTED CASES OF HEMORRHAGIC FEVER AND HEPATITIS VIRUSES 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. Evidence abounds for HFVs but the causative agents are not fully discerned. Molecular and serological tools to diagnose Lassa fever, Yellow fever and other viral haemorrhagic fevers (VHF) and research programmes identifying VHF agents, as well as estimating their public health relevance rarely exist in Ghana. Reports on the prevalence ratio of viral hepatitis (VH) in Ghana is one in six and this was consistent with a recent data in a study. There are no guidelines for the screening of viral markers for viral hepatitis; testing is left to the local health institutions to implement based on high index of suspicion. Routine screening for HAV and/or HEV are not
performed. However, reported fatal cases of HEV in pregnant women in southern Ghana have been documented. This study sought to establish the prevalence of VHF's and VH in Northern Ghana. On reports and geographical locations of border countries with confirmed VHF cases, 16 health facilities were chosen as sentinel sites. Patient consenting and meeting the case definition were recruited and sampled. Virus detection and characterization by serological and molecular techniques was done for viral agents associated with VHF. Laboratory analyses were conducted on 276 serum samples. Investigations with RT-PCR assays for all the clinical specimens were found negative for VHF virus types, Lassa, Crimean Congo, Yellow fever, Dengue, Ebola, Marburg, and Rift Valley. Anti-Lassa fever IgG antibody titers were recorded for one case (titer ≥ 1:20) and 8 cases of anti-Dengue type-2 IgG (titer ≥ 1:80). Two cases exhibiting specific IgG (titer 1:1280 and 1:1280) and IgM (titers 1:20 and 1:20) against Chikungunya virus were found. Viral nucleic acid were however detected for viral hepatitis agents including; 21 (7.6%) for Hepatitis A; 58 (21.0%) for Hepatitis B, and 23 (8.3%) for Hepatitis C viruses. Anti-HEV IgM antibodies in all serum samples evaluated were 62 (22.5%). Our findings do not indicate a significant presence of VHF agents in Northern Ghana. However, the data generated suggest that VH infections, which often share clinical symptoms with VHFs are widespread, illustrating the need for differential diagnosis to be implemented.

**NON-POLO HUMAN ENTEOVIRUSES ASSOCIATED WITH RESPIRATORY INFECTIONS IN PERU (2005-2010)**

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Human enteroviruses (HEVs) are known to cause respiratory tract infections and are classified into four groups (A-D) and 106 different serotypes. Little is known about the various HEVs' role in respiratory infections in South America. This study describes the epidemiology and phylogenetic characterization of non-polio HEV respiratory infections in patients with influenza-like illness enrolled in a passive surveillance network in various regions of Peru. Oropharyngeal swabs and epidemiological data were collected from participants after obtaining verbal consent. Viral isolation was performed in cell culture and identified by immunofluorescence assay. Serotype identification of HEV isolations was performed using commercial monoclonal antibodies. Identification of non-serotypeable isolations was carried out by reverse transcriptase-PCR, followed by sequencing for genotype determination. Between 2005 and 2010, we analyzed a total of 24,240 samples. We identified at least one respiratory virus in 41.1% of samples (9971/24240); of those, HEV was found in 173, for a prevalence of 0.7% (173/24240). Our results revealed a clear predominance of HEV-B species, 92.5% (160/173). No isolations of HEV-C and HEV-D were found. The mean age and standard deviation for patients in Peru between 1999 and 2011. Patients with fever >38°C and signs and symptoms compatible with viral infection were enrolled in a clinical-based febrile surveillance study in sites across Peru (Cusco, Junin, Lima, Loreto, Madre de Dios, Piura, and Tumbes). The identification of Group C viruses was made initially by viral isolation using Group C hyperimmune ascitic fluid in an indirect immunofluorescence test. Microneutralization tests, considered the most specific serological assay available, were used to distinguish among closely related Group C viruses. Between 1999 and 2011, 61 Group C isolates were recovered from approximately 32,000 acute-phase febrile serum samples. All of the Group C viruses identified were isolated from patients in the jungle cities of Iquitos, Yurimaguas, and Puerto Maldonado. Isolates serologically consistent with Caraparu virus (62.3%), Murutucu/ Marituba (34.4%) and Itaqui virus (3.3%) were identified. These results demonstrate the sustained transmission of and human exposure to distinct Group C viruses across Peru. Serological data will be paired with full-genome sequences to further explore the evolutionary relationships among Group C viral strains.

**FIELD TRIAL FOR RIFT VALLEY FEVER CLONE 13 VACCINE IN LIVESTOCK FARMS IN KENYA**

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We carried out a field trial of Rift Valley Fever (RVF) Clone 13 vaccine, produced by Onderstepoort Biological Products, South Africa, to determine its safety and efficacy in commercial livestock in Kenya. Experimental studies have suggested that Clone 13 is a superior RVF vaccine when compared to the currently available Smithburn strain vaccine, in terms of safety and efficacy, and Clone 13 has the potential for testing that can differentiate infected and vaccinated animals (DIVA). General health observations, injection site reactions, and rectal temperature were assessed by monitoring all animals for the first 3 days and on day 14 post-vaccination. Blood specimens were obtained on days 0, 14, 28, 56, 183 and 366 after vaccination and sera tested for the presence of IgM and IgG antibodies against RVF virus. The individual farm animal husbandry and reproduction management system was maintained for the study animals. A total of 404 animals (cattle, sheep, goats) located at three commercial farms in Kenya were enrolled in the study. Of these, 170 (42.1%) animals were not pregnant whereas 234 (57.9%) were at either early or late pregnancy. Of the 404 animals, 195(48.3%) were inoculated with RVF Clone 13 vaccine, and the remaining 209 (51.7%) animals inoculated with saline as placebo. Eleven (2.7%) study animals were lost to follow up after being sold following accidental trauma or lost to predation. Most animals maintained good health following vaccination with no adverse events except for 20 sheep (4.9%) that developed...
uncomplicated mild nasal discharge 1 to 3 days post vaccination confirmed as bacterial infections; all recovered. One cow was treated for pneumonia after birth. Of 234 pregnant animals that delivered, 231 (98.7%) had live births including 31 cattle, 102 sheep (including 10 twins), and 98 goats (including 26 twins); however, one (0.4%) abortion and two (0.8%) still births occurred in goats. No teratogenicity was observed in any offspring. Both IgM and IgG antibodies were detected in vaccinated but not unvaccinated animals. In conclusion, the RVF Clone 13 vaccine was found to be safe for use in cattle, sheep, and goats including animals in early and late pregnancies. The vaccine also appears to have good efficacy as demonstrated by detection of antibodies against the virus.

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IDENTIFICATION OF A NOVEL ORTHOBUNYAVIRUS ISOLATED FROM CULEX (MELANOCONION) PORTESI MOSQUITOES FROM IQUITOS, PERU

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Vector-borne pathogens are among the most important emerging and re-emerging viruses that cause epidemics in humans and livestock. In this study, we describe the isolation and molecular characterization of a novel orthobunyavirus (genus Orthobunyavirus, family Bunyaviridae) isolated from Culex (Melanoconion) portesi during entomological surveillance for arboviruses in the city of Iquitos, located in the Amazon basin of northeastern Peru. Mosquitoes were collected by CO2-baited CDC light traps, identified to species, pooled, triturated, and inoculated onto mosquito (C6/36) and mammalian (Vero 76) cell cultures. In one pool of Culex (MeI) portesi collected in November 2009, evidence for orthobunyavirus infection was detected by cytopathic effect and indirect immunofluorescent assay in inoculated Vero 76 cells using polyclonal antibodies against Group C orthobunyaviruses. Hyperimmune ascitic fluid against a range of Group C viruses, including Apeu, Itaqui, Murutucu, Marituba, Oriboca, and Caraparu viruses, were unable to neutralize the virus isolate in microneutralization assays. Full genome sequence was generated by random amplification and pyrosequencing and compared with sequences available in GenBank. While the highest sequence similarity was with Group C viruses, nucleotide (<75%) and amino acid identity (<70%) was low for S, M, and L segments compared with previously reported viruses. Based on serological and molecular results, we conclude that this isolate is a novel member of the Group C orthobunyaviruses. Future studies will be necessary to determine the prevalence and possible human health impact of this newly-identified virus.

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IMMUNE RESTORATION DISEASE AND CHANGES IN CD4+ T-CELL COUNT IN HIV-INFECTED PATIENTS DURING HIGHLY ACTIVE ANTIRETROVIRAL THERAPY AT ZEWDITU MEMORIAL HOSPITAL, ADDIS ABABA, ETHIOPIA

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Highly active antiretroviral therapy (HAART) improves the immune function and decreases morbidity, mortality and opportunistic infections (OIs) in HIV-infected patients. However, since the use of HAART, immune restoration disease (IRD) has been described in association with many OIs. Our objective was to determine the proportion of IRD, changes in CD4+ T-cell count and possible risk factors of IRD in HIV-infected patients. A retrospective study of all HIV-infected patients starting HAART between September 1, 2005 and August 31, 2006 at Zewditu memorial hospital HIV clinic, Addis Ababa, Ethiopia was conducted. All laboratory and clinical data were extracted from computerized clinic records and patient charts. A total of 1166 HIV-infected patients with mean ± SD age of 36 ± 9.3 years were on HAART. IRD was identified in 170 (14.6%) patients. OIs diagnosed in the IRD patients were tuberculosis (66.5%, 113/170), toxoplasmosis (12.9%, 22/170), herpes zoster rash (12.9%, 22/170), Pneumocystis jirovecii pneumonia (4.1%, 7/170), and cryptococcosis (3.5%, 6/170). Of the 170 patients with IRD, 124 (72.9%) patients developed IRD within the first 3 months of HAART initiation. Low baseline CD4+ T-cell count (odds ratio [OR], 3.16, 95% confidence interval [CI], 2.19-4.58) and baseline extra pulmonary tuberculosis (OR, 7.7, 95% CI, 3.36-17.65) were associated with development of IRD. Twenty nine (17.1%) of the IRD patients needed to use systemic anti-inflammatory treatment where as 19(11.2%) patients required hospitalization associated to the IRD occurrence. There was a total of 8 (4.7%) deaths attributable to IRD. In conclusion, the proportion and risk factors of IRD and the pattern of OIs mirrored reports from other countries. Close monitoring of patients during the first three months of HAART initiation is important to minimize clinical deterioration related to IRD.

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DISTRIBUTION OF KILLER CELL IMMUNOGLOBULIN-LIKE RECEPTORS (KIR) GENES IN AN ADMIXED PERUVIAN POPULATION

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Killer cell immunoglobulin-like receptors (KIR) are glycoproteins located on the surface of NK cells. These receptors are classified into two groups according to their cytoplasmic domain, which transduces inhibitory or activating signals, and consequently modulates NK cell function and most likely the susceptibility to diseases or infections. We studied the distribution of KIR genes in 363 Peruvian HTLV-1-infected individuals using two ethnic classification methods: 1) a questionnaire, which defined the participants as Andean (both parents born in the Andes) or Mestizo (only one parent born in the Andes); and b) ancestry informative markers (AIM), which allowed classifying the whole population into three groups according to their ethnic admixture proportions. DNA was obtained from blood samples of each individual and KIR genotyping was carried out using PCR-SSP. No significant differences were observed in gender and age according to the Andean/Mestizo classification, whereas significant differences were found when the ethnic admixture proportion criterion was applied. The frequency of KIR2DS3, KIR2DS4 and KIR2DL3 were statistically different between Andeans and Mestizos. When using ethnic admixture proportion, significant differences were observed for KIR2DL1 and KIR2DS4s in addition to those genes, among the three groups defined. No significant differences were detected in haplotypes and inhibitory-activating KIR genes using either the questionnaire or AIM-based classification. AIM helps minimizing both the bias in ethnic group definition and the effects of population stratification, and therefore should be used in order to avoid false results when searching for gene-disease associations in admixed populations.

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SEROPREVALENCE OF SELECTED ARBOVIRUSES IN IJARA AND MARIGAT DISTRICTS, KENYA


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Arboviruses are transmitted by arthropods; humans become infected during blood meal by infected mosquitoes, ticks and sandflies. Arboviruses have been well characterized in many industrialized countries, but there are many knowledge gaps in developing nations. Entomological surveys conducted so far have demonstrated circulation of arboviruses of significant public health importance in Aedes, Anopheles and Culex species in vast populations in Kenya, suggesting the presence of competent vector systems for many of the arboviruses. The human population involvement in the transmission of these viruses has however not been demonstrated. This study sought to determine the seroprevalence of a range of arboviruses including Chikungunya, Dengue, Sindbis, Sandfly Naples, Sandfly Sicilian, Uganda S, West Nile and Zika viruses in Ijara and Marigat. About 41% of patients’ serum samples was used to test for antibodies to each of the viruses listed. All the samples were tested by IgG ELISA. A total of 351 patient serum samples were analyzed, of these 193 (54.9%) were male while female were 158 (45.1%), and age range was between 3 and 73. The overall positivity for the arboviruses was 53/351 (15.1%). The arboviruses prevalence in Marigat was 7% (10/143) while Ijara was 21% (43/208). Uganda S virus was the most prevalent with 10%, followed by West Nile virus 6%, Sindbis 5%, Dengue 2%, Chikungunya 1.1%, Sandfly Naples 0.2%. Antibodies against Sandfly Sicilian and Zika viruses were not detected. This is the first documentation of antibodies against Sandfly Naples virus in the sub-Sahara Africa. This study has shown the evidence of past exposure of the selected arboviruses in human population in the two sites. This information together with vectors data will strengthen efforts to develop focused preventive actions to stop transmission and create awareness among clinicians to help improve patients’ management in the region.

LONG-TERM CLINICAL, IMMUNOLOGIC AND VIROLOGIC FOLLOW-UP IN A COHORT INFECTED WITH MAYARO VIRUS

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Alphaviruses, such as Venezuelan equine encephalitis virus and chikungunya virus, are mosquito-borne pathogens possessing the potential of causing explosive epidemics with chronic sequelae in those infected. Much less is known about Mayaro virus (MayAV), an alphavirus endemic to the Amazon basin that causes fever, malaise, and joint pain in the acute setting. Long-term studies of this virus are rare and limited to case reports. Starting in January 2011, as part of a collaborative febrile surveillance project with the Peruvian Ministry of Health in the Amazon cities of Iquitos and Yurimaguas, we enrolled 17 patients with acute MayAV infection confirmed by isolation (11), PCR (6), or ≥4-fold change in IgM titer (17). Most patients had more than one positive assay. In addition to their acute visit, patients were also evaluated at 20 days (±10 days), 3 months (±10 days), and 6 months (±15 days). At each follow-up visit, a detailed interim history was taken and a physical examination was performed. In addition, serum was obtained in order to evaluate IgM and IgG titers and urine was collected and will be evaluated with PCR. Preliminary results show that while IgG levels persist at high levels at 6 months, IgM titers remain elevated in most patients at 3 months, but return to zero in the majority at 6 months. Fourteen of seventeen patients had arthralgias at the acute visit, 3 of 17 (18%) at 20 days, 7 of 16 (44%) at 3 months, and 6 of 12 (50%) at 6 months. Distal joints, specifically of the hand and ankle, were the most commonly affected. Malaise, present in 17 of 17 (100%) at the acute visit, persisted in 2 of 17 (12%) patients at 20 days, 2 of 16 (13%) at 3 months, and 0 of 12 (0%) at 6 months. Our investigation will continue to follow these patients at one and two year visits, and we will also investigate immunologic and virologic findings that correlate with long-term morbidity.

POST-EPI DEMIC SEROPREVALENCE OF RIFT VALLEY FEVER VIRUS AMONG SOMALI VILLAGES IN NORTHEASTERN KENYA

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In endemic areas, Rift Valley fever virus (RVFV) is a significant threat to both human and animal health. Goals of this study were to measure human anti-RVFV seroprevalence in a high-risk area following the 2006-2007 Kenyan Rift Valley Fever (RVF) epidemic, to identify risk factors for RVFV exposure, and to monitor for sequelae of RVFV disease. We conducted a large cross-sectional village cluster survey among residents aged 1-85 years in 6 villages in Ijara District, Northeastern Province, Kenya: Tumtish (N=190, 47 households), Matarba (N=289, 86 households), Gorahindi (N=289, 86 households), Gedilun (N=237, 63 households), Golalbele (N=85, 27 households), and Sabenale (N=64, 20 households). Participants underwent questionnaire administration, physical exam, vision testing, and blood collection for RVFV testing. One thousand one hundred seven individuals were tested for RVFV exposure via RVFV IgG ELISA; 667 or 60% were women and 631 or 57% were children aged 1-15 years. Overall, 173/1111 or 16% (CI95% 13.78-18.42) of local residents were RVFV seropositive. Seroprevalence varied by village: Tumtish (27/190, 14%, CI95% 9.59-20.02), Matarba (37/242, 15%, CI95% 11.00-20.27), Gorahindi (53/291, 18%, CI95% 15.63-24.99), Gedilun (32/237, 13.5%, CI95% 9.27-18.05), Golalbele (15/87, 17%, CI95% 9.98-26.84), and Sabenale (10/64, 15.6%, CI95% 7.26-26.86). Visual impairment (defined as ≥20/20) was much more likely in the RVFV-seropositive group (P<0.0001). Our results highlight significant variability in RVFV exposure in six neighboring villages having very similar climate, terrain, and Somali populations. In concordance with previous studies, RVFV seropositivity was associated with poor visual acuity. Further analysis of questionnaire data will elucidate primary risk factors for RVFV exposure.

REVISITING ALBERT SABIN’S RESEARCH ON HUMAN DENGUE INFECTION

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There is no good animal model for dengue, one of the most important emerging tropical infectious diseases. As a result, we have relied on
DENGUE VIRUS INFECTION IN THE SKIN: DENDRITIC CELLS AND THE ROLE OF MOSQUITO SALIVA

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Dendritic cells (DCs) that reside in the skin serve as sentinels of the immune system. The 4 dengue virus serotypes (DENV-1-4) are transmitted to humans by mosquito bites. DENV infection confers life-long protection to the immune system. However, primary DENV infection confers life-long protection to the immune system. A detailed analysis of the Sabin experiments will be presented.

DENGUE VIRUS-SPECIFIC HUMORAL AND T CELL RESPONSES IN NOVEL HUMANIZED MICE

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Dengue is a mosquito borne viral disease of humans, and animal models that recapitulate human immune responses and/or dengue pathogenesis are needed to understand the pathogenesis of the disease. We recently described an animal model for dengue virus (DENV) infection using humanized NOD-scid IL2rγ−/− mice (NSG) engrafted with cord blood hematopoietic stem cells (HSC). We sought to further improve this model by co-transplantation of human fetal thymus and liver tissues into NSG (BLT-NSG) mice. Enhanced DENV-specific antibody titers were found in the sera of BLT-NSG mice compared to human cord blood HSC-engrafted NSG mice. Furthermore, B cells generated during the acute phase and in memory from splenocytes of immunized BLT-NSG mice secreted DENV-specific IgM antibodies with neutralizing activity. We have characterized a panel of human monoclonal antibodies (MAbs) from B cells in BLT-NSG mice during acute DENV infection and in convalescence. Human T cells in engrafted BLT-NSG mice secreted IFN-gamma in response to overlapping DENV peptide pools and HLA-A2 restricted peptides.
BLT-NSG mice will provide a much-needed platform to assess human immune responses to DENV vaccines and the effects of prior immunity on subsequent DENV infections.

TRANSMISSION OF DENGUE TO MOSQUITOES DURING PERIODS OF LOW VIREMIA

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Our understanding of dengue transmission dynamics is largely driven by hospital and/or symptomatic case data. It has been suggested that higher viremia levels, as observed in patients with more severe disease, may result in a higher probability of uptake by a vector, thus enhancing transmission. Comparatively little attention has been paid to the role of lower level viremic and possibly asymptomatic cases in dengue transmission. Such inapparent infections may account for the majority of dengue infections. Additionally, as dengue transmission occurs at a highly focally spatial level (households, schools, workplaces), the transmission to mosquitoes from such low viremias has not been sufficiently investigated. Accordingly, we investigated the rate of acquisition, subsequent dissemination and transmission of dengue virus serotype 2, strain 1232 at low viremia levels by Aedes aegypti (Rockefeller) mosquitoes from a permissive mouse model. Cohorts of mosquitoes kept for up to fifteen days post exposure to viremic mice were allowed to feed on naïve mice, and were then tested for virus infection and dissemination. Critically, at viremias as low as 1x10^4 and 1x10^5 pfu/ml, mosquitoes acquired virus infections, with predicted transmission rates of 7-18%. Our findings suggest that during lower viremia levels, asymptomatic (or prepatent) cases may account for an important proportion of the transmission potential to mosquito vectors.

GENOME-WIDE PATTERNS OF INTRA-HUMAN DENGUE VIRUS DIVERSITY REVEAL ASSOCIATIONS WITH VIRAL PHYLOGENETIC CLADE AND INTER-HOST DIVERSITY

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Analogous to observations in RNA viruses such as Human Immunodeficiency Virus, genetic variation associated with intra-host dengue virus (DENV) populations has been postulated to influence viral fitness and disease pathogenesis. Previous attempts to investigate intra-host genetic variation in DENV characterized only a handful of viral genes or limited numbers of full-length genomes. We developed a whole-genome amplification approach coupled with deep sequencing to capture intra-host diversity across the entire coding region of DENV-2. Using this approach, we sequenced DENV-2 genomes from the serum of 22 Nicaraguan individuals with secondary infection and captured ~75% of the DENV genome in each sample (range 40-98%). We identified and quantified variants using a highly sensitive and specific method, and determined that the extent of diversity was considerably lower than previous estimates. Significant differences in intra-host diversity were detected between genes and also between immunogenically-distinct domains of the Envelope gene. Interestingly, a strong association was discerned between the extent of intra-host diversity in a handful of genes and viral clade identity. Additionally, the abundance of viral variants within a host, as well as the impact of viral mutations on amino acid encoding and predicted protein function, determined whether intra-host variants were observed at the inter-host level in circulating Nicaraguan DENV-2 populations, strongly suggestive of purifying selection across transmission events. Our data illustrate the value of high-coverage genome-wide analysis of intra-host diversity for high-resolution mapping of the relationship between intra-host diversity and clinical, epidemiological and virological parameters of viral infection.

DAILY HANDHELD ULTRASONOGRAPHY PERFORMED BY CLINICIANS CAN DETECT SUBCLINICAL PLASMA LEAKAGE AND IDENTIFY DENGUE PATIENTS AT RISK FOR DENGUE SHOCK SYNDROME

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Plasma leakage is the critical feature of severe dengue infection. Timely detection of plasma leakage is important to identify those at risk for dengue shock syndrome (DSS). While presently available clinical and laboratory (e.g. hematocrit) markers have only low sensitivity, ultrasonography may improve timely detection of plasma leakage. Unfortunately, limited availability in resource poor settings and costs are hurdles for widespread implementation. In recent years, however, affordable handheld ultrasound devices have become available that can be operated by non-radiologists. We studied the possibility to detect plasma leakage and to identify patients at risk for DSS by non-radiologists using serial handheld ultrasonography in a prospective cohort of Indonesian patients with laboratory proven dengue. A total number of 66 patients were enrolled, of whom 44 were classified as non-DSS and 11 as DSS. At enrollment, subclinical plasma leakage in the form of ascites or pleural effusion was already detected in 26% of the patients. Presence of ascites or pleural effusion at enrollment had a positive predictive value of 35% for the development of DSS, and a negative predictive value of 90%. At enrollment, 55% of DSS cases already had detectable plasma leakage and this increased to 91% during the subsequent days. Gallbladder wall edema was most pronounced in DSS patients and often preceded ascites and/or pleural effusion. The findings of handheld ultrasonography corresponded well with conventional ultrasonography made by a certified radiologist during the critical phase of the infection. Serial hematocrit and albumin measurements, as recommended by WHO guidelines, failed to identify plasma leakage. In conclusion, serial handheld ultrasonography performed by clinicians is a sensitive tool to detect plasma leakage in dengue, in contrast to existing markers such as albumin and hematocrit. Detection of subclinical plasma leakage and/or an edematous gallbladder wall can identify patients at risk for DSS, and these patients merit more intensive monitoring of circulatory status and intravenous treatment. The introduction of more affordable handheld ultrasound devices, operated by shortly trained clinicians, can increase the clinical implementation of ultrasonography in dengue, especially in resource poor countries which are mostly affected by this devastating disease.
ELQ-300 FOR TREATMENT AND PREVENTION OF MALARIA

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ELQ-300 is a novel antirespiratory compound with low nanomolar IC50 values against blood stages of Plasmodium falciparum and P. vivax, including drug resistant laboratory strains and field isolates. It has, so far, proven impossible to generate ELQ-300 resistant mutants using single step methodology, with a demonstrated resistance frequency far improved over atovaquone. The in vitro potency, combined with the high metabolic stability, results in spectacular oral efficacy with a curative blood stage dose of 1 mg/kg against P. berghei or P. yoelii infected mice in standard 3 and 4 day tests. Interestingly, the molecule is also exquisitely potent against exo-erythrocytic stages and impacts not only liver schizonts, but also blocks stage V gametocyte development and inhibits ookinete formation in the mosquito midgut. Although its aqueous solubility is limited, ELQ-300 exhibits high oral bioavailability at therapeutically relevant doses with extended half-lives in rodents and dogs. Impressively, in vivo doses of 0.03mg/kg result in formal causal prophylaxis and killing of all P. berghei liver schizonts; furthermore this same dose results in complete inhibition of P. berghei oocyst formation in a mouse feeding study thus totally inhibiting sporogony and demonstrating a 100% block of transmission. ELQ-300 has high in vitro selectivity over human cytochrome bc1 and it is without cytotoxicity (10µM) against a panel of mammalian cell types. It was not inhibitory against a large safety and selectivity target panel of receptors, amine transporters and ion channels, including the hERG channel, nor is the compound genotoxic as assessed in a 5-strain Ames and in vitro micronucleus assays. Given the low predicted dose in patients, a long predicted human half-life, and potent activity against blood and exo-erythrocytic stages of parasite development, ELQ-300 offers the hope of a new molecule for the treatment, prevention and, ultimately, eradication of malaria.

POTENTIAL EFFICACY OF CITOCOLINE AS ADJUNCT THERAPY FOR CEREBRAL MALARIA

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During cerebral malaria (CM), sequestration of parasitised erythrocytes, platelets and leucocytes within the cerebral microvessels, coupled with cytokine overproduction leads and/or blood brain barrier (BBB) disruption and/or intercellular junction opening. We evaluated the efficacy of citocoline (CTC), a membrane stabilising agent already registered for use in ischemic stroke and anti-histamine as adjunct therapy to enhance the recovery from CM. Initial trials in Plasmodium berghei ANKA-infected mice (which develop a lethal syndrome 7 days post-infection) showed that treatment with CTC alone (1g/kg, from day-4 to day-7) doesn’t enhanced survival after day-7. After day-14, CTC in combination with a sub-effective dose of artemisinin (40 mg/kg) enhanced survival from 20% (artemisinin alone) to 75% (art+CTC). CTC is a very well tolerate compound, registered by the FDA as a nutritional supplement for children. It could be thus very easy to use in public health structures. These data support development of studies in human to address interest of membrane protector as adjunct therapy during Malaria.

PATHWAYS TO HEMOLYTIC TOXICITY OF PRIMAQUINE: EVALUATION OF POTENTIAL HEMOTOXIC METABOLITES OF PRIMAQUINE AND AMINOPHENOL ANALOGS IN VITRO ON NORMAL AND GLUTOSE 6-PHOSPHATE DEHYDROGENASE DEFICIENT HUMAN ERTHROCYTES

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Recent malaria treatment measures have resulted into significant decrease in cases of Plasmodium falciparum, but P. vivax cases are steadily rising. Primaquine (PQ) is the drug of choice for radical cure of relapsing P. vivax malaria. However, utility of PQ has been limited primarily due to hemolytic toxicity in the G6PD deficient populations. The redox active metabolites generated through CYP mediated pathways are responsible for hemolytic toxicity of PQ, but the identity of these metabolites has still remained elusive. The phenolic metabolites reported in in vitro metabolism and experimental animal studies have been suggested as the potential hemotoxic metabolites. In view of these, 5-hydroxy primaquine (5-HPQ), 8-N hydroxyl 6-methoxyaminoquinoline (NHMAQ) and some aminophenol analogs were evaluated in vitro on normal and G6PD deficient human erythrocytes. Hemolytic response was monitored with multiple biochemical markers namely, methemoglobin, reactive oxygen species (ROS) and depletion of reduced glutathione (GSH). The PQ metabolites and aminophenol analogs produced differential hemotoxic response on normal and G6PD deficient human erythrocytes. 5-HPQ and NHMAQ produced a robust increase in methemoglobin and ROS, and the responses were similar in normal and G6PD deficient erythrocytes. However, the metabolites depleted GSH only in G6PD deficient erythrocytes. This differential response may explain selective susceptibility of G6PD deficient individuals to hemolysis during treatment with PQ. SHFQ produced about 3 fold higher methemoglobin accumulation and more prominent depletion of GSH than NHMAQ. However, NHMAQ generated about three-fold higher ROS signal compared to 5-HPQ. The 2-aminophenols generated more prominent hemotoxic responses than 4-aminophenols, while 3-aminophenols were non-toxic. 4-Methyl and chloro substitutions potentiated the toxicity, while 4- and 5-nitro substitutions attenuated the toxicity of 2-aminophenols. A pattern of structure toxicity relation observed in hemotoxic response of aminophenols may be useful for designing PQ analogs with better therapeutic index.

AN IN VIVO GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G6PD)-DEFICIENT MOUSE MODEL TO PREDICT HEMOLYTIC TOXICITY OF CANDIDATE 8-AMINOQUINOLINE (8-AQ) ANTI-MALARIAL DRUGS

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Many of 8-aminoquinoline (8-AQ) compounds investigated to treat relapsing malaria are hemolytic in subjects with glucose-6-phosphate dehydrogenase deficiency (G6PDD). As 8-AQ drugs are the only treatment for relapsing Plasmodium vivax malaria, research studies to develop non-hemolytic 8-AQs are very important, and an in vivo model is desperately needed to test the hemolytic potential of newly developed 8-AQ drugs. We have “proof of concept” for a G6PDD-deficient (G6PDD) mouse model with a similar degree of G6PD deficiency as in the human African type.
A population (10-15% of normal G6PD activity); this model mimics the 8-AQ drug-induced hemolysis in human G6PD individual. This mouse model was validated using two known hemolytic 8-AQs, i.e., primaquine and pamaquine, and two known non-hemolytic drugs, chloroquine and mefloquine. Major hemolytic parameters evaluated were decreased red blood cell counts; increased reticulocyte counts; Heinz body formation; and decreased haptoglobin level in serum. Mice given the hemolytic drugs consistently displayed a hemolytic response, whereas those treated with chloroquine and mefloquine showed no significant hemolytic response. In this study, we assessed the effects of various dose levels of tafenoquine using this G6PD mouse model. Tafenoquine was given orally at several doses (13.3, 7.5 or 2.5 mg/kg/day) for 3 days; or (40, 30, 20, or 10 mg/kg.day) for 1 day. A known hemolytic drug, primaquine, was dosed at 8.8 mg/kg/day for 3 days, as the positive control. Tafenoquine at the 100% causal prophylaxis efficacy dose (CP-ED100, 2.5 mg/kg/day for 3 days, or 10 mg/kg for one day) demonstrated no hemolytic toxicity in our G6PD mouse model, whereas primaquine at 1 ED100 (8.8 mg/kg/day for 3 days) displayed a hemolytic response. Higher doses of tafenoquine, above the ED100 (e.g. 13.3 mg/kg/d for 3 days, or 40 mg/kg for 1 day), were shown to induces hemolysis in G6PD mice. We conclude that tafenoquine treatment at the ED100 dose is safer than treatment with PQ at the ED100 dose.

A PHASE I STUDY TO INVESTIGATE THE HAEMOLYTIC POTENTIAL OF TAFENOQUINE IN HEALTHY SUBJECTS WITH GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY (TAF110027)

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Tafenoquine (TQ) is an 8-aminoquinoline (8-AQ) with a half life of 2-3 weeks currently in development as a single dose treatment for the radical cure of Plasmodium vivax malaria. 8-AQs cause haemolysis in glucose-6-phosphate dehydrogenase (G6PD) deficient individuals; common in malaria regions. As a first step to investigate TQ's haemolytic potential quantitatively we have commenced a dose escalation Phase I safety study. We recruited healthy female volunteers (Hb >12 g/dl) who were heterozygous for the G6PD Mahidol mutation assessed by PCR-RFLP with red cell G6PD enzyme activity 40-60% of normal (based upon testing n=39 males defining a local median value of 11.5 IU/gHb). Based on previously published results, G6PD negative control subjects were Mahidol negative with >90% G6PD activity. A priori we defined a dose escalation strategy based upon the number of subjects reaching a dose limiting toxicity (DLT) defined as an absolute decrease of ≥2.5 g/dL Hb or ≥7.5% in haematocrit: should 3 or more subjects reach DLT in any given cohort dose escalation will stop. We have completed dosing cohorts of 6 normal and 6 deficient subjects with single 100 mg and 200 mg doses. 1/6 of the heterozygote subjects in the 100 mg cohort, and 2/6 in the 200 mg cohort reached DLT. Median maximum fall in Hb in both cohorts was 1.6 g/dL (range 0.9 - 2.4 in the 100 mg cohort, range 1.3 g/dL - 3.1 g/dL in the 200 mg cohort). An increase in bilirubin was common (5/6 in 100 mg cohort, 3/6 in 200 mg cohort) and by day 12 all subjects had exhibited a reticulocyte level of at least 2.2%. In G6PD normal subjects smaller Hb falls were observed (range 0.6 to 2.1 g/dL) but were not attributed to hemolysis. Additional relevant clinical and laboratory data will be presented. TQ is a promising new therapy for radical cure of P. vivax in G6PD normal subjects. Our data demonstrate that in non-anaemic G6PD deficient heterozygotes with 40-60% G6PD enzyme activity TQ regularly causes haemolytic anaemia, which appears to be dose-related, and has been of mild to moderate degree with a dose of 100-200 mg.

USING HUMAN BLOOD STAGE PLASMODIUM FALCIPARUM INFECTION TO DEFINE THE ACTIVITY OF LICENSED AND EXPERIMENTAL ANTIMALARIAL DRUGS

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The growing awareness of slow clearance as a potential marker of incipient failure of artemisinin antimalarials has highlighted the need for better defining the clearance kinetics of parasites following drug therapy. Most previous studies have used parasite counts as determined by slide estimation to determine clearance. Such data are of suboptimal quality to precisely define the inherent activity of various antimalarials. We have undertaken a series of four studies in human volunteers experimentally infected with blood stage parasites of Plasmodium falciparum where the clearance kinetics of parasitemia have been closely monitored by serial quantitative PCR. Data will be presented on the kinetics of clearance, presented as the parasite reduction ration (PRR) in these four cohorts following treatment with artemether-lumefantrine, atovaquone-proguanil, pyrimethamine-sulfadoxine and mefloquine. Benchmarking the pharmacodynamic activity of both licensed and experimental antimalarials and correlating these data with the pharmacokinetic profile of the drugs will provide a much needed evidence base for the development of new antimalarials as well as improving understanding drug activity and the development of drug resistance.

A POPULATION PHARMACOKINETICS ANALYSIS OF OZ439 DISPOSITION IN PATIENTS

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OZ439 is a synthetic trioxolane undergoing clinical investigation and being developed by MMV as a potential anti-malarial therapy. OZ439 has shown in vivo a potential for a single dose cure in Plasmodium falciparum malaria while showing in vitro and in vivo models marked prophylactic potential, extended half-life and oral bioavailability, and increased metabolic stability. Data from Phase I study in healthy volunteers and the on-going Phase IIa have confirmed OZ439 to be safe and tolerable. The pharmacokinetics (PK) of OZ439 will help determine its potential as a single dose cure. PK data from the on-going Phase IIa investigation in patients was analysed using a non-linear mixed effects approach. In this study single doses of OZ439 have been orally administered to patients. The optimal structural model was a 2 compartment model with oral absorption. The oral absorption had a lag time associated with it. Inter-individual variability was attributed to the central volume of distribution, clearance and lag time. The observed PK was linear with a clearance of 65.7 L/hr and an elimination half-life of 53 hours; thus the PK supported OZ439's potential as a single dose cure. This population PK model will aid in predicting the time course of OZ439 at different doses and will be updated as more data is collected. This will include determining the covariates of the PK parameters in order to characterise the effects of age, co-administered drugs, and ethnopharmacology. A major benefit of characterising the PK in this manner is the ability to use these parameters.
in PK/PD models, to make population predictions of OZ439’s efficacy as an anti-malarial, to confirm the suitability of OZ439’s PK for a single dose cure and to design future clinical trials.

A CONSERVED PLASMODIUM PROTEIN REGULATES MEROZOITE PRODUCTION DURING INTRAERYTHROCYTIC SCHIZONY

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A critical aspect of Plasmodium biology is its ability to multiply exponentially during different stages of its life cycle. Both within the vertebrate and invertebrate hosts, the parasite undergoes a rapid increase in population through asexual reproduction, which is critical for its transmission and disease pathogenesis. In the human blood stages, P. falciparum typically produces an average 20 merozoites per schizont, which upon release, infect new host erythrocytes and continue to exponentially increase in numbers. Although this is a crucial step in maintaining infection in the human host, mechanisms controlling merozoite production are still poorly understood in Plasmodium. Here, we report the identification of a conserved Plasmodium protein, critical for regulating merozoite production in the intraerythrocytic stages of P. falciparum. piggyBac insertion into the coding sequence of this protein attenuates the parasite intraerythrocytic growth rate. Understanding the functions of this protein will provide novel insights into a crucial component of parasite biology.

GENOME-EDITING THE MALARIA PARASITE PLASMODIUM FALCIPARUM

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Malaria afflicts 225 million people worldwide and its most lethal etiologic agent, Plasmodium falciparum, is evolving to resist the latest-generation therapeutics. Tools for genome-directed study of such resistance and ways to overcome it, however, are sorely lacking. Here we report rapid and targeted genetic engineering of this parasite, using zinc-finger nucleases that produce double-strand breaks in a user-specified locus and trigger homology-directed repair. Targeting an integrated gfp locus, we obtained a homogeneous population of knockout parasites in an unprecedented 15 days. Moreover, ZFNs engineered against pfCRT produced parasites that carry a panel of investigator-defined point mutations and acquired antimalarial drug resistance. The efficiency, versatility and precision of this approach enable genome editing of this human pathogen to meet the challenge of substantially reducing the burden of disease.

GLOBAL IDENTIFICATION OF PALMITOYLATION SITES IN PLASMODIUM FALCIPARUM SCHIZONTS

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Protein palmitoylation, the addition of a 16-carbon saturated fatty acid to cysteine residues, is a major post-translation modification that is used in all eukaryotes to regulate protein function. Like phosphorylation, palmitoylation is a reversible event, and can serve to regulate protein localisation, stability, and activity either stably or in response to specific stimuli. Previously only three Plasmodium falciparum proteins were known to be palmitoylated, but by combining two established and orthogonal purification approaches we have recently identified more than 300 palmitoylated proteins in late blood-stage P. falciparum parasites, including palmitoyl-proteins involved in protein secretion, drug resistance, signalling, development, cytoadherence and invasion. This suggests that palmitoylation is likely to regulate multiple Plasmodium-specific biological processes, but in order to drive specific biological hypotheses it is critical that we identify which specific sites within each palmitoyl-protein are modified. Like phosphorylation, palmitoylation sites can largely not be predicted using primary sequence data alone, with the exception of cysteines immediately downstream of myristoylation sites. We have now developed a novel protocol that can identify specific palmitoylation sites, and have used it to identify more than 1000 palmitoylation sites in P. falciparum blood stage parasites, some of which are now being followed in functional studies. Given the scale at which this modification is used in blood stage parasites, palmitoylation should be considered alongside phosphorylation as a major regulatory mechanism and potential target for intervention.

DISCOVERY OF CONSERVED PLASMODIUM ANTIGENS ON THE SURFACE OF RED BLOOD CELLS USING DNA APTAMERS

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During its intraerythrocytic cycle, the human malaria parasite Plasmodium falciparum remodels the host red blood cell (RBC) plasma membrane with a poorly defined collection of antigenically diverse proteins. While these proteins play roles important for parasite survival and virulence they are exposed to potential drugs and immune effectors in blood for ~24 hours as parasites develop. It is not clear, however, whether any of these proteins are structurally conserved enough to provide a novel target for vaccine or drug development. Here we describe a novel DNA aptamer selection scheme to probe for epitopes on the parasitized RBC surface that are conserved between geographically distinct parasite isolates. Our scheme isolated 14 aptamers that tightly bind parasitized RBCs (with low-nanomolar dissociation constants) and possess no affinity for non-parasitized RBCs. Three aptamers recognize all laboratory-adapted clones and field isolates of P. falciparum, P. vivax and P. knowlesi tested yet show no affinity for the murine malaria parasites, P. berghei and P. chabaudi. Moreover, some of these aptamers efficiently kill blood-stage parasites in vitro in a dose-dependent and sequence-specific manner. Current work is focused on identifying the aptamer binding to membrane extract targets and elucidating their role in parasite survival. Discovery of a protein or epitope conserved between the major species of human malaria parasites may have implications for drug and vaccine development and validates our aptamer selection scheme as a powerful tool for antigen discovery.
CIRCULATING ANTIGEN LEVELS MAY PROVIDE A MONITORING TOOL FOR HUMAN
NEUROCYSTICERCOSIS

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Antigen levels may serve to monitor NCC patients after antiparasitic treatment, 
failures had persistent or increasing antigen levels. Urine antigen detection 
during the initial two weeks of therapy. Urine being a non-invasive sample, we wanted to evaluate 
clinical circumstances, urine levels have been shown to correlate with 
in a few months after successful antiparasitic treatment. Also, under 
circumstances, urine levels have been shown to correlate with 
levels. Urine being a non-invasive sample, we wanted to evaluate 
whether it could be used as a follow up tool to determine early changes 
in levels of circulating antigen, during the initial two weeks of therapy. Thirty-one patients, 18 men and 13 women, aged 18 to 80 years, with 
neurocysticercosis demonstrated by MRI and confirmed by antibody 
seroLOGY on EITB, had urine samples collected at baseline and at days 1, 
3, 5, 7, 9, 11, and 13. These samples were processed using a monoclonal antibody-based AgELISA (B158/B60). Sensitivity of the AgELISA was 81% 
(25/31) both in serum and in urine. There was a very high correlation 
between baseline serum and urine levels. Antigen levels did not become 
negative in this short period and had different trends, mostly stable, or 
reducing. In some patients, antigen levels decrease by more than 10 
times along two weeks. Patients who were later shown as treatment 
failures had persistent or increasing antigen levels. Urine antigen detection 
may serve to monitor NCC patients after antiparasitic treatment, 
particularly in those who are antigen positive at baseline.

BASELINE AND EARLY POST-ANTIPARASITIC TREATMENT URINE ANTIgen LEVELS IN NEUROCYSTICERCOSIS

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Precise detection of cysticercosis which paved the way to the development 
of a single cyst is located in brain. Scanners are also poorly available in 
 countries. In Madagascar seroprevalence of cysticercosis (in blood) can 
reach 20% in population of the highlands. However biological methods 
used in blood can give high level of false positivity due to extra-cerebral localisation of the cysts, and can be in same time poorly sensitive when a single cyst is located in brain. Scanners are also poorly available in 
tropical countries leading treatment of the patients with anti-elmhythms on behalf of ELISA anti-Taenia solium results. We thus urgently need to 
 improve diagnostic of NCC. We analysed cellular and serological immune 
response in adult patients suffering from recent seizures, with or without 
images of NCC on CT-Scan. We used both reference glycosylated proteins 
and liquid of cysts (LC) as antigen for lymphocyte proliferation tests (LPT) 
and for serological analysis. In the same time we compared anti-Taenia 
isotypes (IgE, IgA, IgD, IgG) in blood and in cerebrospinal fluid (CSF). 
LPT using LC but not glycosylated protein was found to be an accurate 
method to detect cysticercosis which pave the was to the development of 
new strategy of test for this disease. In the same time isotype analysis 
enhanced clearly local secretion of antibodies in CSF and IgD was more 
accurate to detect this proliferation than IgA or IgE. An overall analysis of 
these data will be presented.

ENHANCED CORTICOSTEROID USE IN THE TREATMENT OF PARENCHYMAL NEUROCYSTICERCOSIS REDUCES SEIZURE OCCURRENCES WITHOUT A REDUCTION IN EFFICACY OR AN INCREASE IN SIDE EFFECTS

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Although corticosteroids are commonly employed to reduce treatment 
induced seizures in the treatment of parenchymal neurocysticercosis 
(NCC), the benefits of their use and exactly how and when to use 
them has not been rigorously studied. In an open randomized trial, two 
groups of 31 patients with one or more viable parenchymal cysts were 
treated with 15 mg/kg albendazole for 14 days and either the standard 
dexamethasone regimen at 6 mg/day for 10 days, (Arm 1) or for 28 days at 8 mg/day with a 14 day taper (Arm 2). Subjects were required to be on 
antiseizure medication with proven effective blood levels. The number of 
seizures and persons with seizures were compared from 1-360 days with 
the interval of 11-42 days as the primary outcome, efficacy at 180 days by 
MRI and side effects from 1-360 days. Number of seizures (mostly partial 
seizures) and people with seizures were increased in Arm 1 compared 
to Arm 2 during the trial but did not reach significance for the primary 
outcome 11-42 day interval (p=0.623). There was a similar increase of 
generalized seizures in Arm 1 but too few occurred in either Arm to be 
analyzable. However, from days 1-10 and 11-21 the number of partial 
seizures (p=0.016 and p=0.016 respectively) and people with seizures 
(p=0.013 and p=0.020 respectively) were significantly increased in Arm 
1 compared to Arm 2. There was also a significant increase of partial
seizures (p=0.036) and persons with seizures (p=0.041) in Arm 1 during the entire course of the trial, mostly due to early events. By a number of measures efficacy including percent reduction in cysts cure rates, and subjects requiring retreatment, was similar between the Arms. Although there were more adverse events in Arm 1, mostly due to neurologically based changes, none of the events reached significance. Enhanced corticosteroids significantly decreased treatment induced seizures compared to a standard regimen without a decrease in efficacy or undue increase in side effects. Effects were due predominately to a decrease in seizures within the first 21 days.

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TWO LARGE EPILEPSY SURVEYS IN A CYSTICERCOSIS-ENDEMIC REGION IN TUMBES, PERU

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Epilepsy is one of the older diseases of mankind. The prevalence of epilepsy worldwide added to inadequate treatment and late intervention results in chronic morbidity and considerable mortality in poor populations. Neurocysticercosis (NCC), a helminthic disease of the central nervous system, is one of the leading causes of seizures and epilepsy. Taking advantage of a large cysticercosis elimination program, we performed two wide scale cross-sectional studies in 58 rural communities of the Northern coast of Peru to assess the prevalence and characteristics of epilepsy and epileptic seizures in this cysticercosis-endemic region. Two studies were conducted between 2006 and 2007, involving 20,610 individuals. An initial epilepsy screening survey was followed by a phase of medical evaluation, followed by interview with a neurology specialist. A total of 17,452 individuals older than 2 years (86.41%) consented to participate. The overall prevalence of epilepsy was 17.25/1000, and that of active epilepsy was 10.8/1000 inhabitants, without marked differences between surveys. The prevalence of epilepsy by age increased after age 25 years and dropped after age 45. Only 45 out of 188 (23.94 %) patients with active epilepsy (30/107 and 15/81 from 2006 and 2007 respectively) were taking antiepileptic drugs. All of them were receiving sub-therapeutic doses. The seroprevalence of antibody against T. solium in individuals with epilepsy was approximately 40% in both studies. In the first survey there was no statistically significant difference in overall seroprevalence between individuals with and without epilepsy. The proportion presenting strong antibody reactions (4-7 bands by EITB) was however five times higher in individuals with epilepsy than in individuals without epilepsy. In the second survey, the seroprevalence as well as the proportion presenting strong antibody reactions were significantly higher in individuals with epilepsy. Brain CT showed NCC-compatible images in 109/282 individuals with epilepsy (39%). All individuals with viable parasites on CT were seropositive. Prevalence of epilepsy in this cysticercosis endemic region is high and NCC is an important contributor to it.

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PREVALENCE OF EPILEPSY IN 60 VILLAGES OF THREE PROVINCES OF BURKINA FASO

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Taenia solium cysticercosis is considered as an emerging infection in Sub-Saharan Africa. Results from a previous pilot study in Burkina Faso showed that half of people with epilepsy in two villages where pigs were raised had neurocysticercosis. The aim of this study was to estimate the lifetime prevalence of epilepsy in 60 villages located in three Provinces of Burkina Faso. This is the baseline cross-sectional component of a large community randomized-control trial which took place between February 2011 and January 2012. The provinces of Nayala (10 villages), Boukiemèdè (30 villages) and Sanguié (20 villages) were selected for inclusion in the study. In each province, all departments where pigs were raised (30 of 31) were selected. Within each department, two villages meeting the eligibility criteria were selected at random. In each village, one person was selected at random from each of 10 (with sows), 30 (with piglets) and 40 (with or without pigs) randomly-selected concessions (a grouping of several households). A total of 60 of 80 participants in each village were asked for their consent to provide a blood sample to test for the presence of antigens to the larval stages of T. solium using an ELISA test. A total of 4,970 individuals, aged 6 to 99 years (median of 30 years), were interviewed with a screening questionnaire for epilepsy. Screened positive participants were examined by a physician to confirm the diagnosis of epilepsy. Preliminary estimates of the unweighted lifetime prevalence of confirmed epilepsy were 3.0%, 1.0% and 3.4% in Boukiemèdè, Nayala and Sanguié, respectively. At the village-level, the lifetime prevalence showed important variation, ranging from 0% (in 7 villages, including 4 villages in Nayala Province) to 8.8% in one village of Sanguié. Although these data are being analysed to account for the clustered nature of the sampling strategy, they provide evidence that the prevalence of epilepsy varies spatially in rural Burkina Faso.

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CYST STAGE AND DIMENSION INFLUENCE SEROLOGICAL RESPONSE IN HEPATIC ECHINOCOCCUS GRANULOSUS INFECTION

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Diagnosis of hepatic cystic echinococcosis (CE) is based primarily on ultrasound (US) and serology. The latter has a minor role as it cannot distinguish between active and inactive cysts and its sensitivity and specificity are poor and influenced by many variables. Furthermore, most clinical studies on serology of CE do not take into account the cyst stage and this impairs their usefulness in clinical decision making. We investigated retroactively a cohort of patients diagnosed with CE who were seen in our clinic from May 2000-June 2012, by looking at the correlation, if any, of their cyst stage as seen on US and their serological test results (IHA and ELISA) results. Cysts were stratified by stages (active, transitional and inactive cysts, WHO IWGE) and by dimension (Sm 5 cm, M 5-10 cm and L≥ 10 cm). Of the 339 patients evaluated in 812 visits, 249 had 1 parasitic cyst and 90 had a non parasitic cyst. IHA and ELISA were positive in 87 and 83% of active cysts, 90 and 93% of transitional and 60 and 50% of inactive cysts and post-surgical residual cavities, respectively.
Specificity of both tests was 99%. A statistically significant difference (p < 0.0001) for median values of IHA and ELISA was found between active and inactive cysts, active and residual cavities and transitional and inactive cysts. Both tests were positive in 58% of examinations, specifically in 81% of active cysts, 90% of transitional cysts and 51% of inactive cysts. Both tests were negative in 99% of non-parasitic cysts. Serological response is also influenced by cyst dimensions. Both tests were positive in 76% of L, 62 and 48% of M and S cysts respectively, with statistically significant differences between serological response among L and S cysts and M and S cysts. Serological response to hepatic CE with routinely used tests is influenced by dimension and biological activity of the cyst. Future clinical studies on the use of serology in CE should take this into account.

**TH17/TREG IMBALANCE IN PATIENTS WITH LIVER CYSTIC ECHINOCOCOSIS**

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Echinococcosis is a chronic parasitic infectious disease regulated by T cell subsets. CD4+CD25+FoxP3+ regulatory T (Treg) cells and Th17 cells have been described as two distinct subsets and have the opposite effect on inflammation. Th17/Treg balance controls inflammation and may play important role in the pathogenesis of immune evasion. To assess whether this balance was broken, we detected Th17/Treg functions in different levels including cell frequencies, related cytokines secretion and key transcription factors in patients with cystic echinococcosis and healthy controls. The results demonstrated that patients with cystic echinococcosis revealed significant increase in peripheral Treg number, related cytokines (IL-10 and TGF-B1) and transcription factor (Foxp3) levels and moderate decrease in Th17 number, related cytokines (IL-17 and IL-23) and transcription factor (RORYt) levels as compared with controls. Results indicated that Th17/Treg functional imbalance exists in patients with chronic cystic echinococcosis, suggesting a potential role for Th17/Treg imbalance in the pathogenesis of immune evasion in echinococcosis.

**USE OF SMARTPHONES IN HEALTH AND DEMOGRAPHIC SURVEILLANCE**

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Health and Demographic surveillance systems (HDSS) can provide essential information in areas where routine vital registration is absent or incomplete. HDSSs also play an essential role in health intervention studies in such areas. Setting up and running an HDSS poses an operational challenge, and a reliable and efficient platform for data collection and management is a key prerequisite. openHDS is an HDSS data system that provides data entry, quality control, and reporting to support demographic and health surveillance. openHDS has recently been integrated with the Open Data Kit (ODK) software platform for data collection using mobile devices running the Android operating system. The use of direct data entry using smartphones offers a number of advantages: it reduces the workload of the data management team, allows for near real-time quality control, and can provide guidance for the project logistics. Here we present an overview of the openHDS/ODK software platform, and report on the experience of using this platform to set up a HDSS to support a malaria intervention study in Kenya, in the Rusinga Island during the Solarmal Project in collaboration with the ICipe research center in Mbta, Nyanza (Kenya).
LESSONS LEARNED IN IMPLEMENTING LOW-COST EHEALTH TOOLS IN NICARAGUA: SUPPORTING INFORMATION COLLECTION, MANAGEMENT AND USE IN HEALTH CARE DELIVERY AND PUBLIC HEALTH RESEARCH IN LIMITED-RESOURCE SETTINGS

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This is a critical time in the global dialogue about “eHealth”. Investing in efficient, accessible, and cost-effective information and communication technology (ICT) tools can help to improve health outcomes and prevent diseases in low-resource settings. Since 2004, the Sustainable Sciences Institute has been working with various eHealth and mHealth tools to support clinical and epidemiological data management needs for the Pediatric Dengue Vaccine Initiative cohort study, in collaboration with the University of California, Berkeley, and the Nicaraguan Ministry of Health. Beginning in 2009, several collaborative projects were launched in Nicaragua with local partners to adapt, test and implement various ICT tools to improve timely and efficient access to information for key healthcare actors at the primary care level. Work includes implementation at various scales of a web-based electronic medical record system (OpenMRS) for pediatric immunization tracking and prenatal health monitoring and follow-up. In 2010, an open-source web-based Laboratory Information Management System (LIMS) was developed for the National Diagnostic and Reference Laboratory and its regional centers, which is currently in the implementation phase. In 2011, a primary care blood transfusion recipient tracking system was developed, linking the Red Cross blood donation information system with that of the national blood bank commission. In parallel, work with OpenROSA-compliant open-source mobile health technologies including OpenXData, OpenDataKit, CommCare, EpiSurveyor, and FrontlineSMS is ongoing and aims to extend the reach of data collection and reporting tools at both the clinic and community levels. These applications include support of the Behavior Change Communication project working with men in rural areas, pregnancy and child emergency notification systems, rapid notification of communicable diseases, and decision support for community surveillance, all using phones as primary data collection instruments. Critical lessons learned include engaging primary stakeholders early and often in the iterative processes of design, implementation and evaluation of these interventions. This helps to ensure that changes in work flow and information flow facilitated by ICTs are incorporated in a sustainable way to support health system strengthening.

MORTALITY TRENDS FROM 2003 TO 2009 AMONG ADOLESCENTS AND YOUNG ADULTS IN RURAL WESTERN KENYA USING A HEALTH AND DEMOGRAPHIC SURVEILLANCE SYSTEM

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Targeted global efforts to improve survival of young adults need information on mortality trends; contributions from health and demographic surveillance system (HDSS) are required. Retrospective analysis of deaths among adolescents (15-19 years) and young adults (20-24 years) was conducted using census and verbal autopsy data in rural western Kenya under HDSS. Mid-year population estimates were used to generate all-cause mortality rates per 100,000 population by age and gender, by communicable (CD) and non-communicable (NCD) causes. Linear trends from 2003 to 2009 were examined. In 2003, all-cause mortality rates of adolescents and young adults were 403 and 1,613 per 100,000 population among females, and 217 and 716 per 100,000 among males, respectively. CD mortality rates among females and males 15-24 years were 500 and 191 per 100,000 (relative risk [RR] 2.6; 95% confidence intervals [CI] 1.7-4.0; p<0.001). NCD mortality rates in same aged females and males were similar (141 and 128 per 100,000, respectively; p=0.76). By 2009, young adult female all-cause mortality fell 53% (χ² for linear trend 30.4; p<0.001) and 61.5% among adolescent females (χ² for linear trend 11.9; p<0.001). No significant CD mortality reductions occurred among males or for NCD mortality in either gender. By 2009, all-cause, CD, and NCD mortality rates were not significantly different between males and females, and among males, injuries equalled HIV as the top cause of death. Significant reductions in adolescent and young adult female mortality rates evidence the effects of targeted public health programmes, however, all-cause and CD mortality rates among females remain alarmingly high. Data underscore the need to strengthen programmes and target strategies to reach both males and females, and to promote NCD as well as CD initiatives to reduce the mortality burden among both genders.

MALARIA RAPID DIAGNOSTIC TESTS IN CONTEXT: INSIGHTS FROM THE ACT CONSORTIUM

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The availability of affordable, accurate Rapid Diagnostic Tests (RDTs) for malaria is enabling a shift from presumptive treatment to parasitological confirmation. Questions remain as to where RDTs should be deployed, best practice to support their effective deployment and their potential impact and cost under real-life conditions. Key determinants have been proposed to include the epidemiological setting, prior provider and community experiences and practices and the presence and effectiveness of supporting interventions. This paper will present a framework for considering the complex issues of RDTs as they are implemented in context, focusing on steps along a pathway from initial diagnostic policy choice through uptake and provider “adherence” to test results and on into public health impact and cost-effectiveness. The presentation will draw on the multidisciplinary work of projects within the ACT Consortium from 9 countries in Africa and Asia and present empirical data from these studies and others from the literature. This will include experiences from the introduction of RDTs through public and private sector providers, and at the community level in a variety of epidemiological and health system settings.
DETECTION OF HUMAN MONKEYPOX IN THE REPUBLIC OF THE CONGO FOLLOWING INTENSIVE COMMUNITY EDUCATION

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In October, 2009, interethnic violence in northwestern Democratic Republic of the Congo (DRC) precipitated the movement of refugees across the Ubangi River into neighboring Republic of the Congo (ROC). By the end of January 2010, approximately 114,000 refugees had relocated to cities and villages along the river in ROC, concentrating mainly in areas north of the city of Impfondo, where medical resources are scarce. Monkeypox is an acute viral infection with a clinical course resembling smallpox. It is endemic in northwestern DRC, but appears to occur only sporadically in ROC. The influx of refugees to ROC heightened concerns about monkeypox in the area, owing to the possibility that virus could be imported, or that incidence could increase due to food insecurity and over reliance on bush meat. As part of a broad-based campaign to improve health standards in refugee settlement areas, UNICEF sponsored a program of intensive community education which included modules on monkeypox recognition and prevention. In April and May, 2010, UNICEF, the implementation partner for the program, performed outreach in 25 cities and villages where refugees had congregated. Approximately 65,000 people attended the outreach sessions. In the six months immediately following the outreach, ten suspected cases of monkeypox were reported to health authorities. Skin lesion specimens were collected from 5 of the suspected cases. Laboratory testing confirmed monkeypox virus infection in 2 individuals (one of whom was in a cluster of 4 suspected cases), and one individual was positive for yaws. Analysis of the viral genome of an isolate recovered from 1 of the 2 confirmed cases is highly similar (but not identical) to the virus implicated in a hospital-associated outbreak of monkeypox that occurred in Impfondo, ROC in 2003. It is less similar to a strain isolated from northwest DRC in 2009. Anecdotes collected at the time of case reporting suggest that the outreach campaign contributed to detection of suspected cases by generating a heightened awareness of monkeypox in refugee settlement areas.

HIGH INCIDENCE OF BURN-RELATED INJURIES IN A DENSELY POPULATED URBAN SLUM IN KENYA

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We examined the household incidence of burn-related injuries using a prospective, population-based infectious disease surveillance system consisting of approximately 28,000 individuals living in 6,000 households in the urban slum of Kibera, in Nairobi, Kenya. The study period was 5 years, July 2006–June 2011. A total of 3,072 cases (2,723 individuals) of burn injury were identified with an incidence of 27.9/1000 person years of observation (PYO). Burn incidence among children <5 years of age was 81.5/1000 PYO compared to 21.2/1000 PYO in those ≥5 (p<0.001). Females ≥5 sustained burn injuries at a rate 1.4-fold greater than males ≥5 (24.5 vs. 18.1/1000 PYO; p<0.001). The disparity was greatest for women 18-34 and 35-49 years age, who were 1.9-fold (p<0.001) and 2.1-fold (p<0.001), respectively, more likely to incur a burn injury compared to men of the same age group. Clinical data from a small proportion of all burn cases showed that 82% of burns were due to cooking, the remaining 18% was due to various non-cooking related accidents and other causes (i.e., electrical burns, assault, etc.). Overall burn injury rates from Kibera were 5-fold and 10-fold higher than rates from an urban regional study in Ghana and a national survey in Bangladesh, respectively. Burn injuries may contribute more significantly to increased morbity in the developing world than previously thought and are potentially impacted by urbanization where dense population and unsafe cooking environments may increase the risk.

THE MARKET IMPACT OF AN INTERNATIONAL COLLABORATION FOR QUALITY CONTROL OF RAPID DIAGNOSTIC TESTS FOR MALARIA

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The number of commercial malaria rapid diagnostic tests (RDT) has expanded over the past decade. The dynamic flux of products, together with weak regulation and a lack of consistent data on quality, has made quality-based procurement difficult. Over several years, WHO, TDR, FIND, US CDC, and other partners developed and operationalized malaria RDT Product Testing (PT) and Lot Testing (LT) programmes. Data collected since 2008 provides unique insight into the impact of such a programme on RDT quality and markets. Through open calls for expression of interest (2008-2011) to ISO13485-certified manufacturers, RDTs were submitted for evaluation against panels of low and high density cultured Plasmodium falciparum (Pf) parasites, wild-type Pf and Plasmodium vivax (Pv) parasites, and parasite-negative samples. Similarly, lot testing open to manufacturers and procurers uses the same, though much smaller, sets of Pf, Pv and malaria-negative samples. In 2011, surveys of manufacturer sales (2007-2010) were conducted. To date, three rounds of PT have been performed on 120 products, including 24 resubmissions. The average panel detection score (PDS) against low density samples increased between Rounds 1 and 3, by 9.6% for Pf and 11% for Pv. The average change in PDS at low parasite density for resubmitted products was greater, at 12.7% for Pf and 27.4% for Pv. Based on sales information from 31 manufacturers, the RDT market has increased from 45M in 2008 to 88M in 2010. Sales have shifted towards products with a higher PDS, a surrogate for analytic sensitivity. Lot testing requests have increased by 258%, from 139 in 2008 to 360 in 2011, representing ~50% of lots sold into the public sector; furthermore, LT failure rates have decreased. The malaria RDT evaluation scheme provides data to distinguish between well and poorly performing tests, which in turn informs procurement decisions and enables manufacturers with better tests to expand their markets. The dramatic improvement of resubmitted RDTs indicates that manufacturers can improve product quality when an oversight mechanism is in place.
VALIDATION OF AN AUTOMATED RDT READER AND DATA MANAGEMENT DEVICE IN TANZANIA

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Lack of proper quality assurance is perceived as a significant obstacle to the widespread implementation of RDT-based malaria management strategy, as recommended by WHO. Previous experience has shown that continuous training and/or job aids can abate reduction in diagnostic accuracy of RDT based diagnosis over time resulting from human error. As well, reporting of diagnostic events is very limited, imprecise and slow in most remote areas, impeding proper decision making by control program managers. Fio Corporation has developed a system to address both problems: improving quality of RDT based diagnosis by providing job aids for RDT processing, automated interpretation through digital technology and optional case real time reporting using transmission over cell phone network. A fully blinded study was conducted in Bagamoyo district of Tanzania to test the diagnostic accuracy of the Fio system using SD Bioline malaria Pf/Pan RDT. Patient population consisted of males and females > 1 y.o., with symptoms of acute malaria. Main statistical analysis by a third party included dx performance of RDT interpreted by device (DEV), dx performance of RDT interpreted by experts visual (VIS), and comparison of DEV and VIS. Reference standard: expert microscopy performed at a central location. RT-PCR was used as tie-breaker in discrepant results. 1346 patients were enrolled over a 6 week period. Overall Pf infection prevalence was 11.1%. DEV Sens: 95.3; Spec: 94.9; PPV:70.3, NPV:99.4. VIS Sens: 94.7; Spec: 95.6; PPV: 72.8; 99.3. Percentage concordance between DEV and VIS was 97.8. User errors were documented in 17/29 (59%) of discrepant results. Data from devices reached the Fio Cloud in real-time and could be accessed by PI & study coordinator. The Sens and Spec obtained are similar to other publications. Fio System was shown to deliver an automated high diagnostic performance, as good as expert visual interpretation. The system was found to be user friendly, practical, reliable and accurate. DEV false positives represented <1% of results.

EVIDENCE-BASED ANALYSIS OF WHEN TO SWITCH TO A COMBO MALARIA RAPID DIAGNOSTIC TEST IN LIBERIA

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In West Africa Plasmodium falciparum (Pf) predominates, accompanied by non-Pf species in mixed infections that can account up to 20% of all malaria cases, with relatively few non-Pf monoinfections. Mixed infections have led some countries to select combo RDTs for routine use. In countries where non-Pf monoinfections account for less than 5% of all malaria cases, Pf-only RDTs are preferable as interpretation of tests results is simpler and cost is lower. In 2011 Liberia’s NMCP with support from PMI/IMaD proposed monitoring malaria parasite species to respond to claims from providers saying Pf-only RDTs fail to detect malaria because non-Pf monoinfections were on the rise. To contain cost and ensure sample was representative of the population, parasite species identification (ID) was done on biological material collected during MIS 2011, and owned by the NMCP. Species ID based on MMEQA slides -excluding heavy parasitemias- were assumed to introduce selection bias, overestimating non-Pf. During MIS household parasitemia surveys, in addition to mRDTs, slides for MM and blotted blood for PCR were collected. Blind species identification was done at Liberia’s National PH Reference Laboratory and IMaD Office, with participation of three expert microbiologists (Level 1). MIS 2011 collected blood from 3841 children. A random subsample of 476 slides (15%) were selected for MMEQA and preliminary analysis of species ID based on thick blood films (thin films are better for species ID, but were not available). 70 contained malaria parasities. Two slides out of 70 had mixed infections (Pf+Pm) and 13 had only Pm, the rest were Pf exclusively. If these preliminary results are confirmed by the analysis of species in the whole set of positive and readable slides, this will signify that about one fifth of malaria cases have non-Pf monoinfection. Those are not detectable by Pf-only mRDTs, and there will be need -after using up existing stocks- to switch to a combo mRDT with the added cost of retraining, and printing job aids. Decisions such as switching from Pf-only to combo RDT’s or from ACT to a different medication is expected to be done on an annual basis or less frequently. A sample size of 750 individuals per stratum (i.e. health region) is easily attainable with biennial parasitemia surveys that reach about 4000 children.

FACTORS ASSOCIATED WITH ANTIMALARIAL TREATMENT OF MALARIA PARASITE-NEGATIVE PATIENTS AT HEALTH FACILITIES IN THREE REGIONS OF TANZANIA: 2010-2012

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Over-treatment of malaria is a common problem in many malaria-endemic countries, with parasite-negative patients often receiving an artemisinin-based combination therapy (ACT) or other antimalarials, leading to over-use of ACT, and potentially delaying appropriate treatment, which may have severe consequences. Understanding factors associated with antimalarial treatment of malaria-negative patients is crucial to addressing this problem, and will be of even greater importance if malaria transmission decreases and the fraction of fevers attributable to malaria is reduced further. To understand current treatment practices and identify factors associated with antimalarial treatment of parasite-negative patients, we conducted surveys at 320 health facilities in three regions in Tanzania with varying malaria epidemiology (Mwanza, Mbeya, and Mtwara). Surveys were undertaken in 2010 and 2012 before and after nationwide roll-out of rapid diagnostic tests for malaria (mRDTs). Patients with fever in the previous 48 hours were interviewed following their consultation at the facility. Finger prick blood samples were taken by the study team to test for malaria parasitemia, allowing cross-referencing with any diagnostic test used by facility staff. Data were collected on patient characteristics, previous treatment for fever, and care received at the facility. Health workers were interviewed about their qualifications, training and supervision, knowledge, and facility stocks of antimalarials and mRDTs. At baseline, data was collected on 1739 patients (follow-up data collection ongoing). By study blood slides, 93% tested negative for malaria in Mwanza, 98% in Mbeya, and 79% in Mtwara. Overall, 42% of malaria-negative patients were treated with antimalarials by health facility workers. We will report the results of multivariate regression analyses accounting for the complex sample design to identify patient, health worker and facility-level factors associated with correct management of malaria-negative patients before and after widespread availability of mRDTs to support health worker decision-making. These results will be relevant to the success of mRDT roll-out and the design of interventions to reduce over-treatment of malaria.

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COMMUNITY LEVEL MANAGEMENT OF FEVER IN AFGHANISTAN - THE ROLE OF MALARIA RAPID DIAGNOSTIC TESTS

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In areas of low and seasonal malaria transmission, differential diagnosis of non-specific fever is important for patient care, control of malaria and in treatment and control of non-malarial causes of fever. Afghanistan is endemic for both vivax and falciparum malaria but with a low transmission intensity and dominated by vivax which accounts for 80-90% of cases. Our previous research has shown that malaria is consistently over-diagnosed and treated at the clinical level, but little is known about how community health workers (CHW) treat patients in the community. A cluster randomised trial of malaria rapid diagnostic tests (RDT) was undertaken using 400 CHWs to recruit 2600 patients in two transmission areas of Afghanistan. All CHWs administratively attached to 22 clinics (clusters) received training on management of malaria according to Government and WHO guidelines. Half of the clinics were randomly assigned to the intervention (RDTs), while half used clinical signs and symptoms for diagnosis and treatment. The primary outcome was the proportion of patients appropriately treated and aimed to evaluate whether the intervention resulted in improved targeting of treatment for patients with and without malaria. This included the use of artemisinin combination therapy for the rarely encountered cases of falciparum malaria. The outcome was measured against PCR based diagnosis of malaria to give a gold-standard diagnosis. The accuracy of the RDT and the prescribers’ response to the results was assessed. This presentation will outline the results of the study and discuss implications for policy and practice of fever treatment at community and clinic level in malaria endemic areas outside Africa.

ASSOCIATIONS BETWEEN FIVE DIAGNOSTIC METHODS OF PLACENTAL MALARIA AND LOW BIRTH WEIGHT IN AN AREA OF HIGH MALARIA TRANSMISSION IN TORORO, UGANDA

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The adverse consequences of placental malaria (PM) have been well established. Most studies rely on measures of PM as a surrogate marker of adverse birth outcomes such as low birth weight (LBW). However, there are no consistent standards for defining PM. Determination of a diagnostic standard is essential in order to compare studies using PM as the outcome of interest. This study compared the associations between 5 different definitions of PM and LBW. A total of 565 HIV-uninfected pregnant women were enrolled at delivery and infants were weighed at birth. LBW was defined as less than 2500 grams. Specimens collected included placental blood and tissue for histopathology. Placental blood was used for 3 definitions of PM: 1) positive blood smear (BS) for asexual parasites, 2) positive HRP2-based rapid diagnostic test (RDT), and 3) positive PCR. Placental histopathology was used for 2 definitions of PM: 1) any evidence of asexual parasites or hemozoin pigment, and 2) a quantitative assessment of hemozoin pigment (present in > 50% of 50 high powered fields examined.) The overall prevalence of PM defined by placental BS, placental RDT, placental blood PCR, conventional histopathology, and quantitative assessment of hemozoin pigment was 17.5%, 23.4%, 28.1%, 65.8% and 16.9%, respectively. Placental BS and RDT were significantly associated with LBW (RR=1.72, CI=0.99-2.99 and RR=1.97, CI=1.19-3.26, respectively) whereas placental PCR was not (RR=1.42, CI=0.72-4.18). Any evidence of PM by histopathology was not significantly associated with LBW (RR=1.73, CI=0.72-4.18); however, the quantitative assessment of hemozoin pigment was strongly associated with LBW (RR=3.60, CI=1.75-7.39). Placental histopathology was the most sensitive test for evidence of active or past placental malaria. Placental BS and RDT were also associated with LBW, while placental PCR was not. A definition of PM based on quantitative measure of hemozoin pigment from histopathology specimens may provide the best surrogate measure of adverse birth outcomes.

DETECTION OF PLACENTAL MALARIA AND IMPACT OF RDT SCREENING AND TREATMENT ON PREGNANCY OUTCOMES IN AREAS OF VARIED TRANSMISSION

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The negative effects of malaria infection in pregnancy have long been recognized. Intermittent preventive therapy (IPTp) with sulfadoxine-pyrimethamine (SP) is becoming less effective as parasite resistance increases. New antimalarial medicines for IPTp are being considered, but have disadvantages. Therefore, screening with malaria rapid diagnostic tests (RDTs) may offer an accurate and practical way to identify pregnant women who will benefit from targeted antimalarial therapy. We assessed the association between antenatal (ANC) and intrapartum RDT results and pregnancy outcome in two African clinical settings (Uganda, hyperendemic, and Burkina Faso, seasonal transmission). We enrolled 995 (345 Uganda, 650 Burkina Faso) HIV-negative women in the second or third trimester of pregnancy and followed them to delivery. On the standard IPTp schedule and at the time of delivery, participants’ blood was collected for RDTs, microscopy, PCR and hemoglobin measurement; placental tissue for histology was obtained at delivery. Participants with negative RDT results received SP; those with a positive RDT received artemether-lumefantrine or quinine, and SP. Preliminary data from Uganda show that 123 (45%) participants had positive RDT results at routine ANC visits. There was no significant difference in mean birth weight for mothers who had a positive RDT at time of usual IPTp dose and those who had all negative RDT results (3.10 kg versus 3.13 kg, t=0.93, p=0.35). There were 8 adverse pregnancy outcomes, 7 of which were among women whose RDT results were all negative and who received SP only. At time of delivery there was no difference in maternal hemoglobin (12.12 g/dL in those with RDT positive results and 12.35 g/dL in RDT negatives, t=1.03, p=0.31). Additional data will be presented from both sites on the accuracy of diagnostic testing for malaria during pregnancy and on the potential for malaria RDT screening and treatment of asymptomatic pregnant women during antenatal visits to impact pregnancy outcomes.
TO ASSESS WHETHER INDOOR RESIDUAL SPRAYING CAN PROVIDE ADDITIONAL PROTECTION AGAINST CLINICAL MALARIA OVER CURRENT BEST PRACTICE OF LONG-LASTING INSECTICIDAL MOSQUITO NETS IN THE GAMBIA: A TWO-ARMED CLUSTER-RANDOMIZED STUDY

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Recently, there has been mounting interest in scaling-up vector control against malaria in Africa. It needs to be determined if indoor residual spraying (IRS with DDT) will provide significant marginal protection against malaria over current best practice of long-lasting insecticidal nets (LLINs) and prompt treatment in a controlled study, given that DDT is currently the most persistent insecticide for IRS. A two armed cluster-randomised controlled study was conducted to assess whether DDT IRS and LLINs combined provided better protection against clinical malaria in children than LLINs alone in rural Gambia. Each cluster was a village, or group of small adjacent villages. All clusters received LLINs and half received IRS in addition. 7,800 children, aged 6 months to 14 years, were enrolled and followed for clinical malaria using passive case detection to estimate malaria incidence, the primary endpoint, for two malaria transmission seasons in 2010 and 2011. Exposure to malaria parasites was assessed using light and exit traps followed by detection of Anopheles gambiae species and sporozoite infection rates. Children were surveyed at the start of the study and the end of each transmission season to determine Plasmodium falciparum parasite rates and prevalence of anaemia. Study findings will be discussed in relation to effective malaria control in the Sahel.

UNDERSTANDING THE LOCAL POPULATION STRUCTURE OF PLASMODIUM IN THE CONTEXT OF MALARIA CONTROL AND ELIMINATION

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There is always population structure and malarial parasites are not an exception. The meaning of such structures, however, would depend on the scale and focus of the research. In the context of malaria control, the evaluation of treatment efficacy requires genotyping parasites at the local or regional level to distinguish homologous from heterologous parasites in recurrent infections as well as to get a good knowledge of the haplotypes circulating in the area. Unveiling such dynamics requires a good understanding of the temporal population structure and the accuracy of the information will depend on the adequacy of the markers used. In this study, we determined the minimum number of microsatellites needed to differentiate Plasmodium population clusters and haplotypes pertaining to each cluster in sympatry by using 215 blood samples (107 infected with Plasmodium vivax and 108 infected with P. falciparum) from a population in Tumeremo (Bolivar State) in Venezuela collected between March 2003 and November 2004. We found that malarial parasites undergoes clonal expansions and that such dynamics needs to be taken into account during the onset of drug resistance at a local level. The use of this design could be easily applied in epidemiological studies to differentiate reinfection from recrudescence cases, describe gene flow and identify lineages that area stable in time. This information would be useful in determining specific geographic units of malaria treatment and control.
CLUSTER-RANDOMIZED 12-MONTH STUDY INVESTIGATING THE EFFECT OF COMMUNITY SCREENING AND TREATMENT OF ASYMPTOMATIC CARRIERS OF PLASMODIUM FALCIPARUM MALARIA WITH ARTEMETHER-LUMEFANTRINE ON SYMPTOMATIC MALARIA EPISODES IN CHILDREN AGED <5 YEARS AND HEMOGLOBIN LEVELS IN SUB-SAHARAN AFRICA

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The systematic detection of asymptomatic carriers (ACs) of Plasmodium falciparum by rapid diagnostic test (RDT) and subsequent treatment with artemisinin-based combination therapy has the potential to impact disease transmission. A single-center, controlled, parallel, cluster-randomized study was conducted in Burkina Faso to evaluate the impact at the community level of detecting and treating ACs during 3 community screening campaigns (CSCs) conducted before the rainy season. The two primary endpoints were: the number of symptomatic malaria episodes with a parasite density >5000/µL (SMRC5000) per person-year in infants and children aged <5 years during the follow-up period, and hemoglobin (Hb) level change from day 1 to day 28 of CSC1 in treated vs. untreated ACs >6 months of age. 18 villages were randomized in a 1:1 ratio. In the intervention arm, ACs were identified by RDT and treated with artemether-lumefantrine (AL) or an alternative (if AL was contraindicated). Blood was collected in the intervention arm from all subjects and in the control arm from a randomly selected subset, at CSC1 for Hb measurement and at each CSC for delayed microscopy reading so that subjects (and study personnel) remained unaware of their AC status. Symptomatic malaria episodes were treated with AL or alternative in both arms for the duration of the study. In total, 14,075 subjects entered the study. At study end, the cluster-level mean number of SMRC5000 per person-year in infants and children aged <5 years was similar between the intervention and the control arms (1.69 vs. 1.60; P=0.3482). The mean change in Hb levels from day 1 to day 28 of CSC1 in the intervention arm was 0.53 g/dL vs. -0.21 g/dL in the control arm (P<0.0001). These results show that the systematic detection by RDT and treatment of ACs at the community level did not have a significant impact on disease transmission in the population. Although the change in Hb level between the two arms was statistically significant, it was not deemed to be clinically meaningful.

THE EFFECTIVENESS OF A SINGLE ROUND OF MASS MALARIA SCREENING AND TREATMENT IN SOUTHERN ZAMBIA

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In Zambia the current interventions of insecticide-treated mosquito nets, indoor residual spraying and case management with artesiminin combination therapy are not likely to result in malaria elimination alone. As part of a pilot mass malaria screening and treatment intervention, 10 health facilities in Gwembe and Sinazongwe districts, Southern Province, Zambia were randomly selected to receive a single round of mass malaria screening and treatment preceding the 2012 high malaria transmission season. In December 2011 and January 2012 approximately 50,000 individuals, regardless of symptoms, were tested for malaria parasites by community health workers using ICT Mal PI rapid diagnostic tests. Individuals testing positive were treated with artemether-lumefantrine, the national first line malaria treatment. The single round of mass malaria screening and treatment will be evaluated using a combination of study designs and analyses: 1) a randomized post-only comparison between intervention and control areas of parasite prevalence in children <6 years of age measured through an oversampled malaria indicator survey in April 2012; 2) a randomized post-only comparison between intervention and control areas of parasite prevalence in all individuals measured through the first round of the intervention in June 2012; 3) a pre-post comparison of parasite prevalence within intervention areas (follow-up June 2012); and 4) a randomized longitudinal comparison of monthly outpatient laboratory-confirmed malaria cases recorded from health facilities within the 2 districts. Each method of evaluation has limitations including the lack of a baseline in the randomized post-only comparisons, the lack of a counterfactual in the pre-post comparison, and known biases in health facility routine data. Preliminary results will be available in September 2012.

REACTIVE CASE DETECTION FOR MALARIA ELIMINATION: REAL-LIFE EXPERIENCE FROM AN ONGOING PROGRAM IN SWAZILAND

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Reactive case detection, whereby households and neighbors of passively detected cases are screened and treated, is being implemented in a number of Plasmodium falciparum malaria eliminating countries. This type of surveillance is designed to take advantage of the spatial clustering of infection and is primarily used to target the asymptomatic infectious pool. A crucial, yet not well understood, aspect of reactive case detection is the size of the screening radius employed around each index case. Using nationwide reactive case detection data from Swaziland, collected between December 2010 - March 2012, analyses were conducted to explore the relationship between the probability of detecting a case and distance to the passively detected index case. Results show that infection is highly clustered at the household level, with the odds of detecting a secondary case being six to seven times higher within the index household than in households located either up to 100m or over 100m away. The probability of detecting a case outside the index household did not appear to be associated with whether another case was detected inside the index household. From an operational perspective, the data suggest that using a screening radius of 1 km is extremely challenging with high coverage unlikely to be achievable in resource constrained settings. Together, these results suggest that future reactive case detection in Swaziland could be made more efficient by focusing screening on a smaller radius or on the index household itself.
THE ROLE OF LUTZOMYIA INTERMEDIA SANDFLY SALIVA ON THE EARLY EVENTS OF LEISHMANIA BRAZILIENSIS INFECTION

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Leishmania parasites are transmitted to the mammalian hosts by the bite of phlebotomine sandflies. During this process, not only parasites but also sandfly salivary products are delivered to the host. Leishmania braziliensis is the etiological agent responsible for cutaneous and mucocutaneous leishmaniasis throughout Brazil and the parasite is transmitted by Lutzomyia genus of sandflies. The objective of this study was to investigate the influence of sandfly saliva on the development of the immune response to L. braziliensis infection. Previously, immunization with sandfly salivary gland extract (SGS) from L. longipalpis was shown to protect against infection while the opposite effect was observed following L. intermedia preimmunization. To understand the mechanisms involved in these differences, we analyzed the impact of L. intermedia preimmunization on the innate immune response. First, we characterized the cellular infiltrate in response to SGS inoculation in the presence or absence of parasites. Next, we examined the cellular recruitment and gene expression profiles in mice preimmunized with L. intermedia compared to mice given PBS as a control. The global effect of preimmunization with L. intermedia SGS on gene expression was subjected to microarray analysis, revealing a distinct set of IFN-inducible genes that were upregulated in response to immunization with SGS; however, these genes were silenced at the time analyzed in mice given L. braziliensis suggesting the parasite is modulating the dermal microenvironment creating a niche for parasite persistence.
Parasitological positive were 34/574 cattle, 2/108 goats and 1/21 dogs. PCR analysis recorded an infection rate of 66/300 (22%) in cattle, for T. brucei and 30 (45.4%) of 66 were positive by SRA LAMP. PCR analysis recorded 16/108 (14.8%) infection for T. brucei but negative by SRA LAMP in Goats. PCR recorded 4/11 T. brucei infections in dogs which were all negative by SRA LAMP. From Southern Tanzania, a total of 404 animals were screened which included 202 cattle, 85 sheep, 10 dogs, 5 donkeys and 102 goats. Microscopic analysis recorded 4 animals infected by T. congolense types of trypanosomes. PCR analysis of 69 animal blood out of 404, recorded 38/69 (55%) positive for T. brucei types. All animal species were equally affected by the T. brucei types as infection in cattle was 33/48 (68.7%); 2/6 for sheep and one each (1/5) for donkeys, goats and dogs. All T. brucei positive samples from southern Tanzania were negative by SRA LAMP. Results from this study indicate that livestock especially cattle may play an important role in the epidemiology of HAT and control of the vector, Glossina, supplemented with treatment of animals is an important measure for control of human infective trypanosomes, and HAT epidemiology. Of importance is the finding that many animals were positive for T. brucei, which in some cases like northern and western sites could be of zoonotic importance as confirmed by SRA LAMP. Presence of human serum resistance associated (SRA) gene confirms the presence of human infective trypanosomes that cause the T. brucei rhodesiense form of HAT.

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**TOWARDS TRYPANOSOMA CRUZI LINEAGE-SPECIFIC SEROLOGY FOR CHAGAS DISEASE**

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Chagas disease, caused by the protozoan Trypanosoma cruzi, remains an important parasitic disease in the Americas. It can be fatal in the acute phase, but life-long chronic infection may be asymptomatic, or lead to debilitation and death by cardiac and/or intestinal complications. Genetically diverse, T. cruzi is classified into the intra-species lineages TcI-TcVI, displaying disparate geographical distributions and ecologies. The varying disease outcomes may be linked to parasite lineage, and complicated by mixed infections. The work presented here addresses the development of lineage-specific serology to identify an individual’s history of exposure to T. cruzi lineages. The molecular diversity of the parasite surface antigen TSSA was analysed across a panel of reference biological clones encompassing T. cruzi genetic and ecological diversity, revealing lineage-specific B-cell epitopes. We demonstrate here the capacity of synthetic peptides based on the TcIIVI/VI common epitope to be recognised by antibodies in human sera from Brazil, Chile, and reported for the first time, Ecuador. Further, we report the first TcII- and TcIV-specific serology, from experimental murine models. A genomic approach to identify T. cruzi lineage-specific epitopes can be used successfully in developing a differential serology to investigate an individual’s history of T. cruzi lineage exposure, and lead to a greater insight into the link with Chagas disease outcome. Overall, this approach represents a potential new tool in Chagas disease epidemiology.

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**COMPARATIVE GENOMICS AND PHYLOGENOMICS OF THE PROTOZOAN PATHOGEN, TRYPANOSOMA BRUCEI**

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The protozoan pathogen, Trypanosoma brucei is the causative agent of Human African Trypanosomiasis (HAT) which affects mainly poor rural populations across sub-Saharan Africa. T. brucei is separated into three subspecies based on the disease forms they cause: T. b. gambiense - which causes a chronic form of HAT, T. b. rhodesiense - which causes an acute form of HAT and T. b. brucei - which causes the livestock wasting disease Nagana. We conducted whole genome sequencing of 16 isolates from across the distribution of T. brucei, followed by a referenced alignment to the annotated TREU927 T. brucei genome and identified 352,505 single nucleotide polymorphisms (SNPs) across the genome. Selection and recombination were estimated to provide a comparative genomic framework across the T. brucei genome. In addition, to test the validity of subspecies designations and competing evolutionary hypotheses in T. brucei, we developed a phylogenomic framework of 9,500 neutrally evolving, independent and unique sequence loci from 500 - 5,500 base pairs in length. Using species tree methods, we estimated the phylogeny of the T. brucei complex to determine relationships between T. brucei subspecies and identify the ancestral lineage within the species complex.

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**ROLE OF THE CHROMATIN REMODELING ENZYME HDAC1 IN LEISHMANIA AMAZONENSIS INFECTION: IMPLICATIONS FOR HOST TRANSCRIPTION REPRESSION**

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Leishmania parasites subvert important host cell signaling pathways involved in the control of the infection. NF-kB is an important transcriptional factor which modulates the expression of genes involved in the immune response. Recent results from our group demonstrated the activation of NF-kB transcriptional repressor homodimer (p50/p50) in L. amazonensis-infected macrophages, treated or not with LPS. As a result of this homodimer complex activation, we observed the down-regulation of the expression of nitric oxide synthase (iNOS) in infected macrophages treated with gamma Interferon. Besides the activation of transcriptional factors, chromatin epigenetic modifications are pivotal regulators of gene transcription. Chromatin remodeling proteins such as deacetylase histones (HDAC) are involved with transcriptional repression and may be associated with transcriptional factors, forming large repressor complexes. In this work, we have studied in details the iNOS transcriptional repression during L. amazonensis infection through the analysis of iNOS promoter occupancy by p50/p50 NF-kB complex and the participation of HDAC 1 in these events. We have found that the increased occupancy of p50/p50 iNOS promoter depends on PI3K/Akt pathway in L. amazonensis infected cells. Consistent with transcription repression, we have detected an increase in HDAC 1 mRNA and protein levels, as well as an increased activity of total histone deacetylase in infected macrophages. We have verified a relevant reduction of L. amazonensis amastigote growth in macrophages silenced for HDAC1 expression. We also verified the mRNA iNOS increased levels in infected macrophages during HDAC1 silencing, showing the participation of this deacetylase in iNOS promoter regulation. In fact, we have observed an increased occupancy of HDAC1 in NF-kB promoter-binding site and a decreased occupancy of acetylated histone 3 (lys 9). These results indicate that important epigenetic modifications associated with p50/p50 NF-kB homodimer are taking place in infected macrophages.

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MONOCYTE-DERIVED TNF-α AND METALLOPROTEINASE 9 IN PATIENTS WITH CUTANEOUS LEISHMANIASIS

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Cutaneous leishmaniasis (CL) caused by Leishmania braziliensis is characterized by the presence of one or more ulcerated lesions with elevated borders. High levels of IFN-γ and TNF-α are detected in these patients and these proinflammatory cytokines are known to play a role in the pathogenesis of CL, by inducing tissue damage. Upon infection with Leishmania or in presence of SLA, monocytes from CL individuals produce high levels of TNF-alpha involved in recruitment of monocytes. Recent studies have shown that circulating monocytes constitute a heterogeneous population based on expression of CD14 and CD16, these cells can be divided in classical (CD14+CD16−), intermediate (CD14+CD16+) and non-classical (CD14−CD16+) monocytes. Intermediate and non-classical monocytes are known to migrate to inflamed sites and secrete inflammatory mediators, and high frequency of these cells has been associated with pathogenesis of many inflammatory diseases. TNF-α can mediate the pathology of the disease through various mechanisms including induction of nitric oxide, expression of metalloproteinases (MMPs) and increased cytotoxicity. MMP-9 is a zinc-dependent enzyme that degrades collagen and has been associated with skin inflammatory diseases. Although the mechanism underlying ulcer development in CL is not known, it’s likely that MMP-9 contribute to tissue damage. Thus, our goal was to investigate the contribution of sub-populations of monocytes to TNF-α and MMP-9 secretion in CL patients. We found that early after infection (pre-ulcerative phase) the frequency of intermediate and non-classical monocytes is elevated in blood of CL patients. Also, while intermediate monocytes produced more TNF-α in response to Leishmania, non-classical ones were the main source of MMP-9 in most CL patients. Similarly, the biopsy study revealed that non-classical monocytes were the main MMP-9 producing cells. These results show that monocytes subpopulations contribute differently to the immunopathology observed in CL patients.

UTILITY OF A WUCHERERIA BANCROFTI SPECIFIC WB123-BASED IMMUNOASSAY FOR USE AS A SURVEILLANCE TOOL FOLLOWING CESSION OF MASS DRUG ADMINISTRATION IN A W. BANCROFTI-ENDEMIC AREA OF MALI

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Significant progress has been made toward the goal to eliminate lymphatic filariasis (LF) by 2020 though the tools for monitoring control success and certification of transmission interruption need to be refined. Recently modified WHO guidelines for transmission assessment surveys (TAS) have recently been proposed to guide decisions about stopping mass drug administration (MDA), but the tools for post MDA surveillance are likely to involve antibody testing. To assess the utility of antibody testing in a target (6-7 year olds) population, we assessed antibody reactivity to Wb123, a Wuchereria bancrofti (Wb)-specific antigen that is expressed early in parasite development and has been shown to be a sensitive and specific marker of exposure to Wb infective stage larvae (L3). Wb123 antibody was compared to calibrated thick smear of midnight blood. 298 children 6-7 years old from two villages in Mali one year following the cessation of 5 rounds of MDA were assessed. Using bloodspots for Wb123 antibody levels and a Wb123 luciferase immunoprecipitation assay systems (LIPS), only 1 of the 298 (0.3%) children tested were positive for anti-Wb123 antibody, a similar prevalence was seen on night blood smears (Wb microfilaria prevalence 0.3%). These data suggest that Wb123-specific antibody testing in children can be a sensitive and specific tool for monitoring transmission following MDA cessation. Given the prevalence of 0.3% (well less than 1%) continued yearly follow-up of prevalence in 6-7 year olds will provide insight into the continued utility of Wb123 immunassays for Wb transmission assessment following MDA not only in this area of Mali, but throughout Africa where co-incident filarial infections have limited the use of other recombinant antigen-based immunoassays.

HOST CHOICE BY ONCHOCERCIASIS VECTORS AND ONGOING TRANSMISSION IN AREAS UNDER IVERMECTIN CONTROL

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The ability of mathematical models to predict intervention impact on vector-borne diseases will be affected by whether the proportion of bloodmeals taken on humans depends on vector and/or host density. Empirical data on onchocerciasis transmission and vector host choice in areas which have received prolonged vector control and mass annual ivermectin treatments will enable locality- and vector-specific prediction of Onchocerca transmission. Seven study sites in four regions of Ghana were visited from 2009 to 2011 in both rainy and dry seasons, to study variation in blackfly and host densities, and host choice. Surveys of wild birds and mammals, households and domestic animals were conducted. Blackflies (15,466, 85% Simulium damnosum s.l.) were collected by host-dependent and host-independent methods, assessed for parity, and stored for molecular and morphological analysis for identification of fly and Onchocerca species, and bloodmeal origin. The size of human populations varied from 188 to 5,202; of domestic animals from 489 to 11,143; and the number of bird species from 31 to 61. Blackfly biting rates ranged from 0 to 298 bites/person/day, and parity rates from 18 to 27% (wet season) and from 30 to 46% (dry season). Three of the villages had levels of L3 larvae/1,000 parous flies above the WHO threshold for morbidity and transmission control (range 1.4 to 115.1 L3/1,000 parous flies) despite annual distributions of ivermectin for up to 23 years in one village. In these villages exposure to infective L3 larvae was between 0.04 and 3.66 L3/person/day. Flies had fed on a range of hosts, predominantly humans and pigs. Onchocerca spp. other than O. volvulus were recorded. Results indicate that blackflies have multiple blood hosts and that active transmission is still occurring despite annual or biannual ivermectin treatment. Such data will inform control programmes about the feasibility of, and the duration of ivermectin treatment required for, elimination of onchocerciasis.

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MOSQUITO-PARASITE INTERACTIONS AND IMPLICATIONS FOR FILARIASIS TRANSMISSION IN PAPUA NEW GUINEA

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In Papua New Guinea, filariasis is transmitted by members of the Anopheles punctulatus group of mosquitoes while culicines, major vectors in neighboring regions of the Pacific, are considered unimportant for transmission. In a diverse vector environment such as Papua New Guinea, it is likely that not all species contribute equally to transmission. Transmission potential can be influenced by vector competence to W. bancrofti as well as vector-host and vector-parasite interactions. To test this hypothesis, we exposed multiple vector species to microfilaricem blood of varying densities to measure time to development of infective-stage larvae (L3s) as well as prevalence and intensity of infection. At lower mf densities (30-60 mf/20ml) 30% of An. farauti s.s. harbored L3s (mean intensity 2.0) and 7% of An. punctulatus harbored L3s (mean intensity 1.0). At higher densities (130-160 mf/20 ml), 83% of An. farauti and 28% of An. punctulatus were permissive to the development of L3s with a mean intensity of 4.6 and 3.1 respectively. The extrinsic incubation period was equal in both species. In Culex annulirostris, no L3s were observed and development was halted at the first or second larval stage.

To put this into the context of transmission in Papua New Guinea we also investigated mosquito parity rates, mosquito biting behavior and availability of microfilariae in peripheral blood. Both An. farauti s.s. and An. punctulatus had comparable age structures. Although An. farauti s.s. is a more competent vector, this species might have a lower capacity to transmit filariasis because of asynchrony between peak biting times and W. bancrofti periodicity. An. farauti s.s. has a peak biting time of 1900h, five hours earlier than the peak density of circulating microfilaria. As a result, An. farauti s.s. is exposed to approximately 33% of the mf that are available at the peak biting time for An. punctulatus. Filariasis elimination efforts can be greatly enhanced by the integration of vector control; however, a greater understanding of the influence of vector behaviors and vector-parasite dynamics on transmission is necessary to inform these strategies.

THE COST-EFFECTIVENESS OF DOXYCYCLINE THERAPY FOR THE CONTROL OF HUMAN ONCHOCERCIASIS IN AREAS CO-ENDEMIC WITH LOIASIS

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The control of onchocerciasis in Africa is based on annual or biannual community-directed treatment with ivermectin (CDTI). However, CDTI is contraindicated in areas where loiasis is co-endemic, including large parts of central Africa, because of the risk of severe adverse effects (SAEs) associated with rapid microfilarial killing and blockage of brain vasculature leading to encephalopathy. An alternative strategy in these areas is to treat with doxycycline, which given daily for 4-6 weeks, is macrofilaricidal against Onchocerca volvulus, causing sustained reductions in adult worm and microfilarial loads. Crucially, Wolbachia is not present in Loa loa, which mitigates the risk of SAEs. Furthermore, the feasibility of achieving high levels of coverage and compliance with a six-week course of mass-distributed doxycycline and its long-term impact has been demonstrated in Camerooun. An onchocerciasis transmission model (EpiOncho) is used to show that community-directed treatment with doxycycline (CDTD) is approximately twice as effective in preventing cases of O. volvulus infection compared with CDTI and more than twice as effective in reducing levels of transmission. Moreover, CDTD is about as cost-effective as CDTI in loiasis co-endemic areas. This is partly because CDTD can be delivered less frequently than CDTI, and partly because of the high cost associated with implementing the additional monitoring and surveillance components of CDTI, which attempt to minimise the occurrence and impact of SAEs. We conclude that CDTD is a safe, viable and cost-effective alternative to CDTI for the control of onchocerciasis where loiasis is co-endemic.

ARE FIVE ROUNDS OF ANNUAL MASS DRUG ADMINISTRATION NECESSARY FOR LYMPHATIC FILARIASIS (LF) TRANSMISSION INTERRUPTION? LA TORTUE, HAITI 2012

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The World Health Organization recommends five years of annual mass drug administration (MDA) in areas where Lymphatic Filariasis (LF) prevalence exceeds 1%, followed by a Transmission Assessment Survey (TAS) in 6–7 years to determine if transmission has been interrupted. It is not clear if 5 rounds of MDA are necessary where the initial antigen prevalence is >1%, but <10%. LF mapping performed in Haiti in 2001 showed that 73% of country’s implementation units (IUs) fell within this prevalence range. For a country with limited resources, guidelines stating that ≤5 rounds of MDA are ample in low prevalence areas would allow for a re-evaluation of transmission within the vast majority of the country. This may result in stopping MDA, and refocusing efforts and resources on the remaining 27% of the IUs. In 2002 the prevalence of LF in La Tortue, Haiti was found to be 6%, and 2 rounds of MDA were performed, ending in 2005. A follow-up convenience sample of >1600 persons of all ages in 2006 found a prevalence of 0.6%. We performed a modified school-based TAS in 11–12 year old children on La Tortue to determine if transmission has been interrupted. Using Survey Sample Builder, we calculated a sample size of 909 children from 32 randomly selected schools with a critical cut-off of 11. After informed consent, we collected blood for ICT and filter paper testing, and GPS, demographic, migration, and risk factor data. We sampled 1082 children from 29 schools. A total of 7 children were positive for LF (prevalence 0.645%, 95% confidence interval: 0.389–0.901), below the critical threshold for transmission. Of the 7 positive children, only 1 child had migrated to the island. Data are being collected from the final three schools and filter papers are being run for antibody testing. Our results suggest that LF prevalence on La Tortue is below the accepted threshold for transmission interruption, and MDA does not need to be restarted. These findings suggest that 5 rounds of MDA may not be necessary in areas of Haiti where the initial prevalence was <10%.
OPTIMISM FOR LYMPHATIC FILARIASIS ELIMINATION: A CASE STUDY OF TANDAHIMBA DISTRICT, SOUTHERN TANZANIA

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Lymphatic Filariasis is endemic in almost all districts in Tanzania. The National Lymphatic Filariasis Elimination Program strategy includes interruption of transmission via Mass Drug Administration (MDA) and morbidity control. Tandahimba District had five annual rounds of MDA with Mectizan and Albendazole, and the coverage was above 65% at each round. Baseline data were collected in 2002, and sentinel site data were collected after three and five rounds of MDA in 2006 and 2008, respectively. Results from four sentinel sites indicated that the microfilaria (mf) prevalence decreased from 6.8% before MDA to 0.4% after five rounds of MDA. As part of a multi-country survey, a first Transmission Assessment Survey (TAS) was conducted in 2009 by following the newly developed Global Guidelines for Monitoring and stopping MDAs. In 2011, two years after stopping MDA, a second TAS was conducted. Sampling for TAS was based on Enumeration Areas (hamlets), with a cluster-sample household survey of 6-7 year-old children. Circulating filarial antigens (CFA) were detected using Immunochromatographic Test cards (ICT). Each positive ICT case was traced and examined at night for Microfilariaemia (mf). In 2009, a total of 1558 children from 69 hamlets were tested for CFA. Ten (0.64%) were positive and only one of these (10%) was mf positive. The follow-up TAS conducted in 2011 involved 1605 children and only 9 (0.56%) were ICT positive. None of these were night blood mf positive. The findings from the TAS surveys indicated that the ICT prevalence was well below the critical cut-off value of 2% for stopping MDA, as defined in the new WHO guidelines. On the basis of the significantly decreased LF transmission in Tandahimba District, it was decided to discontinue MDA and to intensify surveillance. This is the first district in Tanzania to have reached the critical cut-off point for stopping MDA.

GOOD PROGRESS TOWARDS THE ELIMINATION OF LYMPHATIC FILARIASIS IN BANGLADESH

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Bangladesh has a long history of lymphatic filariasis (LF) caused by the parasite Wuchereria bancrofti, and is estimated to have 70 million people at risk of infection, with up to 10 million suffering from various forms of clinical deformity. The National LF Elimination Programme was one of the first to start the elimination process in 2001 with mass drug administration (MDA) using albendazole and diethylcarbamazine (DEC) in endemic areas. Of the 19 districts implementing MDA, five districts have received >5 rounds of MDA and sentinel sites have shown <1% microfilaria (MF). Therefore, to determine if transmission has been interrupted, the new WHO Transmission Assessment Survey (TAS) was carried out in Meherpur, Barguna, Patuakhali, Rajshahi and Dinajpur districts. A school-based survey was undertaken with 6 and 7 year old children as the target population. LF prevalence was measured using Immunochromatographic test (ICT), with sample sizes and critical cut-off numbers calculated using the Sample Survey Builder. The TAS was carried out over a two month period, using trained field teams. The number of children sampled ranged from 1556 to 1692, with cut-offs of 18 and 20 respectively. In total 9 children were found to be ICT positive. No positive cases were found in Meherpur and Patuakhali, however, seven positive cases were found in Dinajpur and one positive case in both Barguna and Rajshahi districts. These results indicate that LF transmission has been interrupted and MDA can stop in these districts. This success may be attributed to high MDA coverage facilitated by Government support, timely and coordinated efforts of the Programme and successful partnerships. This is promising for Bangladesh, however, it will be critical to develop and maintain a systematic post-MDA surveillance strategy to fully confirm the interruption of transmission and reach its goal of LF elimination by 2015.

COMPARISON OF HEALTH FACILITY AND COMMUNITY-BASED ESTIMATES OF SOIL TRANSMITTED HELMINTH INFECTION IN NUEVA SANTA ROSA, GUATEMALA - 2010

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Soil transmitted helminth (STH) infections are associated with significant morbidity as well as decreases in cognitive function and growth retardation. In most countries with a significant STH burden, school-age children (SAC) are at greatest risk of infection, but preschool age children (PSAC) may also be infected. Data on STH prevalence by age is lacking for most of Guatemala and there is little information on PSAC. Data on community prevalence of STH is generally gathered through community-based surveys, but these are expensive and coverage is limited. We explored the use of STH prevalence data generated from stool testing for diarrhea surveillance in Guatemala as a proxy for community-based surveys. Sentinel surveillance for diarrhea (>3 loose stools in a 24 hour period) is conducted in selected peripheral Health facilities in the county of Nueva Santa Rosa (NSR). For comparison, we estimated community prevalence between July and August 2010 by collecting stool samples from residents >1 year of age in randomly selected households in NSR, irrespective of their history of diarrhea. Stool samples were tested for Ascaris sp., Trichurus sp., and hookworm using the Kato-Katz method. Individuals positive for any of the three parasites were considered infected. Facility-based surveillance data included 643 stool samples from 776 cases of diarrhea in 2010; 19 (3%) were positive for STH. Facility-level prevalence was highest among SAC (6%), though 4% (10/267) of PSAC and 2% (3/135) of adults were also infected. The community survey included 324 residents, and 41 (13%) were infected with STH. Prevalence was highest among SAC (18%, 19/104), though 13% (7/54) of PSAC and 9% (15/166) of adults also tested positive. A larger proportion of the health facility STH cases were in PSAC compared to the community survey (53% vs. 17%) and the age distribution was significantly different (P<0.01). Our findings indicate that the use of stool samples from health facility patients presenting with diarrhea underestimates community-level STH burden and skewes the age distribution of cases towards PSAC, possibly because more young children were brought to health facilities for diarrhea. However, both data sources indicate that both PSAC and SAC should be targeted for treatment to prevent negative outcomes associated with STH infection. Furthermore, consideration should be given to treating adults as possible reservoirs of household infections.
SOIL-TRANSMITTED HELMINTHS IN URBAN SCHOOL-AND PRE-SCHOOL-AGED CHILDREN: PREVALENCE AND MORBIDITY IN KIBERA, NAIROBI

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Soil-transmitted helminth (STH) control programs face an increasing need to assess urban transmission. At the same time, while most prevalence studies and World Health Organization (WHO) deworming recommendations focus on school-aged children (SAC), STH burden and potential treatment benefit among pre-school-aged children (PSAC) is less known. We conducted a study of pediatric STH infection prevalence and morbidity in the Kibera informal settlement in Nairobi, Kenya. 899 SAC (5-14 years) and 293 PSAC (6-59 months) were randomly selected as index children from the enrollment registry of the community-based surveillance platform run by the CDC’s International Emerging Infections Program in Kibera. Data from index children include a target of 3 stools tested by the Kato-Katz method for STH ova, anthropometry, hemoglobin, and family-reported febrile, diarrheal or respiratory illness. For SAC, sibling stools were tested for STH. Results from subjects with at least 1 stool (n=493 PSAC, 1225 SAC) were analyzed for STH prevalence and differences between age groups. In index children (n= 212 PSAC, 509 SAC), any STH infection was tested for correlation with anemia (based on age and altitude hemoglobin cutoffs as per WHO) and moderate or severe stunting, wasting or underweight as per WHO. All statistical testing was by chi-square. Prevalences were: any STH 42.3% (PSAC 39.0%, SAC 43.6%, p=0.88), Ascaris 25.3% (PSAC 25.8%, SAC 25.1%, p=0.76), Trichuris 27.8% (PSAC 21.9%, SAC 30.1%, p<0.01); hookworm <0.1%, any co-infection 10.9% (PSAC 8.7%, SAC 11.8%, p=0.06). Prevalence of anemia was 20.9% and of any malnutrition, 21.7%. no correlations with STH infection were found. STH infection is common in this population. PSAC and SAC have similar STH infection prevalences and both should be considered in control plans. Assessment of sibling STH infections as risk factors for index child infection and correlation of STH infection with micronutrient deficiencies and reported child illnesses will be discussed.

SCREENING FOR STRONGYLOIDES INFECTION IN AN IMMIGRANT POPULATION IN BRONX, NEW YORK

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Chronic infection with Strongyloides may transform into a fatal illness as a result of immunosuppression or HTLV-1 co-infection. The aim of this study was to define whether a routine screening program using serology for Strongyloides in immigrants from endemic regions is beneficial. Screening was conducted from 2004 to 2012, in inpatient and outpatient settings, at Jacobi Medical Center, Bronx, New York. Strongyloides serology was performed by serum ELISA. Blood cell counts performed in all patients. If a positive serology was detected, IgE level, HTLV-1 serology and stool ova/parasite were performed when feasible. A total of 631 individuals (317 male [50.2%]) were screened, mean age of 56±17 years. No differences related to age/sex were found between sero-positive and negative patients The majority of patients were from Puerto Rico (21.9%), Jamaica (15.4%), Dominican Republic (6.5%), Mexico (5.4%), Guyana (4.4%), Bangladesh (3.8%), Ecuador (3.6%). Eighty-nine percent of patients were screened during inpatient admissions. IgG antibodies were detected in 86 (14%) patients. Mean time from immigration was 25±18 years, with no difference between the 2 groups. There was no difference regarding history of walking barefoot in home country, asthma, steroid use, complaints of abdominal pain or skin rash. Ser-positive patients were more likely to have eosinophilia (absolute count >500 cells/ml) compared to ser-negative patients (p<0.01). Elevated IgE level (mean 522±634 UI/ml) was observed in 32 (67%) patients who screened positive; 16/26 (62%) sero-positive patients with a normal eosinophil count had elevated IgE levels. Stool exams were performed in 51/86 positive patients and had 6 larval. Co-infection with HTLV-1 was found in 4/57 (7%) sero-positive patients. Those with positive serology were more likely to have eosinophilia and 62% of those without eosinophilia had elevated IgE. Immigrants with either eosinophilia or elevated IgE are candidates for routine screening, despite long-term residency in the USA.
EVALUATION OF ALTERNATIVE DIAGNOSTIC METHODS FOR SCABIES AND STRONGYLODIASIS

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Scabies and strongyloidiasis are neglected tropical diseases which are responsible for considerable morbidity worldwide, primarily in disadvantaged and overcrowded communities. Scabies is a highly infectious skin disease caused by infestation of the skin with the ectoparasitic mite Sarcoptes scabiei. Up to 300 million people worldwide estimated to be afflicted at any time. In addition the scabies mite causes mange in many livestock, companion and wild animal populations. Strongyloidiasis is caused by infection with the parasitic nematode Strongyloides stercoralis and is associated with high mortality in immunosuppressed individuals. Each of these diseases is endemic in many remote Indigenous communities in the tropical north of Australia. A mass drug administration trial for the control of scabies and strongyloidiasis was conducted in a remote Indigenous community in the Northern Territory of Australia. Diagnosis of scabies was made by a clinical evaluation of participant’s skin. Diagnosis of strongyloides in children was made by direct microscopic examination of faecal samples followed by agar plate culture and formalin sedimentation. This study aimed to investigate: i) methods for the diagnosis of strongylopidiasis which are more logistically appropriate for use in remote and resource poor settings and; ii) improved methods of diagnosis of scabies compared with clinical diagnosis which lacks sensitivity. PCR based methods were trialled for the identification of S. scabiei in skin samples and of S. stercoralis in faecal specimens. S. scabiei gene specific PCR was more sensitive than microscopic examination for the detection of S. scabiei in skin samples and is highly specific. PCR targeting the 18S rRNA gene was at least as sensitive as microscopy and agar plate culture for the detection of S. stercoralis. Thus PCR may be a useful alternative to the clinical diagnosis of scabies and when parasitological analysis of fresh faecal samples is the field is logistically difficult.

ASYMMETRICAL ISOTHERMAL AMPLIFICATION METHOD FOR GENOTYPING MUTATIONS, IN HUMAN SOIL-TRANSMITTED HELMINTHS, THAT HAVE BEEN ASSOCIATED WITH BENZIMIDAZOLE RESISTANCE

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Soil-transmitted helminths (STHs), Ascaris lumbricoides and Necator americanus, are gastrointestinal nematodes causing human morbidity in tropical areas of the world. Benzimidazole (BZ) drugs, albendazole and mebendazole have been used extensively for large-scale treatment of STHs. A growing concern is that extensive use of anthelmintics to control human parasites is likely to exert selection on parasite populations as has occurred in gastrointestinal nematodes of livestock. The egg reduction rate has been used to monitor drug efficacy and to detect the development of resistance in the field. This assay is very insensitive for the detection of low levels of drug resistance. Previous molecular assays for putative resistance mutations have been based mainly on sequencing. However, sequencing is a time consuming and complicated procedure, not suitable for routine clinical use or for resource constrained situations. Therefore, development of simple, rapid and cost-effective molecular tools for detecting BZ resistance, that could be adaptable to field conditions would be very helpful for sustainable control of STHs. We developed a novel genotyping assay based on the Smart Amplification Process (SmartAmp2) to detect mutations of the β-tubulin isotype 1 gene associated with BZ resistance under isothermal conditions without PCR amplification.

This isothermal method uses asymmetrical primers and the mismatch-binding protein MutS to prevent mismatch amplification giving high specificity. For experimental development, real-time PCR monitoring of the amplification was achieved within 40–60 min with suppression of the mismatch amplification. Wild-type and mutant plasmids were employed to develop and optimize the assay. The assays were applied to analyze fecal samples of eggs and larvae using full-match and mismatched primer sets. A SmartAmp2 assay was developed for genotyping the mutations in the β-tubulin gene in A. lumbricoides and N. americanus and the reliability of the method was validated using the conventional PCR method. Work is being conducted to use end point detection system to enable this technique to detect mutations associated with BZ resistance in the field.

THE EFFECTS OF HOST DIETARY FACTORS ON CURE RATE AND FECAL EGG REDUCTION FOLLOWING SINGLE DOSE ALBENDAZOLE (400 MG) AMONG SCHOOL-AGE CHILDREN INFECTED WITH HOOKWORM IN THE KINTAMPO NORTH DISTRICT OF GHANA

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Ghanaian school-age children (n=141) from five communities previously identified as having high prevalence of hookworm infection and reduced cure rates were enrolled in a cross-sectional study investigating host predictors of treatment response. Household surveys were used to collect dietary patterns, socioeconomic and demographic information, and other health indicators. Infection status and dietary diversity data were assessed to identify modifiable host factors that might affect treatment response. Prevalence of hookworm infection was 56% (79/141). Those positive for hookworm were treated with single-dose albendazole (400 mg). Immediately prior to treatment, a brief screening questionnaire was administered to identify recent dietary patterns and diarrhea. Consumption of more diverse food groups in the twenty-four hours prior to treatment, as indicated by dietary diversity scores above the median, was associated with better rates of hookworm clearance after treatment (48.2% cure rate) compared to those consuming fewer food groups (12.5% cure rate) (χ2; p < 0.01). Dietary protein showed a stronger effect than dietary diversity: all children below the population median of protein food groups remained infected with hookworm following treatment, and children above the median had a 42% cure rate. Individuals who had not eaten six hours prior to treatment had better drug responses and were 5.0 times more likely to experience higher egg reduction rates than those who ate in the six hours prior to treatment (p < 0.05). Dietary diversity also significantly impacted fecal egg reduction rates in treated individuals. Those with higher dietary diversity were 3.1 times more likely to be in the highest category of egg reduction rate than those with lower dietary diversity (p < 0.05). These findings provide new data on the relevance of dietary patterns in affecting cure rates and egg reduction rates following single-dose albendazole therapy. Further work is needed to determine whether these factors could be used to improve operational effectiveness of mass drug administration.
for a broader profile of parasite antigens compared to RAS. Consistent sporozoite challenge. Importantly, ITI-induced CD8 T cells exhibit specificity and memory CD8 T cell responses leading to complete protection upon cessation. Further, ITI mice resist challenge with blood-stage parasites. Collectively, our data show the cellular basis for potent cross-stage immunity elicited by ITI depends on the induction of CD8 T cell responses and exposure to blood-stage parasites.

**DEVELOPMENT OF TRYPSANOSOMA CRUZI GENETICALLY ATTENUATED KNOCKOUT LINES WITH POTENTIAL USE AS TRANSMISSION BLOCKING VACCINES**

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Chagas disease, caused by the protozoan Trypanosoma cruzi, is the most important parasitic burden in Latin America. There are no effective vaccines to prevent this infection. In endemic areas, dogs are important sources of infection for the insect vector and therefore represent a critical control point for T. cruzi transmission. A transmission-blocking vaccine for dogs would greatly reduce the prevalence of T. cruzi infection in the canine and consequently, in the human population. Live attenuated parasites can be used as experimental vaccines. In this work we report on the generation of T. cruzi attenuated lines (KOS: Serine/threonine protein phosphatase like-protein; KO10: Hypothetical protein; KO121: Protein kinase and ECH: Enoyl-CoA hydratase/sorcerase family protein) by disruption of genes, whose products are predicted to be critical for parasite replication in mammals. We evaluated whether C57BL/6 mice immunized with these attenuated parasites would develop protective immune responses that would prevent the establishment of vector transmissible infection upon rechallenge with T. cruzi. Mice immunized with any of the attenuated lines elicited strong T. cruzi-specific CD8+ T cell responses. However, the frequencies of T. cruzi-specific CD8+ T cells in mice immunized with the KO10 line decreased to undetectable levels in the blood after ~70 days post immunization (dpi). At 300 dpi, parasite-specific CD8+ T cells from mice immunized with KOS and ECH showed relatively high expression of the central memory marker CD127 and low expression of recent activation marker KLRG1 compared with their wild type and KO121 counterparts. The magnitude and the phenotype of T. cruzi-specific CD8+ T cells suggest that these lines could be ideal for a transmission blocking vaccine for dogs. Current studies are focused on determining parasite persistence and on whether mice immunized with these attenuated lines and rechallenged with a virulent T. cruzi strain will not develop blood parasite levels sufficiently high to infect the insect vectors and therefore block the transmission of the infection.

**IMMUNOLOGICAL BASIS OF SUPERIOR PROTECTION FOLLOWING INFECTION-TREATMENT IMMUNIZATION COMPARED TO IRRADIATED SPOROZOITE IMMUNIZATION**

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Immunization of both humans and rodents with either radiation-attenuated Plasmodium sporozoites (RAS) or infection-treatment sporozoite immunization (ITI) elicits sterilizing anti-malarial immunity against subsequent sporozoite challenge. In rodent models, protection following RAS or ITI requires the induction and activity of parasite-specific CD8 T cells. Here we show that both ITI and RAS vaccinations elicit parasite-specific CD8 T cells exhibiting equivalent expression of molecules associated with T cell activation, inhibition, migration, and survival. However, compared to RAS, ITI requires fewer immunizing sporozoites to elicit sterilizing immunity. Moreover, single dose ITI induces larger effector and memory CD8 T cell responses leading to complete protection upon sporozoite challenge. Importantly, ITI-induced CD8 T cells exhibit specificity for a broader profile of parasite antigens compared to RAS. Consistent with this, we find that sterilizing protection following ITI is associated with a short duration (4-6 days) and low magnitude (up to 4-7%) level of blood-stage breakthrough parasitemia shortly following chloroquine cessation. Further, ITI mice resist challenge with blood-stage parasites. Collectively, our data show the cellular basis for potent cross-stage immunity elicited by ITI depends on the induction of CD8 T cell responses and exposure to blood-stage parasites.

**IDENTIFICATION OF NOVEL HIGHLY PROTECTIVE PREERYTHROCYTIC ANTIGENS FOR MALARIA VACCINE DEVELOPMENT**

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Malaria is the most devastating parasitic disease affecting humans. There is no licensed malaria vaccine. Efforts to develop an effective malaria
vaccine have been limited by the small number of known antigens, which represent < 0.5% of the 5300 encoded proteins in the Plasmodium falciparum genome. Most vaccines under clinical evaluation today contain only one antigen and are only partially protective. The most advanced candidate, RTS,S, provides about 50% protection. Thus, there is a great need for an effective malaria vaccine that could provide robust protection and contribute to the control and eventually eradication of malaria. Our vaccine rationale is based on the fact that immunization with radiation-attenuated sporozoites (RAS) provides high level (>90%) protection against sporozoite challenge in both mice and humans, and this protection is dependent on the induction of CD8+ T cells targeting multiple antigens expressed in the pre-erythrocytic stages of the parasite life cycle. The circumsporozoite protein (CSP) is one of the targets of protective T cell responses induced by RAS immunization. However, other unknown antigens clearly contribute to protection. The goal of our research was to identify novel antigens that are the targets of protective T cell responses in mice immunized with protective regimens of RAS. We have identified several new and highly protective pre-erythrocytic stage antigens using a novel high-throughput genomics screening approach. Our antigen discovery system utilizes an array of adenovirus vectors carrying 300 highly expressed P. yoelii pre-erythrocytic genes with identifiable P. falciparum orthologues. In the antigen discovery screen, antigen presenting cells were infected with individual adenovectors from the array and then mixed with splenocytes from mice immunized with protective regimens of RAS. We prioritized antigens based on the frequency of CD8+ T cell recall responses to each of the 300 antigens. We selected 20 antigens that recalled the most robust T cell responses and tested their capacity to protect mice from a P. yoelii sporozoite challenge. Outbred CD1 mice were immunized with a DNA prime - Ad boost regimen and sterile protection was measured following sporozoite challenge. Several of the prioritized antigens induced higher levels of protection than CSP. The P. falciparum orthologues of these antigens are being considered for advancement to clinical development.

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ANTIBODY DYNAMICS AFTER ACUTE MALARIA INFECTION IN CHILDREN

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Naturally acquired antibodies directed against Plasmodium falciparum are acquired slowly with repeated infections and protect against malaria disease. However children have been shown to generate short-lived anti-malaria antibodies. While this may be due in part to a less mature immune system, malaria infection may also exert immuno-modulatory effect. Our goal was to examine children’s antibody signature after acute clinical malaria in order to categorize stability of responses. Children (n=89, mean age 25 months (range 1 - 66 mo)) were recruited from Chulaimbo sub-District Hospital in western Kenya upon presentation with a febrile illness. Participants diagnosed with acute malaria provided a venous blood sample, were treated with 6-doses of CoArtem14 (Artemether/Lumefantrin), and examined 4 weeks later (recovery) when another blood sample was drawn. Plasma samples were examined for the prevalence and magnitude of anti-malaria antibodies by a) lumexin multiplex serology to 13 malaria antigens and b) functional antibody-mediated growth inhibition of cultured parasites. We found that total IgG to MSP-1α (3D7, FVO, and FUP) and PfCelTOS declined from acute to recovery time points. No change was detected in IgG levels to AMA1 (3D7 or FVO), PfCSP, EBA140, EBA175, EBA181, and SERAS (two variants). In contrast, functional sialic-dependent pathway inhibitory antibodies and global growth inhibition antibodies against W2mef were boosted after an episode of symptomatic malaria. MSP1α invasion inhibitory antibodies and global growth inhibition antibodies against PID10 remained unchanged after infection. In addition, we found that IgG to measles decreased after an episode of malaria. Total IgG did not however change significantly between the time points. Thus, acute clinical malaria infections differentially influence the maintenance of anti-malaria antibodies as well as have potential detrimental consequences for immunity against measles.

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RHOPTRY NECK PROTEIN 2 IS PRODUCED IN OOOCYST-DERIVED SPOROZOITES AND REQUIRED FOR SALIVARY GLAND INVASION

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During apicomplexan parasite invasion, it is revealed that tight junction is formed between parasite and target cells, which confers parasite move inside cells. Recently in Plasmodium merozoite, it was reported that rhoptry neck protein 2 (RON2) is secreted to the tight junction and RON2 and AMA1 interaction is important during erythrocyte invasion. However, ron2 disrupted transgenic parasite has never been successfully generated presumably because RON2 is essential for proliferation in the blood stage. Here, using Plasmodium berghei, we showed that RON2 is produced also in sporozoites and localized to rhoptry. Interestingly, ron2 transcription is restricted to midgut sporozoites, however RON2 protein can be detected through sporozoite migration from midguts to salivary glands. To elucidate sporozoite RON2 function, we generated sporozoite stage specific RON2 silencing transgenic parasites by swapping ron2 promoter to merozoite specific promoter. The number of sporozoites collected from
We also present evidence through comet assays for methyl
Plasmodium falciparum dependent manner by PfRPA1S, another RPA homologue in exchange activity but its function was negatively regulated in a dose-
of bacterial homologue SSB in initiating homologous pairing and strand homologous linear dsDNA in the presence of 0.5mM CaCl2. We also
PfRad54 accelerated PfRad51 mediated pairing between ssDNA and its
Plasmodium falciparum homologues of Rad54 and Replication protein-A (RPA).
of PfRad51 in the presence of putative interacting partners, especially
vitro, as reported previously. Here, we evaluated the catalytic functions
Plasmodium falciparum homologue of exchange reaction during recombination and DNA repair. Previously, our
homologue Rad51 play a central role in homologous DNA strand
exploiting the recombination machinery and its molecular characterization
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Stromal cell-derived factor (SDF)-1α (CXCL12) is a pleiotropic chemokine with diverse functions including induction of anti-pathogen immunity and inhibition of erythropoiesis. In murine malaria, increased expression of SDF-1α promotes control of parasitemia. Although several studies indicate that SDF1A genetic variation regulates outcomes in the context of HIV-1 infection, hematopoiesis, and cancer, the role of genetic variability in SDF1A in Plasmodium falciparum infections has not been explored. The effect of SDF1A (C-1002T) variation was therefore, investigated in Kenyan children (2.0-38.0mos., n=873) residing in a holoendemic P. falciparum transmission region of western Kenya. Children were stratified into aparasitemic (n=212) and parasitemic (n=661) groups with parasitic children being further categorized into SMA (hemoglobin, HB<5.0g/dL; n=236) vs. non-SMA (HB≥5.0g/dL; n=425), high-density parasitemia (HDP; ≥10,000 parasites/µL; n=477) vs. low-density parasitemia (LDP; ≤1,000 parasites/µL; n=184), and reticulocyte production index (RPI≥2.0) vs. (RPI<2.0). Multivariate logistic regression modeling controlling for age, gender, bacteremia, glucose-6-phosphate dehydrogenase, alpha-thalasemia, and sickle cell and HIV-1 status did not show any significant associations between carriage of C-1002T genotypes and SMA, RPI≥2.0, and HDP. However, carriage of the CC genotype was associated with protection against the acquisition of P. falciparum infection compared to the TT genotype (Odds ratio, OR, 0.311; 95% CI, 0.115-0.842; P=0.022). These results demonstrate that although variation at 1002 in the SDF1A promoter appears to protect against acquisition of P. falciparum infection, this variant may not affect malaria disease outcomes once an individual becomes infected.

ROLE OF PFRAD54 AND REPLICATION PROTEIN A IN RAD51-
MEDIATED DNA STRAND EXCHANGE AND REPAIR OF DNA
DAMAGE INDUCED BY MMS IN PLASMODIUM FALCIPARUM
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Exploiting the recombination machinery and its molecular characterization in the malaria parasite would provide mechanistic understanding of recombinational rearrangements leading to immune evasion via antigenic switching, a major impediment in developing an effective vaccine against these protozoan parasites. Bacterial RecA protein and its eukaryotic homologue Rad51 play a central role in homologous DNA strand exchange reaction during recombination and DNA repair. Previously, our lab has shown that PFRad51, the Plasmodium falciparum homologue of Rad51, exhibited ATPase activity and promoted DNA strand exchange in vitro, as reported previously. Here, we evaluated the catalytic functions of PFRad51 in the presence of putative interacting partners, especially P. falciparum homologues of Rad54 and Replication protein-A (RPA). PFRad54 accelerated PFRad51 mediated pairing between ssDNA and its homologous linear dsDNA in the presence of 0.5mM CaCl2. We also present evidence that recombinant PFRPA1L protein serves the function of bacterial homologue SSB in initiating homologous pairing and strand exchange activity but its function was negatively regulated in a dose-
dependent manner by PIRPA1S, another RPA homologue in P. falciparum. We also present in vivo evidence through comet assays for methyl methanesulfonate (MMS)- induced DNA damage in malaria parasites

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A SINGLE NUCLEOTIDE POLYMORPHISM IN THE PROMOTER
OF STROMAL CELL-DERIVED FACTOR (SDF)-1A (C-1002T) IS
ASSOCIATED WITH PROTECTION AGAINST PLASMODIUM
FALCIPARUM INFECTION IN KENYAN CHILDREN
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GENOMIC DIVERSITY AND EVOLUTIONARY HISTORY OF
PLASMODIUM VIVAX
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Most studies of genetic diversity in Plasmodium vivax have focused on microsatellites or selected loci and do not provide a genome-wide perspective. We have sequenced the genomes of ten P. vivax field isolates