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;
Ndyomugyenyi, Richard; Magnussen, Pascal; Clarke, Sian E.

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particular environment. For instance the higher prevalence of *E. histolytica* / *E. dispar* and *Blástocystis* 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**

Dalila Y. Martinez, Francisco Bravo, Eduardo Gotuzzo

Univesidad Peruana Cayetano Heredia, Lima, Peru

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 amoebic infections.

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**EVALUATION IN VITRO OF THE ANTI AMEBOIC EFFECT OF TWO FLAVONOIDs: EPICATECHIN AND KAEMPFEROL**

Sindy Galicia-Vega1, Elizabeth Barbosa-Cabrera1, Luis Escarenño-Ramírez2, Adriana Jarillo-Luna1, Victor Rivera-Aguilar1, Rafael Campos-Rodriguez1, Judith Pacheco-Yepez2

1Postgraduate and Research Section, Superior Medicine School, National Polytechnic Institute, Mexico City, Mexico, 2Morphology Sciences Coordination, Superior Medicine School, National Polytechnic Institute, Mexico City, Mexico, 1Microbiology UBIPO, Fes-Iztacala, Universidad Nacional Autonoma de Mexico, Mexico City, Mexico

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 695 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 4 h, 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**

Vanessa Pineda, Dayra Alvarex, Kadir Gonzalez, Ana Maria Santamaria, Carlos Justo, Chystrie Rigg, Jose E. Calzada, Azael Saldana

Gorgas Institute for Health Research, Panama, Panama

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 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 isolates from fecal samples collected in the indigenous community of Ipéti Choco, Distrit 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). Assemble 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 assemblies 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 Ipéti Choco community. However, the zoonotic potential of giardiasis under the observed epidemiological scenario needs further studies.
ROLE OF REACTIVE OXYGEN SPECIES AND ANTIOXIDANT ENZYME CAPACITY DURING EXPERIMENTAL AMOEBOIC LIVER ABSCESS

Luis Escareño-Ramírez1, Teresita Cruz-Hernandez1, Sindy Galicia-Vega1, Alexander Kormanovski1, María E. Quintanar-Quintanar2, Luz M. Cárdenas-Jaramillo1, Rafael Campos-Rodríguez1, Judith Pacheco-Yepez1

1Postgraduate and Research Section, Superior Medicine School, National Polytechnic Institute, Mexico City, Mexico, 2Pharmacology Coordination, Superior Medicine School, National Polytechnic Institute, Mexico City, Mexico, 1Morphology Sciences Coordination, Superior Medicine School, National Polytechnic Institute, Mexico City, Mexico

In an amoeobic liver abscess (ALA), polymorphnuclear 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 7 d 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 12 h) and a decrease (at 48 h) in SOD in the abscess in both the lesion area (P=0.008) and hepatic parenchyma (P=0.009), an elevated (at 12 h; P=0.03) and a diminished (at 7 d; 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.

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

Daouda Ndiaye, Mouhamadou Ndiaye, Baba Dieye, Yaye Die Ndiaye, Lamine Gueye, Beckenbauer Diatta, Babacar Faye, Jean Louis Ndiaye, Roger Tine, Omar Ndir

Cheikh Anta Diop University, Dakar, Senegal, Dakar, Senegal

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 be carried out in 1255 patients tested, we found a 79%, and in the third S2, using arterial blood at 3 weeks of interval, we found a 72%.

T. gondii infection was confirmed in 1255 patients. 1255 patients were tested, we found a prevalence of 8,7% and 0% for (IgM+IgG) 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.

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

Kofi D. Kwofie1, Anita Ghanash1, Joseph H. Osei1, Helena Quaynor1, Samuel Obied2, Eric H. Frimpong2, Irene Ayi3

1Noguchi Memorial Institute for Medical Research, Accra, Ghana, 2Obstetrics and Gynaecology Department, Korle-Bu Teaching Hospital, Accra, Ghana, 3Department of Clinical Microbiology, Kwanre Nkrumah University of Science and Technology, Kumasi, Ghana

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 also 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 (3.94%) 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.

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

Sandra Rios

University of Antioquia, Envigado, Colombia

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 ticks 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 fenomeño. 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

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DEGRADATION AND UTILIZATION OF COMPLEX CARBOHYDRATES BY TRICHOMONAS VAGINALIS

Andrew Brittingham, Ryan D. Huffman, Lauren D. Nawrocki, Tyler J. Nielsen, Wayne A. Wilson
Des Moines University, Des Moines, IA, United States

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, we can use 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 possesses 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.

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


1U.S. Naval Medical Research Unit No.6, Lima, Peru, 2Instituto Nacional de Salud del Niño, Lima, Peru

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, 95%, p<0.001, Blastocystis ratio: 1.34, 95%, p<0.001). Sequencing analysis of trichomonad 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.

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CONGENITAL TOXOPLASMOSIS IN BRAZIL: MODELING THE COST OF MATERNAL SCREENING

Eileen Stillwagon, Larry Sawyers

1Gettysburg College, Gettysburg, PA, United States, 2American University, Washington, DC, United States

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 sanitation 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 due to 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.
However the difficulty is to define a technical procedure usable i) to

Cyclospora cayetanensis

malnutrition and opportunists, we started studies on four pathogens:

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Moreover, in tropical areas other causes of immune-depression can occur

seem to be higher than in Europe, but are not well documented.

hosts and are described in travellers coming back from their trip with

tritherapies. Some of these parasites could affect immune-competent

were mostly described in Europe after emergence of the HIV outbreak

years. 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, COWP, 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?

Guillaume Roux1, Fabiola Gosinary1, Gisele Raherinampinaina2, Rindra Randremanana1, Jeanine Hollanjovony1, Eliosa Hariniaina1, Annie Robinson1, Ronan Jambou1

1Institut Pasteur de Madagascar, Antananarivo, Madagascar, 2Tsaralalana children’s Hospital, Antananarivo, Antananarivo, Madagascar, 3Regional Reference Hospital Antsirabe, Antsirabe, Madagascar

Opportunistic intestinal parasites (cryptosporidia, microsporidia, etc.) were mostly described in Europe after emergence of the HIV outbreak but their prevalence fall down dramatically with effective antiretroviral tritherapies. Some of these parasites could affect immune-competent hosts and are described in travelers 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 process but associations of cryptosporidias and microsporidias have already been found in several patients.

ELECTRON MICROSCOPY CHARACTERIZATION OF DIENTAMOeba FRAGILIS VIRUS LIFE CYCLE

Gouri B. Banik1, Debra Birch2, Damien Stark1, John T. Ellis2

1University of Technology, Sydney, NSW, Australia, 2Macquarie University, Sydney, NSW, Australia

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 an icosahedral 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 excerted through eocytosis. 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.
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 (C57BL6 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.

### A Study of Mussel Perna Perna Infected with Cryptosporidium Spp. Intended for Human Consumption Indicating Environmental Contamination

Teresa Cristina Bergamo Bomfim, Geisi Ferreira Maríñê, Marcelo de Freitas Lima

Universidade Federal Rural do Rio de Janeiro, Rio de Janeiro, Brazil

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 feces 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 analyses, 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. Mussles 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 18SSU 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 for at least one of the study sites. It was possible to identify three species *C. andersoni*, *C. meleagris* 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 *Cryptosporidium* 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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**Malnutrition is Associated with Increased Mortality in Adult Medical Inpatients at a Regional Referral Hospital in southwestern Uganda**

Stephen B. Asiimwe1, Christopher C. Moore2

1Mbarara University of Science and Technology, Mbarara, Uganda
2University of Virginia, Charlottesville, VA, United States

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
TB were significantly associated with mortality (p < 0.05). In the multivariate analysis, only malnutrition (adjusted OR 3.7, 95% CI 1.6-8.6, p = 0.002) and abnormal temperature (adjusted OR 2.0, 95% CI 1.0-3.9, p = 0.04) remained independently associated with mortality. Our findings indicate that malnutrition contributes strongly to mortality in acutely ill hospitalized adults in SSA. Additional research is urgently needed to better understand the pathophysiology of malnutrition in acutely ill hospitalized adults in SSA and ways to mitigate its effect.

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**TOXOPLASMA MYOCARDITIS IN IMMUNOCOMPETENT REQUIRING CARDIAC TRANSPLANTATION**

Mahesh Menon  
Nepean Hospital, Penrith, Sydney, Australia

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, polymyositis, 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.

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**IDENTIFICATION OF THREE CANDIDA AFRICANA STRAINS IN SENEGAL**

Doudou Sow  
Parasitology- Mycology/UCAD, Dakar, Senegal

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. kefyr 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.

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**ENTAMOEBA HISTOLYTICA MENINGOECEPHALITIS IN A SAMOAN TODDLER WITH ACUTE ONSET SEIZURES**

Lucy M. Goh, James R. Marrone, Lana Y. Filippo, Kamlesh Kumar, Amelia L. Mejia, Tagiliiima I’Atala, Saipale Fuimaono, Seini Biukoto  
LBJ Tropical Medicine Center, Pago Pago, American Samoa

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 Samoa 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.

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

Eric Ogola1, Sergio Recuenco2, Michael Niezgoda3, Gilchrist Lokoel1, Alex Gentile A4, Samuel Oketch1, Titus Kwambai1, Godfrey Bigogo1, Peter Oremo1, Barry Fields5, Joel Montbombery5, Charles Rupprecht2, M. Karuki Njenga2  
1Kenya Medical Research Institute, Kisumu, Kenya, 2Centers for Disease Control and Prevention, Atlanta, GA, United States, 3Kenya Field Epidemiology and Laboratory Training Program, Nairobi, Kenya, 4George Washington University, Washington, DC, United States, 5Ministry of Public Health and Sanitation, Nairobi, Kenya

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, 201(35.3%) 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 new onset seizures. Suspicion for CNS amebic infection in a child with intestinal amebiasis and meningitis 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 Entamoeba 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 Samoa 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.

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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 dyspnoea 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

Ijeoma Ejigbi1, Manfred Amany2, Data Data Santorino2
1Emory University, Atlanta, GA, United States, 2Mbarara University of Science and Technology, Mbarara, Uganda

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

Austine B. Orinde1, Tabitha Kimani2, Esther Schelling3, Jared Omolo1, Gideon M. Kikuvi1, Karuki M. Njenga3
1Ministry of Public Health and Sanitation, Nairobi, Kenya, 2International Livestock Research Institute, Nairobi, Kenya, 3Swiss Tropical and Public Health Institute, Basel, Switzerland, 4Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya

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 morbidty 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 informant in the public health 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

Yordanos B. Molla
Brighton and Sussex Medical School, Brighton, United Kingdom

Podocoiosis is geochemical elephantiasis of the lower legs that affects barefoot individuals exposed to red clay soil of volcanic origin. Podocoiosis can be prevented, early forms of the disease can be treated, and disease progression can be curbed. Podocoiosis is an important public health problem in Africa, Central America and northern India. The aim of this study was to assess the burden of podocoiosis 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, a detailed structured interview was conducted on all cases identified. The prevalence of podocoiosis 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 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 (62% 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 podocoiosis and associated morbidities such as ALA, mossy lesions and open wounds in northern Ethiopia. Predominance of cases at early clinical stage of podocoiosis indicates the potential for reversing the swelling and calls for disease prevention interventions.

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ACCESS TO TREATMENT FOR CHAGAS DISEASE IN MEXICO: A POLICY ANALYSIS

Jen Manne1, Callae S. Snively1, Janine M. Ramsey2, Michael Z. Levy3, Till Bärnighausen1, Michael R. Reich1
1Harvard School of Public Health, Boston, MA, United States, 2Instituto Nacional de Salud Pública, Tapachula, Mexico, 3University of Pennsylvania, Philadelphia, PA, United States

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 antitypanosomal 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.

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

Simone Y. Asare1, Elijah Paintsil2, Kwandwo Koram3, Frank Atuguba3, Victor Asoala4, Debbie Humphries1
1Yale School of Public Health, New Haven, CT, United States, 2Yale School of Medicine, New Haven, CT, United States, 3Noguchi Memorial Institute for Medical Research, Accra, Ghana, 4Navrongo Health Research Center, Navrongo, Ghana

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 aged 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) 17.1% of the matched clinical malaria cases consumed meat compared to only 8.1% of the matched clinical malaria controls. Children who had consumed meat within the 12 month recall period from the administration of the nutritional history questionnaire had an increased risk of being clinical malaria cases compared to children who did not consume meat during the same period. (OR=3.33, 95% CI (.92-12.11), p=.067) Parasitemia concentrations were significantly higher in cases when compared to controls (Wilcoxon Rank-Sum Test p-value < .001); the mean P. falciparum density for matched clinical malaria controls was 6,531.3 parasites/μl of blood compared to 42,199.0 parasites/μl of blood for the matched clinical malaria cases. Higher parasitemia levels are statistically associated with clinical malaria case status; very low food security and meat consumption may affect clinical malaria outcomes among children in the Kassena-Nankana District (KNK).

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ADDRESSING THE NEGLECTED TROPICAL DISEASE PODOCONIOSIS IN NORTHERN ETHIOPIA: LESSONS LEARNED FROM A NEW COMMUNITY PODOCONIOSIS PROGRAM

Sara M. Tomczyk1, Gail Davey2, Abreham Tamiru3
1London School of Hygiene and Tropical Medicine, London, United Kingdom, 2Brighton and Sussex Medical School, Brighton, United Kingdom, 3International Orthodox Christian Charities, Debre Marcos, Ethiopia

Despite its great public health importance, few control initiatives addressing podoconiosis (non-filarial elephantiasis, a geochemical neglected tropical disease) exist. In June 2010, the first podoconiosis program in Northern Ethiopia, consisting of prevention, awareness, and care and support activities, began in Debre Marcos, Northern Ethiopia. This study aims to document and disseminate the lessons learned from a new community podoconiosis program in Debre Marcos. We used a content analysis approach to examine and evaluate data from a series of sources. These sources include conducted interview transcripts, a focus group discussion transcript and secondary sources including monitoring and evaluation field reports, observation notes, and research obtained from a literature review. Themes were identified and grouped into matrix tables. Overall, sixteen program steps were identified and grouped into 6 domains: Initial preparation, training and sensitization, foundation building, treatment activity implementation, awareness, and follow-up. Emphasis is placed on the need for baseline data, effective training, local leadership, experience-sharing, mass-awareness, cross-cutting sector issues (i.e. water and waste management), and integration with government health systems. Related successes and challenges are also described, as are stakeholder roles and misconceptions and socio-cultural challenges affecting the program start-up. Many of the identified successes and challenges are relevant to the aim of the podoconiosis program to be sustainable and community-led. Much of this information has already been used to improve the Debre Marcos program. We also anticipate that the domains and steps identified will be useful in guiding new programs in other settings where podoconiosis is highly prevalent. We hope to encourage partnerships and collaboration among podoconiosis stakeholders in future growth and disease control expansion.

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CHRONIC LIVER DISEASE IN HEMODIALYSIS

Osama El-Minshawy1, Twafik Ghabra, Eman El Bassuoni
1University of Tabuk School of Medicine, Tabuk, Saudi Arabia

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**

Nicholas H. Carter¹, Elizabeth E. Dawson-Hahn¹, Michael P. Koster¹, Margarette Blaise-Jean²

1Brown University, Warren Alpert School of Medicine, Providence, RI, United States, 2Foundation St. Luc, Tabarre, Haiti

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**

Joseph P. Shott¹, Bakary S. Diarra², Moussa B. Kanoute², Charles Luswata³, Aissata Ongobia², Kassoum Kayenta², Silvia Portugal⁴, Jacqueline Moebius⁵, Boubacar Traore², Alassane Dicko², Ruth D. Ellis², Michal Fried⁵, Peter D. Crompton⁴, Patrick E. Duffy⁵

¹Office of the Director, Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States, ²Mali International Center for Excellence in Research (ICER), University of Sciences, Techniques and Technologies (USTT), Bamako, Mali, ³Medical Science and Computing, Inc. (MSC), Rockville, MD, United States, ⁴Laboratory of Immunogenetics, Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States, ⁵Laboratory of Malaria Immunology and Vaccinology, Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States

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**

Hans J. Overgaard¹, Maria Ines Mátiz², Juan Felipe Jaramillo², Victor Alberto Olano², Sandra Lucia Vargas², Diana Sarmiento², Neal Alexander³, Audrey Lenhart⁴, Razak Seidu⁵, Thor Axel Stenström⁶

¹Norwegian University of Life Sciences, Ås, Norway, ²Universidad El Bosque, Bogota, Colombia, ³London School of Hygiene and Tropical Medicine, London, United Kingdom, ⁴Liverpool School of Tropical Medicine, Liverpool, United Kingdom

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 diarrhea 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

Claire Fuller, Sitche Mey, Christoph Bendick

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

Emma Weldon, Pamela Martey

The morbidity and mortality associated with accidental chemical ingestion are preventable. A sudden increase in the numbers of children admitted to Okomfo Anoyke 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.

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INSUFFICIENT IODIZED SALT COVERAGE AT COMMUNITY LEVELPOSES A RISK FOR INDIVIDUAL-LEVEL IODINE DEFICIENCY: THE FOURTH THAI NATIONAL HEALTH EXAMINATION SURVEY 2009

Wit Wichaidit, Virasakdi Chongsuvivatwong, Rassamee Sangthong, Wichai Aekplakorn

Thailand was re-classified by the World Bank as an upper-middle income country in 2010. However, iodine deficiency disorder (IDD) remains a significant public health problem due to lack of robust control on salt iodization. This study evaluated the coverage of salt iodization at household and community levels, and their association with iodine deficiency in Thai children. The fourth Thai National Health Examination Survey (NHES IV) was a nationally representative cross-sectional survey conducted in 2009 by three-stage stratified sampling. Children aged 1-14 years were sampled for the study. Children’s primary caretaker was interviewed about food intake using a food frequency questionnaire and a 24-hour food recall. Urine iodine of children was tested to measure iodine level. Data were analyzed with descriptive statistics and multi-level logistic regression using R software and epicalc and lmer4 packages. A total of 9035 children were recruited. Nationally, the prevalence of iodine deficiency was 30.8%, with significant regional variations (p<0.0001). Multi-level logistic regression showed that individual-level iodine deficiency was significantly associated with insufficient iodine content in household salt (adjusted OR = 1.22; 95% CI = 1.05 - 1.41) and prevalence of insufficiently iodized salt at sub-district level (adjusted OR = 1.44; 95% CI = 1.09 - 1.91). The analysis was adjusted for for weekly consumption of seafood, age, gender, household income, and maternal education. Despite the high success in economic and social development, IDD is still a serious problem in Thailand. The higher strength of association between iodine deficiency and prevalence of insufficiently iodized salt at the community level implies a need for universal coverage of iodized salt, not just iodized salt consumption at the individual household level at present.
A QUALITATIVE STUDY ON BARRIERS TO CONSISTENT USE OF FOOTWEAR IN WOLAITA ZONE, SOUTH ETHIOPIA: IMPLICATIONS FOR PREVENTION AND CONTROL OF PODOCONIOSIS

Desta A. Alembo1, Emi Watanabe2, Getnet Tadele3, Colleen McBride4, Gail Davey5
1IOCC Podoconiosis Research, Addis Ababa, Ethiopia, 2National Institutes of Health, Bethesda, MD, United States, 3Addis Ababa University, Addis Ababa, Ethiopia, 4Brighton & Sussex Medical School, Brighton, United Kingdom

Effectiveness of prevention and control of podoconiosis 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 podoconiosis 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 podoconiosis 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 podoconiosis in Wolaita district are discussed.

TRAUMA TRAINING COURSES AVAILABLE AROUND THE WORLD: A SYSTEMATIC REVIEW

Fahim F. Pyarali1, Maureen McCunn2, Jeffrey Tillus2, Rebecca M. Speck2, Sheida Bunting2, Adam L. Kushner3, Tarek Razek4, Michel B. Aboutanos5
1University of Texas Medical Branch, Galveston, TX, United States, 2University of Pennsylvania, Philadelphia, PA, United States, 3Columbia University, New York, NY, United States, 4McGill University Health Center, Montreal, QC, Canada, 5Virginia Commonwealth University Medical Center, Richmond, VA, United States

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

Adamu Addissie Nuramo, Desalegn Dalecha
Addis Ababa University, Addis Ababa, Ethiopia

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 <5 years, 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 of age.

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

Dorothea Obiri
University of Ghana, Accra, Ghana

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 endothelial growth factor) or PlGF (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 the 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 Angiopeptin 1 and 2, PlGF, IL-1 and TNF. It is expected that there would be a correlation between the angiogenic and inflammatory factors...
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

Sarah E. Smith1, John T. Ludwig1, Vernon M. Chinchilli1, Khanjan Mehta2, Jose A. Stoute1

1The Pennsylvania State University College of Medicine, Hershey, PA, United States, 2The Pennsylvania State University College of Engineering, University Park, PA, United States

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 telemedicine 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

Rebecca Thomson1, Boniface Johannes2, Charles Festo2, Admirabilis Kalolella2, Mark Taylor2, Katia Bruxvoort1, Sarah Tougher1, Yazoume Ye3, Andrea Mann1, Ruilin Ren1, Barbara Willey1, Fred Arnold3, Kara Hanson1, Catherine Goodman1

1London School of Hygiene and Tropical Medicine, London, United Kingdom, 2Ifakara Health Institute, Dar es Salaam, United Republic of Tanzania, 3ICF International, Washington, DC, United States

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 (PFP) outlets. Within PFP 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 PFP outlets (median $4.93), especially in urban areas ($7.04). Among PFP 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

Kristin Johnson1, Malia Duffy1, John Carper1, Thomas Minior2, Robert Ferns1, Bisola Ojikutu1

1John Snow, Inc., Boston, MA, United States, 2United States Agency for International Development, Washington, DC, United States

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

Gautier H. Ouedraogo1, Seni Kouanda1, Herman Lanou1, Letitia Ouedraogo/Nikiema1, Eli Tiendrebeogo1, Yves Traore2, Blaise Sendo3

1Institut de Recherche en Sciences de la Santé (IRSS/CNRST), Ouagadougou, Burkina Faso, 2Université de Ouagadougou, Ouagadougou, Burkina Faso, 3Public Health Department, Université de Ouagadougou, Ouagadougou, Burkina Faso

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

Rebecca Weintrab1, Sarah Arnquist1, Sophie Beauvais1, Marie Connelly1, Yue Guan1, Aaron VanDerlip1, Keri Wachter2, Aaron Beals1

1The Global Health Delivery Project; Brigham and Women’s Hospital, Division of Global Health Equity, Boston, MA, United States; 2The Global Health Delivery Project; Harvard Medical School, Department of Global Health and Social Medicine, Boston, MA, United States

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 forum is 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 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

Mahamadou M. Ndiath, Aly Guéye, Roger Tine, Jean Louis Ndiaye, Badara Cissé, Babacar Faye, Oumar Gaye

UCAD/Senegal, Dakar, Senegal

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 Ndélieng. 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/sq km. 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 a 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)

Alexander K. Rowe1, Samantha Y. Rowe1, David H. Peters2, Kathleen A. Holloway3, John Chalker4, Dennis Ross-Degnan5

1Centers for Disease Control and Prevention, Atlanta, GA, United States, 2Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD, United States, 3World Health Organization Southeast Regional Office, New Delhi, India, 4Management Sciences for Health, London, United Kingdom, 5Harvard Medical School and Harvard Pilgrim Health Care, Boston, MA, United States

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 +/- supervision + 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.

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The study is being conducted across all centres and is the biggest study to date. It aims to investigate the risk factors for common cancers in India, which can be applied locally. As such, we are currently conducting a case-control protocol design, randomised controlled trials, and medical statistics, and good controls have been 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.

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

Alexander E. Finlayson, Shameeq Sayeed, Mary Foulkes, Raqhib Ali

**INDOX, Oxford, United Kingdom**

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 that is 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 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 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

Martin Bienvenu Somda1, Zakaria Bengaly1, Anne Poinsignon2, Sylvie Cornelie2, Françoise Mathieu-Daupe2, Emilie Thérèse Dama1, Edith Demettre-Verceil3, Franck Remoue2, Antoine Sanon4, Bruno Bucheton5

1URBIO, CIRDES and LAMIVECT, Bobo-Dioulasso, Burkina Faso, 2UMR 224 MIVEGEC, IRD, Montpellier, France, 3FFP CNRS, Montpellier, France, 4LEFA, UO, Ouagadougou, Burkina Faso, 5UMR 177 INTERTRYP and LAMIVECT, IRD/CIRAD, Montpellier, France

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 fly control programs in animal african trypanosomosis. 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

Joseph B. Koroma1, Santigie Sesay2, Mustapha Sonnie3, Mary H. Hodges4, Foday Sahr5, Yoabi Zhang2, Moses Bockarie1

1Centre for Neglected Tropical Diseases, Liverpool School of Tropical Medicine, Liverpool, United Kingdom, 2National Neglected Tropical Diseases Control Programme, Ministry of Health and Sanitation, Freetown, Sierra Leone, 3Helen Keller International, Freetown, Sierra Leone, 4College of Medicine and Allied Health Sciences, University of Sierra Leone, Freetown, Sierra Leone, 5Helen Keller International, Regional Office for Africa, Dakar, Senegal

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% - 4.99%), 1 moderate (5% - 9.99%) and 11 high (≥10%) prevalence. Baseline microfilaraemia (mf) studies using the thick blood film method in 2007/8 indicated that prevalence was <1% in 2 districts (1 had 0%), low in 8 and moderate in 2 HDs. MDA with ivermectin and albendazole implemented by community-selected distributors was piloted in 6 HDs in 2007, extended to 12 HDs in 2008 and another 2 HDs in 2010. 4,385,467 (coverage 70.1%), 4,694,711 (coverage 73.2%) and 4,749,556 (coverage 75.1%) people were targeted in 2008, 2009 and 2010 respectively in 12 HDs. Geographic coverage of villages/urban areas was 100% in all 12 HDs and program coverage was ≥65.0% in all except for 1 HD that had 59.5% in 2008. A study of mf prevalence was conducted in 2011, 6 months after the last MDA, to determine impact of 3 MDAs on mf prevalence in the 12 HDs. The mf prevalence and density was determined using the thick blood film method. A total of 6,023 people ≥5 years were examined, male 3,170 (52.6%) and female 2,853 (47.4%). Overall mf prevalence was 0.30% (95% CI: 0.19 - 0.47%); population mf density was 0.05 ml/ml (95% CI: 0.03 - 0.08 ml/ml) and positive-only mf density was 17.59 ml/ml (95% CI: 15.64-19.55 ml/ml). Compared with baseline data, an overall reduction of 87.5% in mf prevalence, 95.5% in population mf density and 65.0% in positive-only mf density (p<0.0001) was noted. Mf prevalence reduced to 0.0% (100.0% decrease) in 4 HDs and by 70.0-95.0% in 7 HDs. Only 1 of the 12 HDs still had mf prevalence >1% (1.58%) and this district had the highest baseline mf prevalence (6.9%). The results show that after 3 rounds of MDA mf prevalence has decreased to <1.0% in all but 1 of the 12 HDs. The LF elimination programme in Sierra Leone is progressing well and on course to eliminate LF by year 2020 in these 12 HDs.

PROGRESS TOWARDS CONTROL OF SCHISTOSOMA MANSONI INFECTION AFTER THREE ROUNDS OF MASS PRAZIQUANTEL ADMINISTRATION IN SIERRA LEONE

Mary H. Hodges1, Jusufu Paye1, Mohamed S. Bah1, Florence McCarthy2, Abdul Conteh2, Santigie S. Sesay2

1Helen Keller International, Freetown, Sierra Leone, 2Ministry of Health and Sanitation, Freetown, Sierra Leone

Five health districts in Sierra Leone were found highly (≥50%) and two moderately endemic for Schistosoma mansoni. The remaining 7 districts had no or low endemicity (<1%). MDA with praziquantel (PZQ), implemented by health workers (HWs), started in 2009 in 6 districts targeting school-going children and treating 562,980 children (coverage 89%). It was scaled up to include all school aged children (SAC) and at risk adults in 7 districts in 2010 treating 1,831,383 persons (coverage 77%) and in 2011 treating 1,781,037 persons (coverage 82%). To minimize side effects expected in heavily parasitized individuals a pre-PZQ meal was funded, implemented by head teachers and/or communities. A survey was performed in 26 sentinel sites in 2012, 9 months after the third round of MDA in the 7 districts. Fresh stool samples from 50 SAC per site were examined by Kato-Katz method for S.mansoni infections, recorded as eggs per gram of feces (egpg). A total of 1,286 SAC were examined, male 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 129egpg (95% CI: 105.56-152.97egpg). 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 chiefdom 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

Benjamin G. Koudou, Moses J. Bockarie  
Liverpool School of Tropical Medicine, Liverpool, United Kingdom

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 these 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

Deogratias Damas1, Upendo Mwingi1, Andreas Nshala1  
1IMA World Health, Dar Es Salaam, United Republic of Tanzania, 2Ministry of Health and Social Welfare, Dar Es Salaam, United Republic of Tanzania

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 36 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.
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 synergist. ‘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 FILARIASIS ELIMINATION IN LIBERIA

Marnijina Moore\textsuperscript{1}, Dziedzom K. de Souza\textsuperscript{2}, Karsor Kolli\textsuperscript{1}, Fatorma Bolay\textsuperscript{3}, Daniel A. Boakye\textsuperscript{2}, Moses J. Bockarie\textsuperscript{4}

\textsuperscript{1}Neglected Tropical Diseases/Non Communicable Diseases Program, Ministry of Health and Social Welfare, Liberia, Monrovia, Liberia, \textsuperscript{2}Parasitology Department, Noguchi Memorial Institute for Medical Research, University of Ghana, Accra, Ghana, \textsuperscript{3}Liberian Institute for Biomedical Research, Liberia, Monrovia, Liberia, \textsuperscript{4}Centre for Neglected Tropical Diseases/Liverpool School of Tropical Medicine, Liverpool, United Kingdom

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 about the benefits of MDA and encourage volunteers to serve as community drug distributors. In this presentation, the baseline data collection, launching and scaling up of MDA activities in Liberia will be described in detail.

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

Deirdre Hollingsworth\textsuperscript{1}, James Truscott\textsuperscript{1}, Rachel Pullan\textsuperscript{2}, Simon Brooker\textsuperscript{2}, Roy Anderson\textsuperscript{1}

\textsuperscript{1}Imperial College London, London, United Kingdom, \textsuperscript{2}London School of Hygiene and Tropical Medicine, London, United Kingdom

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

Mwelecele N. Malecela\textsuperscript{1}, Upendo J. Mwingira\textsuperscript{1}, Andreas M. Nshala\textsuperscript{3}, Irene Mremi\textsuperscript{1}, Bernard Kilembe\textsuperscript{1}, Donnan Mbando\textsuperscript{1}, E. Michael\textsuperscript{1}

\textsuperscript{1}National Institute for Medical Research, Dar Es Salaam, United Republic of Tanzania, \textsuperscript{2}Neglected Tropical Diseases Control Program, Dar Es Salaam, United Republic of Tanzania, \textsuperscript{3}Ministry of Health and Social Welfare, Dar Es Salaam, United Republic of Tanzania, \textsuperscript{4}Notre Dame University, Notre Dame, IN, United States

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!
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PATENT EXTENSION: IMPROVING THE FDA’S NEGLECTED TROPICAL DISEASES REVIEW VOUCHER

Laureano Mestra

World Health Organization/TDR Clinical R&D Fellow Program, Woodcliff Lake, NJ, United States

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 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 FDAs PRV. Finally, a way to calculate the value of the proposed PEV is explained.

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

Peter U. Fischer1, Yenny Djuardi2, Mark Bradley3, Rahmah Noordin4, Paul Ruecket5, Taniawati Supali2

1Washington University School of Medicine, St. Louis, MO, United States, 2Department of Parasitology, University of Indonesia, Jakarta, Indonesia, 3Global Community Partnerships, GlaxoSmithKline, Brentfort, United Kingdom, 4Institute for Research in Molecular Medicine, Universiti Sains Malaysia, Penang, Malaysia, 5GIZ, Jakarta, Indonesia

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 A. lumbricoides, 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.

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DISCOVERY OF ANTIGENS FOR DIAGNOSIS OF SOIL-TRANSMITTED HELMINTH INFESTATIONS

Jodi Beattie1, David Elsemore2, Laurie Flynn2, Jimmeng Geng3, Jeffrey Bethony3, David Diemert4, James McCarter1, Michael Crawford

1Monsanto Company, Saint Louis, MO, United States, 2IDEXX Laboratories, Inc., Westbrook, ME, United States, 3George Washington University, Washington, DC, United States

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.

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

Darin Evans1, Aseged Taye2, Tekola Tilahun2, Patricia Graves1, Moses Katabarawa1, Frank Richards1

1The Carter Center, Atlanta, GA, United States, 2The Carter Center, Addis Ababa, Ethiopia

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
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 microfilaricide 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

Nana O. Wilson, Wesley Solomon, Mingli Liu, Jonathan Stiles
Morehouse School of Medicine, Atlanta, GA, United States

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 artesminin 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, CCL4, CCL11, 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

Asadu Sserwanga1, Ruth Kigozi1, Anne Gasasira2, Sussann Nasr3, Melody Miles4, Denis Rubahika4, Sarah Staedke5, Moses Kamya6, Grant Dorsey Dorsey7, Arthur Mmpibaza8
1Infectious Disease Research Collaboration, Kampala, Uganda, 2Institute for Health Metrics and Evaluation, University of Washington, Seattle, WA, United States, 3Centers for Disease Control and Prevention, Atlanta, GA, United States, 4The National Malaria Control Program, Ministry of Health, Kampala, Uganda, 5London School of Hygiene and Tropical Medicine, London, United Kingdom, 6Department of Medicine, Makerere University, Kampala, Uganda, 7University of California, San Francisco, San Francisco, CA, United States, 8Child Health and Development Centre, Makerere University, Kampala, Uganda

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 Kambumba (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 11, 15748 children were hospitalized in all three hospitals; Tororo (4949), Jinja (9202) and Kambumba (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 Kambunga. 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

Charwan I. Du Preez, Davis R. Mumbengegwi, Ronnie A. Bock
University of Namibia, Windhoek, Namibia

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 care 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

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eriocarpum, were previously shown to contain classes of antimalarial compounds through phytochemical screening. Growth inhibition studies using cellular infection models of Plasmodium falciparum 3D7 and D10 were carried out to determine anti-plasmodial effects of the extracts. In addition, mechanistic studies were conducted to determine the mode of action of the three plants. Organic extracts of V. capensis, N. costata and D. eriocarpum showed antiplasmodial activity at concentrations ranging from 50-250 µg/mL. Extracts from D. eriocarpum showed the highest activity with an IC50 of 63.17 µg/mL followed by D. eriocarpum ranging from 50-250 µg/mL. Extracts from N. costata showed antiplasmodial activity at concentrations of 93.29 µg/mL and 86.62 µg/mL, respectively. All the plant extracts inhibited haemazoin accumulation with D. eriocarpum exhibiting the highest inhibition. The extracts also inhibited protease activity at the early ring stage where infection of red blood cells was being established and at the trophozoite stage where metabolism of the parasites was increased. These results support the ethno-medicinal uses for these plants as complementary medicine for malaria and provide a basis for further studies to determine the potential of the plants as sources of new antimalarial medicines.

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MALARIA CHEMOPREVENTION IN A HIGH TRANSMISSION SETTING: A RANDOMIZED CONTROLLED TRIAL OF MONTHLY DIHYDROARTESININ-PIPERAQUINE VERSUS MONTHLY SULFADOXINE-PYRIMETHAMINE VERSUS DAILY TRIMETHOPRIM-SULFAMETHOXAZOLE VERSUS NO THERAPY FOR THE PREVENTION OF MALARIA

James A. Kapisi¹, Victor I. Bigira¹, Stephen Kinara¹, Florence Mwangwa¹, Beth Osterbauer¹, Jane Achau¹, Moses Kamya¹, Grant Dorsey²

¹Infectious Diseases Research Collaboration, Kampala, Uganda, ²University of San Francisco California, California, CA, United States

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 where 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 dwk. 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 dihydroartesinin-piperaquine (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, 2.87 episodes PPY among those randomized to monthly SP (PE=7%, 95% CI=1-17-26%), 2.92 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.

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PRESCRIPTION PRACTICES AMONG PUBLIC AND PRIVATE HEALTH FACILITIES FOR UNCOMPROMISED MALARIA IN RURAL GHANA

Vida Ami Kukula
Dodowa Health Research Centre, Accra, Ghana

Malaria control continues to rely on the diagnosis and prompt treatment of both suspected and confirmed cases through the health care structures. The irrational use of drugs is a major problem for present day medical practice with consequences as the development of resistance to wide range of medicines, ineffective treatment, adverse effects and an economic burden on the patients and economies. No study has been published in Ghana on the prescription practices of both private and public health providers. This study compared the prescription practices of private and public health care providers in Dangme West District of Ghana from February to July 2011. The study was conducted in a demographic surveillance site located in the Greater Accra region of Ghana, with perennial malaria transmission. It is prospective study of patients presenting at eleven public and five private health facilities diagnosed and treated with an antimalarial. 4247 patients prescribed an antimalarial were recruited, of these, 2606 (61.4%) and 1641 (38.6%) represented public and private provider attendants respectively. 31.5% of the patients from public providers were blood slide or rapid diagnostic test diagnosed and 62.5% clinically diagnosed compared to 33.5% and 66.5% for private provider attendants. The mean number of drugs per prescription was 4.2 for public and 4.0 for private providers. Public care providers were more likely to prescribe more drugs than private providers with a minimum prescription of 1 for both providers and a maximum of 10 and 7 respectively. Both providers prescribed an analgesic, antibiotic and a multivitamin as top three drugs in addition to an antimalarial. In conclusion, although malaria treatments were varied, there were not great differences between the public and private providers. Monotherapy with artemisinin derivatives were relatively common. A mashwarm of regulatory and non-regulatory interveions, aimed at providers and consumers must be implemented to improve diagnosis and prescription practices of health care providers.

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CLINICAL EFFICACY AND SAFETY OF ARTESUNATE-AMODIAQUINE AND ARTEMETHER LUMEFANTRINE FOR THE TREATMENT OF UNCOMPROMISED MALARIA AND PREVALENCE OF DRUG RESISTANCE MARKERS IN NGAOUNDERE, NORTH CAMEROON

Innocent M. Ali¹, Eric O. Ngongang², Palmer Netongo Masumbe³, Anthony Aju³, Barbara Atogho Tiedeu³, Marie Solange B. Evehe³, Eric A. Achidi³, Wilfred F. Mlbacham⁴

¹Laboratory for Public Health Research Biotechnologies, University of Yaounde 1, Yaounde, Cameroon and Unit for Molecular Microbiology and Immunology, Biochemistry Department, University of Dschang, Yaounde, Cameroon, ²Laboratory for Public Health Research Biotechnologies, University of Yaounde 1, Yaounde, Cameroon, ³Faculty of Health Sciences, University of Buea, Buea, Cameroon, ⁴Laboratory for Public Health Research Biotechnologies, The Biotechnology Centre, University of Yaounde 1, Yaounde, Cameroon

Cameroon switched policy for treatment of uncomplicated malaria to antimalarial to artesunate-amodiaquine (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, www.astmh.org
SAFETY OF ARTEMISININS DURING EARLY PREGNANCY, ASSESSED IN 62 SUDANESE WOMEN

Elhassan Mohamed Ishag1, Ishag Adam Ahmed2, Gamal Khalid Ahmed3

1University of Gezira, Wad Medani Maternity Teaching Hospital, Sudan, 2University of Khartoum, Khartoum, Sudan, 3University of Gadairif, Gadairif, Sudan

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, Gadairif 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 - artemether injections (48), artesunate plus sulfadoxine-pyrimethamine (11) or artesunate 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 artemether 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 UNCOMPPLICATED PLASMODIUM FALCIPARUM MALARIA IN AFRICAN CHILDREN

Johannes Pfeil1, Steffen Borrmann2, Yesim Tozan3

1Childrens Hospital, University of Heidelberg, Heidelberg, Germany, 2Department of Infectious Diseases, Heidelberg University School of Medicine, Heidelberg, Germany, 3Department of International Health, Boston University School of Public Health, Boston, MA, United States

Recent randomized multi-center trials showed that dihydroartesinin-piperaquine (DHAPQ) 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 DHAPQ 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 DHAPQ 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 DHAPQ 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 DHAPQ and 28.1 (95%CI 27.5--28.7) with AL treatment in a scenario

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where 90% of children with recurrent infections have access to early treatment for uncomplicated malaria. The number of deaths is 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 DHAQ as first-line therapy. We conclude that even though the post-treatment prophylactic advantage of DHAQ seems to be relatively small, changing the first-line therapy of uncomplicated falciparum malaria from AL to DHAQ 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 DOSE REGIMEN ON THE RISK OF TREATMENT FAILURE IN PATIENTS DIAGNOSED WITH UNCOMPPLICATED FALCIPARUM MALARIA

Patrice Piola, on behalf of the WWARN AL Dose Impact Study Group

WorldWide Antimalarial Resistance Network, Oxford, United Kingdom

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. In the multivariate model, 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: 23.34-34.01). The fixed dose formulation provides better efficacy results than co-blisters 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

Stephanie Dellicour1, Philippe Brasseur2, Per Thorn3, Oumar Gaye4, Piero Olliaro5, Malick Badiane6, Andreas Stergachis7,Feiko O. ter Kuile1

1Liverpool School of Tropical Medicine, Liverpool, United Kingdom, 2Institut de Recherche pour le Développement (IRD), Dakar, Senegal, 3Thorn IT Services Limited, London, United Kingdom, 4Université Cheikh Anta Diop, Dakar, Senegal, 5UNICEF/UNDP/WB/WHO Special Programme for Research and Training in Tropical Diseases (TDR), Geneva, Switzerland, 6District Medical d’Oussouye, Oussouye, Senegal, 7University of Washington, Seattle, WA, United States

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 out-patient 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

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TREATMENT EFFICACY OF ARTESUNATE-AMODIAQUINE TREATMENT REGIMENS FOR UNCOMPPLICATED FALCIPARUM MALARIA: COMPARISON OF FIXED VERSUS CO-BLISTER FORMULATIONS

Philippe J. Guerin, on behalf of the WWARN ASAQ Dose Impact Study Group

WorldWide Antimalarial Resistance Network, Oxford, United Kingdom

Artesunate-Amodiaquine (AS-AQ) is the first line antimalarial treatment in 25 countries and until 2007 was available in a co-blistered, non fixed dose formulation. A fixed dose combination (FDC) of AS-AQ was introduced in 2007 to optimize the AS: AQ ratio and improve adherence. Current dosing recommendations for the FDC are according to three age ranges with 3 dosing strengths available. To assess the spectrum of total mg/kg dose of AQ administered and compare the effect on treatment efficacy of fixed and non fixed AS-AQ combinations, individual patient level data from clinical efficacy studies of uncomplicated P. falciparum were collated using standard algorithms. Factors associated with PCR adjusted efficacy were evaluated using Cox regression model with shared frailty to account for study effects. Data were available on 5410 patients from 24 studies conducted between 2003 and 2011 (5313 from Africa; 97 from Asia). 20 studies had 28 days follow up and 4 studies were followed up for 35 days or longer. 142 recrudescent cases were reported, the median time to recrudescence was 21 days [IQR: 21-28]. In the multivariate model risk factors for recrudescence were lower age 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).
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.

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

Elizabeth Margret Asiimwe1, Fredrick Kabikira1, Denis Kaiywa1, Sylia Thiam2

1AMREF Uganda, Kampala, Uganda, 2AMREF HQ, Nairobi, Kenya

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.

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

Ehijie F. Enato1, Petra F. Mens2, Augustine O. Okhamafe3, Henk D. Schallig2

1Department of Clinical Pharmacy and Pharmacy Practice, University of Benin, Benin, Nigeria, 2Parasitology Unit, KIT Biomedical Research, Amsterdam, The Netherlands, 3Department of Pharmacuetics & Pharmaceutical Technology, University of Benin, Benin, Nigeria

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.

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EFFICACY OF ARTEUNATE AND ARTEUNATE-AZITHROMYCIN FOR THE TREATMENT OF UNCOMPPLICATED PLASMODIUM FALCIPARUM MALARIA IN VIETNAM

Nguyen X. Thanh1, Trieu N. Trung2, Nguyen C. Phong1, Huynh H. Quang2, Bui Dai1, Dennis Shanks3, Marina Chavchich4, Michael D. Edstein5

1Military Institute of Hygiene and Epidemiology, Hanoi, Vietnam, 2Institute of Malariology, Parasitology and Entomology, Qui Nhon, Vietnam, 3Australian Army Malaria Institute, Brisbane, Australia

Reports of reduced susceptibility of artemesunate 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.
A SURVEILLANCE SYSTEM TO MONITOR THE QUALITY AND AUTHENTICITY OF ARTEMISININ COMBINATION TREATMENTS IN AFRICA AND SOUTHEAST ASIA

Harparkash Kaur1, Albert van Wyk1, Naiefa Malik1, Caroline Lynch1, Shunmay Yeung1, Paul N. Newton2, Prabha Dwivedi2, Dana Hostetler3, Isabel Swamidoss4, Michael D. Green4, Facundo Fernandez5

1London School of Hygiene & Tropical Medicine, London, United Kingdom, 2Centre for Clinical Vaccinology and Tropical Medicine, Churchill Hospital, Oxford University, Oxford, United Kingdom, 3Georgia Institute of Technology, School of Chemistry and Biochemistry, Atlanta, GA, United States, 4United States Centers for Disease Control and Prevention, Atlanta, GA, United States

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

Sumudu Britton1, James McCarthy1, Eloise Thompson2, Bismarck Dinko2, Colin Sutherland3

1Queensland Institute of Medical Research, Brisbane, Australia, 2London School of Hygiene and Tropical Medicine, London, United Kingdom

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 98 whole blood (WB) samples from the Gambia and 25 FP samples from Ghana. Using primers to Plasmodium falciparum (htLAMP-Pf) and Plasmodium genus (htLAMP-Pg) on control samples the assay had a limit of detection of 0.0005% parasitemia (approximately 25 parasites/µL).

Thermal Contrast Significantly Improves the Sensitivity of Lateral Flow Assays for Malaria Diagnosis

Zhenpeng Qin1, Chandy C. John1, Gregory S. Park1, Elissa K. Bulter1, Max von Hohenberg1, David R. Boulware1, Taner Akkin1, Warren C. Chan1, John C. Bischof1

1University of Minnesota, Minneapolis, MN, United States, 2University of Toronto, Toronto, ON, Canada

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 parasitemia 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 parasitemia. 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**

Clarence Mkoba1, Denise Roth Allen2, Emmy Metta1, Admirabilis Kalolella1, Catherine Goodman3, Salim Abdulla1, S. Patrick Kachur2

1Ifakara Health Institute, Dar es Salaam, United Republic of Tanzania, 2Centers for Disease Control and Prevention, Atlanta, GA, United States, 3London School of Hygiene and Tropical Medicine, London, United Kingdom

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**

Felix A. Botchway1, Cecilia Elorm Lekpor2, Seth Amankwah1, William Ababio1, Patience B. Williams1

1University of Ghana Medical School, Accra, Ghana, 2Pathology Department, University of Ghana Medical School, Accra, Ghana, 3Child Health Department, University of Ghana Medical School, Accra, Ghana, 4Hematology Department University of Ghana Medical School, Accra, Ghana

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. Thin and thick 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.
**Addressing Over- and Under-Diagnosis of Malaria in Tanzania: An Evaluation of Large-Scale Implementation of Malaria Rapid Diagnostic Tests (mRDTs) in Three Regions with Varying Malaria Epidemiology**

Admirabilis B. Kalolella1, Katia Bruxvoort2, Rebecca Thomson2, Charles Festo3, Happy Nchinimb1, Matthew Cairns3, Julie Thwing1, Mark Taylor1, Catherine Goodman2, Patrick Kachur1

1Ifakara Health Institute, Dar es Salaam, United Republic of Tanzania, 2London School of Hygiene and Tropical Medicine, London, United Kingdom, 3Centers for Disease Control and Prevention, Atlanta, GA, United States

Artemisinin based combination therapy (ACT) is the first line drug in most malaria-endemic countries, but there are concerns that quality of care remains poor. Patients needing ACT often do not receive it, but there is also considerable over-treatment due to the lack of accurate diagnosis and inappropriate management. In 2010-2012, Tanzania rolled-out malaria rapid diagnostic tests (mRDTs) at government health facilities to improve treatment of febrile illness. Here, we report results of health facility surveys to assess treatment practices before and after mRDT scale up in three regions with varying malaria epidemiology. Patients with fever in the previous 48 hours were enrolled at 320 randomly selected health facilities in Mwanza, Mbeya, and Mtwarara regions in May - October 2010 and March - August 2012. Patients were interviewed following their consultation, and data were collected on patient characteristics, previous treatment for fever, and care received at the facility. Finger prick blood samples were taken by study staff to test for malaria parasitemia. Health workers seeing patients were also interviewed about their training and supervision, knowledge, and facility stocks of antimalarials and mRDTs. At baseline, data were collected on 1746 patients, of which only 15.9% received a diagnostic test from facility health workers. Based on study blood smears, 20.9% tested positive in Mtwara, 6.6% in Mwanza, and 1.6% in Mbeya. An ACT was obtained by 65.8% of patients testing positive by the study blood slide and 39.0% of patients testing negative, meaning that overall only 58.5% of patients received appropriate malaria treatment given their study blood smear result. We will compare these results with those from 2012 to evaluate the success of mRDT roll-out at addressing over- and under-diagnosis of malaria, and the role of stock-outs and health worker practices in addressing these key problems. These data will contribute to enhancing interventions to increase appropriate treatment of patients with and without malaria in Tanzania and other malaria-endemic countries.

**Accuracy of Two Rapid Diagnostic Tests for Diagnosis and Monitoring Treatment of Malaria in a High Transmission Setting in Uganda**

Phoebe K. Mbabazi

Makerere University College of Health Sciences, Kampala, Uganda

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 have 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.

**The Diagnostic Values of Rapid Diagnostic Test Based on Histidine Rich Protein II of CareStartTM Malaria in Detecting Malaria Infection in Pregnant Women San and Kita**

Moussa Djimde, Mohamed Keita, Hamadoun Diakite, Moussa Niangaly, Sidiki Konate, Nouhoum Guindo, Etienne Guirou, Etienne Guirou, Binta Barry, Kassoum Kayentao, Ogobara K. Doumbo

Malaria Research and Training Center, University of Bamako, Mali, Bamako, Mali

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 Sulphadoxine-Pyrimethamine during in vivo testing of 42 days. Result showed both the sensitivity and specificity of 95%. Positive and negative predictive values were of 86% and 98% respectively, and a kappa value was 0.87. There were 5% of false positives and negatives. When tracking in vivo, the proportion of positive 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.

**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**

Albertine K. Lele1, Olivia A. Achonduh1, Joelle Pamen-Ngako1, Joel N. Ambeila1, Ignatius C. Ndong1, Sarah N. Ndive1, Barnabas B. Orang-Ojong1, Theresia M. Njuabe1, Lindsay Mangham2, Virginia Wiseman2, Wilfred F. Mbacham1

1Laboratory for Public Health Research Biotechnologies, Biotechnology Center, University of Yaounde I, Yaounde, Cameroon, 2London School of Hygiene and Tropical Medicine, London, United Kingdom

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.

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

Dennis Adu-Gyasi, Mohammed Adams, Sabastina Amoako, Emmanuel Mahama, Maxwell Nsoh, Seeba Amenga-Etego, Frank Baiden, Kwaku Poku Asante, Sam Newton, Seth Owusu-Agyei
Kintampo Health Research Centre, Kintampo North, Ghana

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 WBCs of patients, an assumed WBCc of 8.0 X 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 absolute 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 x 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.

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

Pongwit Bualombai, Patcharin Boon-in, Ponlawat Ruangsisarak, Kanungrit Congpuong, Aiemumporn Kanchana, Wichai Satimai, Panadda Dhepaksorn
1Bureau of Vector Borne Diseases, Nonthaburi, Thailand, 2Center for National Blood, Bangkok, Thailand, 3Department of Medical Science, Nonthaburi, Thailand

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 immunoclotomatographic test to detect either Plasmodium falciparum or P. 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 glyceraldehyde-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 parasitemia levels. However, at least, this study got an alternative RDT prototype to be validated its feasibility for using in field onward.

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

Richard Nyomugyenyi, Kristian S. Hansen, Sham Lal, Clare Chandler, Anthony K. Mbonye, Pascal Magnusson, Sian E. Clarke
1Vector Control Division, Ministry of Health, Kampala, Uganda, 2ACT Consortium, London School of Hygiene and Tropical Medicine, London, United Kingdom, 3Department of Community Health, Ministry of Health, Kampala, Uganda, 4DBL Centre for Health Research and Development, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark

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

Anthony K. Mbonye1, Clare Chandler2, Kristian S. Hansen2, Sham Laï2, Bonnie Cundill3, Richard Nydomyugenyi3, Pascal Magnusson4, Sian E. Clarke2

1Department of Community Health, Ministry of Health, Kampala, Uganda, 2ACT Consortium, London School of Hygiene and Tropical Medicine, London, United Kingdom, 3Vector Control Division, Ministry of Health, Kampala, Uganda, 4DBL Centre for Health Research and Development,Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark

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

Anna Y. De La Cruz1, Jenny Liu1, Eric Schatzkin1, Karen Schlein1, Sepideh Modrek2, Oladimeji Oladepe2, Dominic Montagu1

1University of California San Francisco, San Francisco, CA, United States, 2Stanford University, Stanford, CA, United States, 3University of Ibadan, Ibadan, Nigeria

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

Noppadon Tangpukdee, Chatnapa Duangdee, Polrat Wilairatana, Srivicha Krudosood

Mahidol University, Bangkok, Thailand

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-smead 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 INTERMITTENT PREVENTIVE TREATMENT DURING PREGNANCY (IPTp) WITH COMBINED USE OF LLNS IN HIGH RESISTANCE AREA TO SULFADOXINE-PYRIMETHAMINE IN CÔTE D’IVOIRE

Offianan A. Toure1, Penali L. Kone1, M’Thanhorou A. Coulibaly1, Tiacoh L. N’Guessan1, Ako A. Ako1, Gbessi E. A1, Baba Coulibaly1, David Koffi1, Sarr Demba2, Jambou Ronan3, Kone Moussa4

1Institut Pasteur, Abidjan, Côte d’Ivoire, 2Department of Infectious Diseases, University of Georgia, Athens, GA, 3Institut Pasteur, Antananarivo, Madagascar, 4UFR Sciences Pharmaceutiques et Biologiques University of Cocody, Abidjan, Côte d’Ivoire

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 Anonkouakouté and Samo 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 Samo, 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% of 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 UNCOMPPLICATED PLASMODIUM FALCIPARUM MALARIA IN ETHIOPIA IN 2004

Moges K. Mekonnen

Ethiopian Health and Nutrition Research Institute, Ministry of Health, Addis Ababa, Ethiopia

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 parasitemia 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

Magatte Ndiaye1, Babacar Faye1, Roger Tine1, Jean Louis Ndiaye1, Aminata Cole Lo1, Annie Abiola1, Yemou Dieng1, Daouda Ndiaye1, Rachel Hallett2, Michael Alifrangis3, Oumar Gaye1

1Service de Parasitologie – Mycologie, Faculté de Médecine, Université Cheikh Anta DIOP; Dakar, Senegal, 2Faculty of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London, United Kingdom, 3Centre for Medical Parasitology, Department of International Health, Immunology and Microbiology, University of Copenhagen, Copenhagen, Denmark

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 CVNKN 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

Baba Dieye1, Daria Van Tyne1, Rachel Daniels2, Amy Bei2, Yaye Dié Ndiaye1, Mouhamadou Ndiaye1, Omar Ndir1, Souleymane Mboup1, Clarissa Valim2, Amanda Lukens2, Dan Milner2, Sarah K. Volkman2, Dyann F. Wirth2, Daouda Ndiaye1
1University Chekh Anta Diop, Dakar, Senegal, 2Department of Immunology and Infectious Diseases, Harvard School of Public Health, Boston, MA, United States

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 approximately 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 ex vivo and in vitro 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.

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

Aminata C. Lo1, Babacar Faye1, Annie K. Abiola1, Magatte Ndiaye1, Roger C. Tine1, Badara Cissé1, Jean L. Ndiaye1, Paul Milligan1, Rachel Hallett2, Colin Sutherland3, Oumar Gaye1,4
1University Chekh Anta Diop of Dakar/Senegal, Dakar, Senegal, 2London School of Hygiene and Tropical Medicine, London, United Kingdom

Chloroquine was until 2002 the most commonly drug used 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 sulphadoxine-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 SVMNK 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

Dyson A. Mwandama
University of Malawi College of Medicine, Blantyre, Malawi

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=0.003). The prevalence of dhps 581 was high among pregnant women at delivery (37% vs 2.6% at ANC and 1.3% in OPD, 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, PFHDPS AND PFHDHR DRUG RESISTANT HAPLOTYPES IN THE SOUTH BUT NOT IN THE NORTH OF CÔTE-D’IVOIRE

Berenger A. Ako1, Shannon H. Takala2, Offianan A. Toure1, Aristide A. Coulibaly1, Landry N. Tiaco1, Eric Adjii1, Coulibaly Baba1, Louis K. Penali1, Simon-Pierre A. Nguetta1, Christopher V. Plowe2

1Institut Pasteur Cote d’Ivoire, Abidjan, Côte D’Ivoire, 2University of Maryland School of Medicine, Center for Vaccine Development, Malaria Section, Baltimore, MD, United States, 3Université de Cocody, Laboratoire de Génétique, Abidjan, Côte D’Ivoire

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 used of CQ and SP. The nationwide coverage of recommended ACTs is not quite effective due in part to disparities in the distribution of heath care 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 Pfdrfr IRIN, and the simple mutant Pfdrps 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

Meera Venkatesan1, Nahla Gadalla2, on behalf of AL/ASQA Molecular Markers Study Group3

1WorldWide Antimalarial Resistance Network, Baltimore, MD, United States, 2Tropical Medicine Research Institute, Khartoum, Sudan, 3WorldWide Antimalarial Resistance Network, Oxford, United Kingdom

The initial reduction in peripheral Plasmodium falciparum parasitemia following artemisinin-based combination therapy (ACT) is driven predominantly by 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 overall 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

Khalid B. Beshir, Rachel Hallett, Teun Bousema, Colin Sutherland

London School of Hygiene & Tropical Medicine, London, United Kingdom

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 of pfcrt as measured by real-time quantitative PCR. The amino acid NFD of pfcrt as measured by real-time quantitative PCR. The amino acid NFD of pfcrt as measured by real-time quantitative PCR. The amino acid NFD of pfcrt as measured by real-time quantitative PCR. The amino acid NFD of pfcrt as measured by real-time quantitative PCR.
Drug resistance is a significant public health issue. Activities and interventions such as DOT in a context where artemisinin combination therapies (ACTs) approach for malaria treatment (especially for Plasmodium falciparum) 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-piperaquine, dihydroartemisinin-piperaquine 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

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

Sara E. Canavati de la Torre¹, Po Ly², Chea Nguon¹, Arantxa Roca-Feltren³, David Sintasath⁴, Maxine Whittaker⁵, Pratap Singhhasivanon⁶

¹Faculty of Tropical Medicine, Mahidol University/Malaria Consortium Cambodia, Phnom Penh, Cambodia, ²The National Centre of Parasitology and Malaria Control, Phnom Penh, Cambodia, ³Malaria Consortium Cambodia/London School of Tropical Medicine and Hygiene, Phnom Penh, London, Cambodia, ⁴Malaria Consortium Asia Regional Office, Bangkok, Thailand, ⁵Australian Centre for International and Tropical Health, University of Queensland, Queensland, Australia, ⁶Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

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 Artesinin-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 VMWW/VMWs, 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 observation of DOTs. VMWs do not have transport for the collection of stool samples. The number of health facilities serve by each VMW is 3 on average, but this could vary from 1 to 5. VMWs are not paid for the transport and this is considered a barrier 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.

**SELECTION OF PLASMODIUM FALCIPARUM STRAINS WITH REDUCED SENSITIVITY TO THE HIV PROTEASE INHIBITOR LOPINAVIR**

Christian Nsanzabana, Philip J. Rosenthal

University of California San Francisco, San Francisco, CA, United States

Some HIV protease inhibitors (PIs) are active against cultured malaria parasites at concentrations that are clinically relevant. Lopinavir acted against multiple laboratory strains of Plasmodium falciparum with an IC₅₀ of 1-3µM, and against two freshly cloned strains from Tororo, Uganda with IC₅₀ of 1.7µM. With standard dosing of lopinavir/ritonavir, lopinavir circulates at ~9-19 µM. Importantly, in an ongoing clinical trial, children treated for HIV infection with a lopinavir-ritonavir-based antiretroviral regimen experienced a 41% decrease in the incidence of malaria compared to children treated with a non-nucleoside reverse transcriptase-based regimen. Impacts of the PI on malaria were likely due principally to pharmacokinetic interactions between ritonavir and lopinavir, but also the direct antimalarial activity of lopinavir. The antimalarial mechanism of action of HIV PIs is uncertain, although it is likely that they act against one or more of the ten plasmoidal aspartic proteases known as plasmspsins. To help to characterize mechanisms of action and resistance, we selected malaria parasites with decreased sensitivity to lopinavir. We cultured the P. falciparum multidrug resistant reference strain W2 and the sensitive strain 3D7 with selective concentrations of lopinavir for fourteen months. Changes in sensitivity were selected only very slowly. The strains obtained after culture for 212 cycles under lopinavir pressure had IC₅₀ of ~10µM for both strains, corresponding to three times the IC₅₀ of the parental strains. We are currently cloning parasites with reduced sensitivity to lopinavir and assessing the stability of the phenotype when drug pressure is removed. Differences between parasites with varied sensitivity will be assessed, including sequencing of plasmspsin genes. Our goal is to determine if alterations in lopinavir sensitivity selected in culture are due to specific genetic changes in plasmspsin genes or other portions of the P. falciparum genome. If alterations mediating changes in lopinavir sensitivity are identified, surveillance for these genetic determinants may help to guide optimal antiretroviral therapy in HIV-infected children at risk of malaria.

**MOLECULAR MARKERS OF ANTIMALARIAL DRUG RESISTANCE IN SOUTH-CENTRAL VIETNAM**

Marina Chavchich¹, Kerryn Rowcliffe¹, Nguyen Dang Kim², Vu Huy Chien³, Nguyen Xuan Thanh², Trieu Nguyen Trung³, Nguyen Chinh Phong³, Nguyen Xuan Thien³, Huyinh Hong Quang³, Bui Dai³, George Dennis Shanks¹, Michael Douglas Edstein¹

¹Australian Army Malaria Institute, Enoggera, Australia, ²Military Institute of Hygiene and Epidemiology, Hanoi, Vietnam, ³Institute of Malariology, Parasitology and Entomology, Qui Nhon, Vietnam

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. artesinin-piperine, dihydroartemisinin-piperine 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, pfdfhr-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 (PVMRD1) POLYMORPHISMS IN THAILAND AND CAMBODIA

Jaymin C. Patel1, Jessica T. Lin2, Oksana Kharabora2, William Rogers3, Jetsumon Sattabongkot4, Ratawan Ubaalee4, Anthony Schuster4, Duong Socheat5, Chansuda Wongsrichanalai6, Jonathan J. Juliano6

1Department of Epidemiology, Gillings School of Global Public Health, University of North Carolina at Chapel Hill, Chapel Hill, NC, United States, 2Division of Infectious Diseases, University of North Carolina School of Medicine, Chapel Hill, NC, United States, 3Naval Medical Research Unit #2, Phnom Penh, Cambodia, 4Department of Entomology, U.S. Army Medical Component Armed Forces Research Institute of Medical Science, Bangkok, Thailand, 5National Malaria Center, Phnom Penh, Cambodia, 6Institut Pasteur du Cambodge, Phnom Penh, Cambodia

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 (pvmrd1) gene to chloroquine resistance and increase in pvmrd1 copy number to reduced susceptibility to mefloquine and other ACT partner drugs. In this study, we compared pvmrd1 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 pvmrd1 mutant SNPs (Y976F and F1076L). In total, a greater proportion of Cambodian isolates harbored the 976F mutation correlated with chloroquine resistance (90% vs. 7.1%, p<0.001), while 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 pvmrd1 polymorphisms in Thailand versus Cambodia. Selection for different Pvmrd1 haplotypes in these two areas has likely been shaped by different drug policies in the two 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-MELFOQUINE COMBINATIONS FOR THE TREATMENT OF UNCOMPROMICATED FALCIPARUM MALARIA IN THAILAND DURING FIVE YEARS OF CLOSED DRUG MONITORING (2006-2010)

Wichai Satimai1, Kanungrit Congpoung1, Pongwit Bualombai1, Arunya Pinyorattanachote2, Kalaya Tunchan1, Somchai Inthanakom1

1Bureau of Vector Borne Diseases, Nonthaburi, Thailand, 2Office of Disease and Prevention No. 11, Nakornnonthararat, Thailand, 3Office of Disease Prevention and Control No. 9, Tak, Thailand, 4Office of Disease Prevention and Control No. 12, Songkla, Thailand

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 (ASM2 and ASM3). 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

Julia Goldberg, Guenther Fink

Harvard School of Public Health, Cambridge, MA, United States

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 [1.299-3.890], p-value 0.004). 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 [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

Abdul Mansudi Manah1, Shamsul Azhar Shah2, Rohaizat Hassaan2, Mohd Yusof Ibrahim3

1Sabah State Health Department, Keningau, Malaysia, 2National University of Malaysia, Kuala Lumpur, Malaysia, 3Sabah State Health Department, Kota Kinabalu, Malaysia

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 was 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
health office from 1st January until 31st July 2008 which met with the inclusion criteria were included in the study. A total of 166 pairs of cases and controls which were matched by age, sex and mukim were analyzed. McNemar Odds Ratio (ORM) 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 ORM, 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.

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

Gary L. Roark1, Patrick J. Hickey2
1CURE Hospital, Niger, Niamey, Niger, 2Tropical Public Health Department of Preventive Medicine and Biometrics, Uniformed Services University of the Health Sciences, Bethesda, MD, United States

Non-falciparum malaria is not a 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 Nigerien population is at significant risk from non-falciparum malaria. Potential reasons for an elevated incidence of non-falciparum malaria in this population are discussed.

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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

Sanie S. Sesay1, Arantxa Roca-Feltrer1, David Lalloo2, Feiko ter Kuile2, Sanjoaquin Miguel1, Dianne Terlouw4
1Malawi-Liverpool-Wellcome Trust Clinical Research Programme, Blantyre, Malawi, 2Liverpool School of Tropical Medicine, Liverpool, United Kingdom, 3Malawi-Liverpool-Wellcome Trust Clinical Research Programme and Liverpool School of Tropical Medicine, Blantyre, Malawi, 4Malawi-Liverpool-Wellcome Trust Clinical Research Programme and Liverpool School of Tropical Medicine, Liverpool, United Kingdom

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 (MIS) 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 MIS. 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.

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

Nagesh (Nash) R. Aragam1, Nabi Nge2, Irving Hoffman1, Feng-Chang Lin1, Colin J. Sutherland2, Jeffrey A. Bailey3, Jonathan J. Juliano1
1University of North Carolina, Chapel Hill, NC, United States, 2London School of Tropical Medicine and Hygiene, London, England, United Kingdom, 3University of Massachusetts School of Medicine, Worcester, MA, United States

Cellular immunity to Plasmodium falciparum circumsporozoite protein (CSP) 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

Christian M. Parobek1, Duong Socheat2, William O. Rogers3, Jonathan J. Juliano4
1School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC, United States, 2National Malaria Center, Phnom Penh, Cambodia, 3Naval Medical Research Unit #2, Phnom Penh, Cambodia, 4Division of Infectious Diseases, University of North Carolina at Chapel Hill School of Medicine, Chapel Hill, NC, United States

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 surface protein (pvcsp) and the merozoite surface protein 1 (pvmmsp1), 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 pvmmsp1 consistently displayed a signature of strong balancing selection. Moreover, interspecies comparisons of orthologous antigens revealed that a subregion within the 42-kd block of pvmmsp1 is under strong selection for non-synonymous nucleotide changes. In contrast to pvmmsp1, 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

Elizabeth T. Rogawski1, Ebbie Chaluluka2, Malcolm E. Molyneux2, Gaoqian Feng2, Stephen J. Rogerson2, Steven R. Meshnick3
1University of North Carolina, Chapel Hill, NC, United States, 2Malawi-Liverpool-Wellcome Trust Clinical Research Program, College of Medicine, University of Malawi, Blantyre, Malawi, 3Burnet Institute, Melbourne, Australia, 4Department of Medicine (RMH/WHH), University of Melbourne, Melbourne, Australia

Fetal anemia is common in malarious 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 decrease 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

Elizabeth N. Githae
KEMRI Wellcome Trust Research Programme, Kilifi, Kenya

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

Jasmin Akter1, Kamala Thriemer2, Wasif Ali Khan3, Sullivan David3, Harald Noedl2, Rashidul Haque1
1International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh, 2Tropical Medicine, Medical University of Vienna, Vienna, Austria, 3Johns Hopkins Malaria Research Institute, Baltimore, MD, United States

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 southeastAsia multiplicity of infection for hypoendemic 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 monolocular 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 REPEATS IN LILONGWE, MALAWI

Natalie M. Bowman¹, Seth Condon¹, Tisungane Mvalo², Francis Martinson², Irving Hoffman¹, Steven R. Meshnick¹, Jonathan J. Juliano¹

¹University of North Carolina School of Medicine, Division of Infectious Diseases, Chapel Hill, NC, United States, ²University of North Carolina Project Malawi, Lilongwe, Malawi

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. Isolation-by-distance among parasites has been suggested in falciparum malaria in the past. The impact of parasite genetic diversity on many critical issues for malaria control remains unclear. Investigating parasite population structure and diversity can help us better understand immunity, response to selective pressures and evolution of the parasite.

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GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY PREVALENCE IN THE GAMBIA

Joseph Okebe¹, Alfred Amambua-Ngwa¹, Jason Parr², Sei Nishimura³, Melissa Daswani¹, Ebako N. Takem¹, Muna Affara¹, Sergin J. Ceesay¹, Davis Nwakamna¹, Umberto D’Alessandro¹

¹Medical Research Council Unit, Banjul, Gambia, ²University of Manchester, Manchester, United Kingdom, ³London School of Medicine and Dentistry, London, United Kingdom

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/g/Hb 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?

Piyaporn Wangroongsarb

Bureau of Vector Borne Disease, Nanthaburi, Thailand

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

Smala Ouédraogo¹, Florence Bodeau-Livinéc², Valérie Briand², Bich-Tram Huynh³, Ghislain Kobto Koura², Achille Massougboji², Michel Cot²

¹Faculty of Medicine in Cotonou, Cotonou, Benin, ²French Institute of Research for Development (IRD), Paris, France, ³Institut Pasteur, Paris, France

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, < 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.

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

Victor I. Bigira1, James Kapisi1, Stephen Kinara1, Florence Mwangwa1, Beth Osterbauer1, Jaffer Okiring1, Barnabas Natamba2, Tamara Clark2, Jane Achan1, Moses Kamya1, Grant Dorsey2

1Infectious Diseases Research Collaboration, Kampala, Uganda, 2University of California San Francisco, San Francisco, CA, United States, 3Division of Nutritional Sciences, Cornell University, Ithaca, NY, United States

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.

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**MALARIA TREATMENT COST IN HEALTH SYSTEM: WHAT IS THE CHILDREN UNDER FIVE YEARS OLD MALARIA PROVIDER COST IN BURKINA FASO (WEST AFRICA)?**

Danielle M. Yubgare Belemsaga

IRSS, Ouagadougou, Burkina Faso

Malaria is the major cause of morbidity in Burkina Faso especially among children under 5 years old. The cost related to the treatment of this disease in the country has not been well documented at the household and health system levels. Knowledge about the cost of treating malaria can affect the health care seeking behaviour of people and the use of different malaria prevention products. This paper estimates the health system cost due to simple or severe malaria with children under 5 years old in order to make available better understanding of the burden of malaria. Data have been collected from the following health facilities: the Nanoro religious district hospital, primary health facilities (5). We reviewed also Medical outpatient (243) and inpatient (122) records. We interviewed 46 Outpatient (OPD) and 10 inpatient (IPD) caregivers. Health system cost was estimated per component drug and lab test, personnel, and building. Malaria was ascertained not by parasitological tests but through fever at primary facilities. Lab test was used at district hospital. The survey was conducted from July to September 2010, during the high transmission season. Simple Malaria unit cost for OPD at facility level was 1.9 USD for medicine, 0.2 USD for lab test, 0.3 and 0.4 USD for nurse at primary centre and district hospital, 0.3 and 1.0 USD for infrastructure at primary and district hospital. For severe malaria, IPD cost at district hospital was 4.5 USD for medicine, 7.2 USD for consumable, 18.7 for lab test, 2.0 for nurse, 6.2 for MD and 4.5 for building. The average cost of treating an episode of the disease including direct and indirect costs for household was 8.5 USD at OPD exit interview and 71.19 USD at IPD exit interview. For simple malaria, drug cost was the highest. Laboratory tests had the highest unit cost of severe malaria followed by consumables and personnel (MD) cost. Simple malaria cost without co-morbidity was 1.57 USD, with one co-morbidity 2.52 USD and with 2-3 co-morbidities 3.96 USD. Severe malaria cost without morbidity was 19.12 USD, with one co-morbidity 23.60 USD and with 2-3 co-morbidities 25.23 USD. In conclusion. malaria cost for health system is higher with co-morbidities. The introduction of prevention measures could reduce the cost of the treatment of malaria. In addition, the better implementation and monitoring of abolition of user fees policies could reduce the morbidity of malaria.

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

Smaila Ouedraogo1, Ghislain Kobo Koura2, Florence Bodeau-Livinec2, Manfred Mario Accrombessi1, Achille Massougbodji1, Michel Cot2

1Faculty of Medicine in Cotonou, Cotonou, Benin, 2French Institute of Research for Development (IRD), Paris, France

Although widely implemented, the effectiveness of anaemia preventive measures (intermittent preventive treatment in pregnancy (IPTp), anti-helminthic and haemactics) 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 at ANV1 and ANV2 under supervision. A treatment dose of albendazole and haemactics 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,
CRP concentrations were also measured. The effectiveness of preventive measures on the risk of anaemia and Hb concentrations was assessed at ANV2 and delivery by comparing the risk factors between ANV1 and after interventions (ANV2 and delivery). Multivariate linear and logistic regressions were used as appropriate. 63.8% of the women were anaemic at ANV1, 64.7% at ANV2 and 40.6% at delivery. The prevalence of malaria decreased from 15.1% at ANV1 to 4.0% at ANV2, and increased again at delivery to 9.6%, malaria infection being associated with a lower mean Hb at ANV1 and delivery. Helminth prevalence decreased from 11.1% at ANV1, to 7.2% at ANV2 and 2.4% at delivery. Iron deficiency stayed high throughout pregnancy (33.3% at ANV1, 36.3% at ANV2 and 30.7% at delivery). IPTp and anti-helminthic treatments were efficacious to clear parasitic infections and to improve haematologic status, whereas the effectiveness of daily iron and folate supplements to correct iron and folate deficiencies and to decrease anaemia was less marked.

**A PILOT SCHOOL SURVEY TO ESTIMATE THE MALARIA BURDEN IN A SETTING WITH DECLINING ENDEMICITY**

Ebako N. Takem1, Joseph Okebe1, Serign Ceessay1, Musa Jawara1, Eniyou Orio1, Muna Affara1, Davis Nwakanma1, Margaret Pinder1, John Townsend1, Alfred Ngwa1, Makie Taal2, Kalifa Saidy2, Momodou Sowe2, Amicoleh Mbaye3, Sulayman Cham3, Wandifa Fatty3, Umberto D'Alessandro1

1Medical Research Council Unit, Fajara, Gambia, 2National Public Health Laboratory, Kotu, Gambia, 3Ministry of Basic and Secondary Education, Banjul, Gambia

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 (axenial forms and gametocytes), 3) PCR detection of malaria infection, and 4) haemoglobin by Hemocue. Three thousand and 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 Plasmodium 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 DURING THE DRY SEASON**

Danielle M. Yugbare Belemsaga

IRSS, Ouagadougou, Burkina Faso

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 Nanoro’s demographic surveillance site in Burkina Faso. A random sampling of household was done. We included household 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. During household survey, the average cost of treating an episode of the disease including direct and indirect costs for household was 7.83USD. During exit interview this cost were estimated 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**

Téné Marceline Yaméogo1, Carole G. Kyele1, Sanata Bamba1, Léon G. Savadogo1, Adama Sanou2, Abdel Aziz Traoré3, Issaka Sombie1, Macaire S. Ouédraogo1, T. Robert Guiguemè1

1Institut Supérieur des Sciences de la Santé, Bobo - Dioulasso, Burkina Faso, 2District sanitaire de Dô, Bobo - Dioulasso, Burkina Faso, 3UFR - SDS, Ouagadougou, Burkina Faso

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 artesimine 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 to 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 to 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-medications 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.

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

Luis F. Chaves1, Akiko Satake1, M. Hashizume2, N. Minakawa2
1Hokkaido University, Sapporo, Japan, 2Nagasaki University, Nagasaki, Japan

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 the all 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.

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

Joaniter I. Nankabirwa1, Bonnie Wandera1, Sarah G. Staedke2, Moses Kamya1, Simon Brooker2
1Makerere University Kampala, Kampala, Uganda, 2London School of Hygiene and Tropical Medicine, London, United Kingdom

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 helminth 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, the majority of infections due to Plasmodium falciparum spp. infection was 30.1%, with 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.

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

Julius Atashili1, Gervais G. Tchinda, Henri-Lucien F. Kamga, Anna L. Njunda, Eric A. Achidi, Peter M. Ndumbe
1University of Buea, Cameroon, Buea, Cameroon

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.

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MALARIA GAMETOCYTE PREVALENCE IN NORTHERN KWAZULU-NATAL, SOUTH AFRICA

Jaishree Raman1, Eric Raswiswi2, Rajendra Maharaj1
1South African Medical Research Council, Durban, South Africa, 2KwaZulu-Natal Provincial Malaria Control Programme, Jozini, South Africa

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 heamolysis 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**

**Kimberly E. Mace**, Abdou Salam Gueye, M. Esther Tassiba, Michael F. Lynch, Alexander K. Rowe

1Centers for Disease Control and Prevention, Atlanta, GA, United States, 2Population Services International, Cotonou, Benin

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 abstraction form as an intervention to improve documentation and quality of care.

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**GENETIC DIVERSITY OF PLASMODIUM FALCIPARUM IN THE THREE MAJOR ECOCOLOGICAL ZONES OF GHANA**

**Anita Ghansah**

Noguchi Memorial Institute for Medical Research, Legon, Ghana

Parasite genetic diversity information is useful for malaria control activities such as drug and vaccine failure rates. Parastise 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 Savannah), Begoro and Bekwai (forest zone) and Cape Coast (Coastal Savannah). 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, 2.062: 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**

**Verner N. Orish**, Onyekachi S. Onyeabor, Nnaemeka C. Iriemenam

1Efia Nkwanta Regional Hospital Sekondi, Western Region, Sekondi, Ghana, 2The Satcher Health Leadership Institute, Department of Community Health and Preventive Medicine, Morehouse School of Medicine, Atlanta, GA, United States, 3Department of Medical Microbiology and Parasitology, College of Medicine of the University of Lagos, Ido-araba, Lagos, Nigeria

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, 1.03-2.65, P = 0.039). In addition, adolescent pregnant girls were more likely to have malaria and anaemia compared to their adult 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.
INTERMITTENT PREVENTIVE TREATMENT OF MALARIA IN PREGNANCY (IPTP): EMPHASIS ON ADEQUATE DOSAGE AND TRIMESTER UPTAKE

Verner N. Orish1, Onyekachi S. Onyeabor2, Nnaemeka C. Iriemenam3

1Effia Nkwanta Regional Hospital Sekondi, Western Region, Sekondi, Ghana, 2The Satcher Health Leadership Institute, Department of Community Health and Preventive Medicine, Morehouse School of Medicine, Atlanta, GA, United States, 3Department of Medical Microbiology and Parasitology, College of Medicine of the University of Lagos, Idu-araba, Lagos, Nigeria

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 MALARIA ENDEMICITY MAP FOR ETHIOPIA USING SEROLOGICAL INDICATORS OF PRIOR EXPOSURE TO PLASMODIUM FALCIPARUM AND P. VIVAX

Ruth Ashton1, Takele Kefyalew2, Alison Rand3, Ashenafi Assefa4, Addis Mekasha5, Gezahegn Tesfaye6, Rachel Pullan3, Richard Reithinger6, Simon Broker7, Chris Drakeley1

1Malaria Consortium, Kampaala, Uganda, 2Malaria Consortium, Addis Ababa, Ethiopia, 3Southern Nations, Nationalities and Peoples Regional State Health Bureau, Hawassa, Ethiopia, 4Malaria Consortium, Kampaala, Uganda, 5United States Agency for International Development, Ethiopia, 6London School of Hygiene and Tropical Medicine, London, United Kingdom

Ethiopia has a diverse ecology and geography that results in spatial and temporal variation in malaria transmission. Using evidence-based strategies to allocate the most appropriate interventions to different populations and transmission settings is crucial to sustaining reductions in malaria burden in Ethiopia, minimising epidemic risk, and eventually attaining elimination. Defining endemicity based on the detection of infection through light microscopy or antigen-detecting rapid diagnostic tests to define endemicity has limitations, due to highly seasonal transmission in many areas and presence of low density infections. Detection of Plasmodium falciparum and P. vivax antibodies was used to examine previous exposure to infection, a proxy to assess local transmission over a period of years. 197 cross-sectional school-based surveys among children aged five to 18 years were conducted in Oromia Regional State during the main transmission season. Key indicators were detection of parasites by light microscopy, and enzyme-linked immunosorbent assay (ELISA) to detect IgG against four antigens: P. falciparum glutamate rich protein (PfGLURP), P. falciparum merezoite surface protein (PfMSP), P. vivax merezoite surface protein (PvMSP) and P. vivax apical membrane antigen (PvAMA).

DEVELOPING A SCHOOL-BASED MALARIA EPIDEMIC DETECTION SYSTEM IN ETHIOPIA

Ruth A. Ashton1, Takele Kefyalew2, Tamiru Mesele3, Esey Batisso4, Gezahegn Tesfaye6, Richard Reithinger5, Simon Broker6

1Malaria Consortium, Kampaala, Uganda, 2Malaria Consortium, Addis Ababa, Ethiopia, 3Southern Nations, Nationalities and Peoples Regional State Health Bureau, Hawassa, Ethiopia, 4Malaria Consortium, Kampaala, Uganda, 5United States Agency for International Development, Ethiopia, 6London School of Hygiene and Tropical Medicine, London, United Kingdom

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

Barbara A. Willey1, Karim Anaya-Izquierdo1, Joanna Armstrong Schellenberg1, Werner Maokola2, Mwajuma Chomba2, Yuna Hamisi3, Mwifadhi Mirsho2, Kizito Shirima2, Fatuma Manzi2, Mary Masanja2, Pedro Alonso3, Hassan Mshinda2, Marcel Tanner2, Ian Douglas1, David Schellenberg1

1 London School of Hygiene and Tropical Medicine, London, United Kingdom, 2Ifakara Health Institute, Ifakara, United Republic of Tanzania, 3Hospital Clinic I Provincial, Barcelona, Spain, 4Swiss Tropical and Public Health Institute, Basel, Switzerland

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 IPT-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 IPT-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

Tuan M. Tran1, Silvia Portugal1, Shanlin Li1, Didier Doumtabe2, Safiatiou D瘤ombo2, Seydou Dia3, Bathily Aboudramane2, Jules Anye4, Abdramane Traore5, Chiung-Yu Huang2, Seidina Diakate2, Rick M. Fairhurst6, Jesus Valenzuela7, Kassoum Kayentoa8, Aissata Ongobo9, Ogobara K. Dumbo9, Boubacar Traore2, Peter D. Crompton1

1Laboratory of Immunogenetics, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States, 2Malaria Research and Training Center, Faculty of Medicine and Dentistry, University of Bamako, Bamako, Mali, 3Biostatistics Research Branch, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States, 4Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States

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?

Michael Coleman1, Mohammed H. Al-Zahraani2, Marlice Coleman1, Janet Hemingway1, Abdiasis Omar1, Adel A. Alsheikh2, Raafat F. Alhakeem2, Ziad A. Memish2

1Liverpool School of Tropical Medicine, Liverpool, United Kingdom, 2Ministry of Health, Riyadh, Saudi Arabia

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
recommends a country should achieve before considering elimination. On this basis alone, the goal of malaria elimination is considered realistic. Moreover, transmission is now limited to the southern regions of Asser and Jazan, bordering the Republic of Yemen, where Plasmodium falciparum is the main cause of malaria and Anopheles arabiensis is the main vector. However, moving from a control to an elimination programme requires a considerable effort to scale up monitoring and evaluation efforts. KSA is adopting state of the art tools to support MSE and decision-making, developed by the IVCC with financial support from the Bill and Melinda Gates Foundation. Using annual data records, we show how trends in malaria in KSA have changed over time due to the impact of vector control as well as climate. Case data for malaria areas are utilised to generate focal risk maps of disease and to determine outbreak alert thresholds. The prospects for malaria elimination in KSA and the relevance of the model being applied will be discussed objectively in relation to other countries pursuing or considering malaria elimination, both in the region and globally.

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

Jaymin C. Patel1, Md. Taqueer Alam1, Patricia Juliao2, Kim A. Lindblade1, Norma Padilla2, Demetrio Gonzalez2, Janey P. Messina3, Michael Esmh4, Steve M. Taylor5, Amanda C. Poe1, Venkatachalaram Udhayakumar1, Antoinette K. Tshefu6, Steven R. Meshnick6

1Malaria Branch, Division of Parasitic Diseases and Malaria, Center for Global Health, Centers for Disease Control and Prevention, Atlanta, GA, United States, 2Laboratorio de Microbiologia, Centro de Estudios en Salud, Universidad del Valle de Guatemala, Guatemala City, Guatemala, 3Military Medical Center of Guatemala, Guatemala City, Guatemala, 4Department of Geography, University of North Carolina at Chapel Hill, Chapel Hill, NC, United States, 5Department of Epidemiology, Gillings School of Global Public Health, University of North Carolina at Chapel Hill, Chapel Hill, NC, United States, 6Ecole de Sante Publique, Faculte de Medecine, University of Kinshasa, Kinshasa, Democratic Republic of the Congo

Malaria is dispersed from place to place by movements of either mosquitoes or humans. A better understanding of the patterns of dispersion will aid in preventing its geographical spread. Using landscape genetics, which combines population genetics and spatial epidemiology, we studied the population and spatial structure of Plasmodium falciparum in the Democratic Republic of the Congo (DRC). The population structure of P. falciparum parasites was characterized among 117 isolates from the DRC, 40 isolates from Guatemala and 12 from Guatemalan United Nations peacekeeping soldiers who visited DRC in 2010 and were found to have P. falciparum parasitemia by either slide microscopy or polymerase chain reaction (PCR) analysis. Seven neutral microsatellite markers were characterized and genetic relatedness was calculated using Nei’s genetic distance (GD) and Slatkin’s RST. Among the seven DRC sites Nei’s GD ranged from 0.26 to 0.92 and RST varied from -0.06 to 0.13. While genetic relatedness largely varied with distance, some geographic barriers to dispersion were noted. Parasites from the Guatemalan soldiers were closely related to parasites found in the DRC (Nei’s GD=0.28) but very different from those found in Guatemala (Nei’s GD=2.02). Similar results were found using Slatkin’s RST (0.07 to 0.20). Clustering of isolates from soldiers and DRC was confirmed by principle coordinate analysis. The results from the study support the epidemiologic findings that the soldiers acquired malaria while they were in northeastern DRC. Molecular tools and population genetics analysis can allow us the opportunity to study the spread of malaria within and between countries. By integrating genetic and spatial information, better models of disease spread can be developed.

EFFECTIVENESS OF MALARIA CONTROL INTERVENTIONS AMONG PREGNANT WOMEN AND CHILDREN UNDER FIVE YEARS IN A RURAL AREA OF BURKINA FASO: A RESULT FROM NOUNA HEALTH AND DEMOGRAPHIC SURVEILLANCE SITE (NHDSST SURVEY)

Maurice YE

Nouna Health Research Center, Nouna, Burkina Faso

Malaria remains a major cause of global morbidity and mortality, with most of the burden being in sub-Saharan Africa though Insecticide-Treated mosquito nets (ITN) have been proved to be one of the most effective intervention to prevent malaria. The 2000 Abuja summit put emphasis on promoting effective prevention methods and management of case for vulnerable groups such as pregnant women and children under five. In this study we aimed to assess the ownership and use of ITNs and access of children under five to artemisinin based combination therapy (ACT). The study took place in a rural area of north-western Burkina Faso, which was characterized as holoendemic. A cross sectional surveys were undertaken in a two samples of population derived from the Nouna Health and Demographic Surveillance Site in 2010. The first was constituted by a sample of 2850 households and the second with a sample of 409 children. Our results were compared to the Abuja indicators agreed upon by the Head of Africa States as target goals to achieve. Overall 89% of households revealed a possession of at least two bed nets among which 47.96% were insecticide treated bed nets. 24.5% of children have slept under ITNs the last night whereas it was 28.4% for pregnant women. 49.7% of children have presented a fever and 32.5% were tested positive for malaria among which 13.7% have been treated adequately with ACT. Malaria was the first cause of death (32.7%). Overall reduction of 22.8% of falciparum malaria prevalence was observed compared to 2006 survey data. In conclusion, many effort remains to achieve universal coverage with ITNs and clinical malaria cases management among vulnerable group so that to reach the Abuja targets and MDGs goals.

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

Ashley Garley1, Elizabeth Patton2, Svetlana Negroustoueva3, Yozoume Ye1

1ICF International, Calverton, MD, United States, 2United States Agency for International Development, Washington, DC, United States

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

Rakiswendé S. Yerbanga

Institut de recherche en Sciences de la santé, Direction Régionale de l’Ouest (IRSS-DRO), Bobo Dioulasso, Burkina Faso

Targeting gametocytes, gametes and/or ookinetes; 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 ookinetes stages of Plasmodium falciparum field isolates in an ex vivo assay; Anopheles gambiae females were membrane fed on gametocytaemic 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 NeemAzal®, 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

Dohyeong Kim1, Kristen Fedak2, Randall Kramer3

1North Carolina Central University, Durham, NC, United States, 2ICF International, Durham, NC, United States, 3Duke University, Durham, NC, United States

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

Nancy Stephen Matowo, Fredros Oketch Okumu

Ifakara Health Institute, Morogoro, United Republic of Tanzania

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

Grace Mugumbate, Stephen Fienberg, Kelly Chibale, Graham E. Jackson

University of Cape Town, Cape Town, South Africa

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 helmintic diseases. Also, the mosquito which transmits the malaria parasite has been 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 is key for pharmacological malaria control 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 =
-10.7 kcal/mol for the receptor. The AKH interacted with Tyr110, Thr129, Gin209, Lys307 and Tyr285 in the receptor binding site. From the ZINC database, about 120 compounds, containing a variety of scaffolds, showed higher binding affinities (highest affinity: ΔGb = -12.5 kcal/mol) for the receptor than AKH-1. These results provide novel scaffolds that could be used for potential treatment of neglected diseases.

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FINDINGS FROM A RAPID QUALITATIVE ASSESSMENT OF ACCESS TO MALARIA PREVENTION AND TREATMENT RESOURCES AMONG BURMESE MIGRANTS IN TAK PROVINCE, THAILAND

Denise Roth Allen1, Eugenie Poirot1, Piayporn Wangroongsarb2, Muhammed Shafique3, Jimee Hwang4, David Sintasath4, Sylwia Meek1, S. Patrick Kachur1
1Centers for Disease Control and Prevention, Atlanta, GA, United States, 2Bureau of Vector Borne Diseases, Ministry of Public Health, Bangkok, Thailand, 3Malaria Consortium Asia, Bangkok, Thailand, 4Malaria Consortium, London, United Kingdom

Recent evidence of the declining efficacy of Thailand’s first line artemisinin-based combination therapy (ACT), mefloquine-artesunate, 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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STOCHASTIC MODELS FOR THE AUTODISSEMINATION OF INSECTICIDES BY MOSQUITOES

Samson S. Kiware
Ifakara Health Institute, Dar es salaam, United Republic of Tanzania

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 (PFP) 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 PFP 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, PFP 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-LARVICIDAL EFFECT AGAINST MAJOR MALARIA VECTORS IN UGANDA

Matthew Lukenge1, Josephine Birungi1, Jonathan Kayondo2, Charles Masembe2, Louis Mukwaya2
1Makerere University Kampala, Kampala, Uganda, 2Uganda Virus Research Institute, Entebbe, Uganda

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

Fadima I. Yaya Bocoum1, Danielle Belemsaga1, Seni Kouanda1, Halidou Tinto1, Alex Adjagba1
1Institut de recherche en science de la santé, Ouagadougou, Burkina Faso
2PATH, Ferney voltaire, France

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 2450FCFA on bed net, but only 6% of bed net were bought. Households spent an average of 910 FCFA on coils and 1768 FCFA 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.

ASSESSING THE EFFICACY OF TWO TYPES OF LONG-LASTING INSECTICIDAL NETS (LLINS) TESTED IN NAMPULA PROVINCE, MOZAMBIQUE

Ana Paula Abilio1, Julieth Morgan2, Samira Sibindy1, Jacinta Luciano1, Ayubo Kampango1, Júlio Matusse1, Elias Machoe1, Maria Pondja1, Dulcısaria Marenjo1, Adeline Chan3
1National Institute of Health-Ministry of Health, Maputo, Mozambique
2President’s Malaria Initiative, Centers for Disease Control and Prevention, Maputo, Mozambique
3Malaria Control Program- Ministry of Health, Maputo, Mozambique
4Malaria Branch, Centers for Disease Control and Prevention, Atlanta, Georgia

Long-lasting insecticidal nets (LLINs) are an important tool to prevent malaria in sub-Saharan Africa by reducing human-vector contact. In line with the new WHOPES LLIN evaluation guidelines we prospectively evaluated LLIN efficacy, including physical durability and insecticidal efficacy, on LLINs distributed during a campaign in October 2008 in Nampula Province, Mozambique. We present here the insecticidal efficacy of Olyset® and PermaNet® LLINs from the first year of follow-up. We tagged 2000 Olyset® and 4000 PermaNet® LLINs and randomly distributed them during the 2008 campaign. The tagged LLINs were then located using GPS during a house-to-house survey one month after the distribution campaign. A random sample of the households (HHS) found to have a tagged LLIN was selected for a three year follow-up evaluation. At one, two and three years after the distribution campaign, the selected HHS were surveyed and tagged LLINs were collected and transported to the Instituto Nacional de Saúde (INS) in Maputo for further evaluation. We assessed unused LLINs as the baseline. We took two day-old non-blood-fed female mosquitoes from an insectary susceptible colony of An. arabiensis to conduct cone bioassay tests on the baseline LLINs and LLINs collected during the first year of follow-up for both brands. Untreated polyester netting (25 cm x 25 cm) was used as negative control. A total of 49 Olyset® and 83 PermaNet® LLINs were collected during the first year of follow up. The knockdown effect was recorded after three minutes of exposure, and mortality was recorded after 24 hours post-exposure. The mortality on the baseline LLINs exposed to An. arabiensis was 100% for both brands. We found 96.9% and 99.7% An. arabiensis mortality after exposure to Olyset® and PermaNet® respectively on the LLIN collected during the first year of follow-up. Our findings show that LLINs retain their insecticidal efficacy after one year in rural Mozambique, although this starts dropping: Olyset® more rapidly compared with PermaNet® but not statistically significantly (P= 0.097). Further assessment after two and three years is on-going and results available later in 2012. These will give understanding of the long-term LLIN effectiveness in this setting.
EFFECTS OF INSECTICIDE RESISTANCE AND CREPUSCULAR BITING BEHAVIOR ON THE COST EFFECTIVENESS OF A MASS, LONG-LASTING, INSECTICIDAL NET DISTRIBUTION: A MODELING STUDY

Olivier J. Briet, Nakul Chitnis
Swiss Tropical and Public Health Institute, Basel, Switzerland

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 range of 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

Rungrawee Tipmontree1, Nardlada Khantiku1, Wichai Satimai1, Muhammad Shafique3
1Bureau of Vector Borne Diseases, Nonthaburi, Thailand, 2Office of Disease Prevention and Control No.1, Chiangmai, Thailand, 3Malaria Consortium Asia, Bangkok, Thailand

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 artesiminin tolerant 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

Suchart Patipong, Siriporn Yongchaitrakul, Wichai Satimai
Bureau of Vector Borne Disease, Nonthaburi, Thailand

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 use 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 ANTIPOLVIRUS AGENTS FROM ZEPHYRANTHES CANDIDA LINDL AND CASSIA SIAMEA LAM

Omonike O. Ogbole1, Johnson A. Adeniji2, Ransay S. Kamdem2, Edith O. Ajaiyeoba1, Muhammad I. Choudhary1, Festus D. Adu1
1Department of Pharmacognosy, Faculty of Pharmacy, University of Ibadan, Ibadan, Nigeria, 2WHO Polio Laboratory, Department of Virology, College of Medicine, University of Ibadan, Ibadan, Nigeria, 3Department of Organic Chemistry, Higher Teachers’ Training College, University of Yaounde I, Yaounde, Cameroon, 4HEI Research Institute of Chemistry, International Center for Chemistry and Biological Sciences, University of Karachi, Karachi, Pakistan

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 poliomyelitis. 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...
MITT colorimetric assay. Linear regression was used to determine IC_{50} and CC_{50} 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 IC_{50} of 1.85×10^{-3}µg/mL and 1.21×10^{-1}µg/mL respectively. Activities were retained in the chloroform fractions of Z. candida and C. siamea with IC_{50} of 1.2×10^{-3}µg/mL and 2.3×10^{-1}µg/mL respectively. Hexane fraction of C. siamea was also comparatively active with IC_{50} of 5.1×10^{-1}µg/mL. Five compounds were isolated from Z. candida, namely; 7-hydroxy-3',4'-methylenedioxyflavan, lycorine, triphlaeridine, β-sitosterol glucoside and stigmasterol. Lupeol, lupenone, betulinic acid, emodin, chrysophanol, psycion and β-sitosterol glucoside were obtained from C. siamea. Lupeol was the most active compound from C. siamea with IC_{50} value of 1.4×10^{-2}µg/mL. Lycorine was the most active compound from Z. candida with IC_{50} value of 5.8×10^{-2}µg/mL. Two compounds; 7-hydroxy-3', 4'-methylenedioxyflavan and triphlaeridine are reported from Z. candida and their anti-poliovirus properties are established. This study proposed 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

Ungke Antonjaya

Eijkman-Oxford Clinical Research Unit, Jakarta, Indonesia

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 scare. 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

Repon C. Paul1, Mahmudur Rahman2, Eric Mast3, Eric Wiesen3, Trudy Murphy3, Minal Patel4, Mizanur Rahman4, Jayantha Liyanage4, Nihal Abeyesinge5, Stephen Luby1

1International Centre for Diarrheal Disease Research, Bangladesh, Dhaka, Bangladesh, 2Institute of Epidemiology, Disease Control and Research (IEDCR), Ministry of Health and Family Welfare, Bangladesh, Dhaka, Bangladesh, 3Centers for Disease Control and Prevention, Atlanta, GA, United States, 4World Health Organization, Dhaka, Bangladesh, 5World Health Organization, SEARO, Delhi, India

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 MALARIA TRANSMISSION DYNAMICS IN WESTERN KENYA

Sidney Ogolla1, Erwan Pirou1, Asito S. Amolo1, Peter O. Sumba1, Nancy Fiore2, Rosemary Rochford2

1KEMRI, Kisumu, Kenya, 2SUNY Upstate Medical University, Syracuse, NY, United States

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: Kisumu District where malaria transmission is holoendemic and Nandi 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.

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**A SURVEY OF ARBOVIRUSES CIRCULATING IN KENYA 2007-2010**

Hellen S. Koka¹, Caroline Ochieng¹, Albina Makio¹, James Mutisya¹, Lillian Musila¹, Edith Chepkorir², Joel Lutomiah³, Rosemary Sang⁴

¹U.S. Army Medical Research Unit - Kenya, Nairobi, Kenya, ²International Centre for Insect Physiology and Ecology, Nairobi, Kenya, ³Kenya Medical Research Institute, Nairobi, Kenya

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, Turkana, Tana Delta, 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 isolated. Four of the Alphaviruses and three of the Bunyaviruses were identified to specie 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 upto 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), Anopheles funestus (3) and Aedes tricholabis (1). Five of these mosquitoes were collected from Garissa, two from Magadi and one from Tana Delta. Alphaviruses were isolated from pools of Cx. univittatus (3), Culex spp (1), Ae. lirudis pools (2), Ae. tricholabis (2), Aedes mcintoshi (2) and Aedes spp (1). Six of these isolates were collected Garissa, two from Naivasha, one from Budalangi and three from Tana Delta. All Flavivirus isolates were isolated from Cx.univittatus mosquitoes, five of which were from Garissa and one from Turkana. Eight Bunyaviruses (4 Bunyamwera, 1 Pongola), 11 Alphaviruses (4 Ndomu, 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, map out risk zones and direct correct diagnosis of febrile illnesses in the human population.

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**SEROLOGICAL EVIDENCE OF NIPAH LIKE VIRAL INFECTION IN PIGS IN BANGLADESH**

M. S. Khan¹, Gary Crameri², Emily S. Gurley³, M. Jahangir Hossain¹, Lin-Fa Wang⁴, Stephen P. Luby⁵

¹International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh, ²CSIRO Livestock Industries, Australian Animal Health Laboratory, Geelong, Australia (AAHL), Geelong, Australia, ³Centers for Disease Control and Prevention, Atlanta, GA, United States

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 (Sas 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.

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**SEROLOGICAL AND MOLECULAR CHARACTERIZATION OF SUSPECTED CASES OF HEMORRHAGIC FEVER AND HEPATITIS VIRUSES IN NORTHERN GHANA**

Joseph Humphrey Bonney¹, Mubarak Osei-Kwasi¹, Theophilus Adiku¹, Jacob Samsom Barnor¹, Meike Hass², Robert Amesiya³, Chris Kubio⁴, Stephan Oelschlaeger⁵, Beate Becker-Ziaja⁵, Lawson Ahadzie⁶, Stephan Guenther⁷

¹Noguchi Memorial Institute for Medical Research, Accra, Ghana, ²University of Ghana Medical School, Microbiology Department, Accra, Ghana, ³Bernard Nocht Institute for Tropical Medicine, Hamburg, Germany, ⁴St. Theresa’s Hospital, Nandom, Ghana, ⁵West Gonja Hospital, Damongo, Ghana, ⁶Disease Surveillance Division, Ghana Health Service, Accra, Ghana

Haemorrhagic Fever (HF) viruses are prevalent in West Africa and have led to outbreaks with considerable morbidity and mortality. Evidence abounds for HF agents 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 and VH in Northern Ghana. Base 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 ≥ 2:20) and 8 cases of anti-Dengue type-2 IgG (titer ≥ 1:80). Two cases exhibiting specific IgG (titers 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 VHF are widespread, illustrating the need for differential diagnosis to be implemented.

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NON-POLIO HUMAN ENTEROVIRUSES ASSOCIATED WITH RESPIRATORY INFECTIONS IN PERU (2005-2010)

Jose L. Huaman¹, Gladys Carrion¹, Julia S. Ampuero¹, Victor Ocaña¹, Maria E. Gamero¹, Jorge Gomez¹, Eric S. Halsey¹
¹U.S. Naval Medical Research Unit-6, Lima, Peru, ²Centro de Salud Pachitea, Dirección Regional de Salud de Piura, Ministerio de Salud, Piura, Peru, ³Dirección General de Epidemiología, Ministerio de Salud, Lima, Peru

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 isolates was carried out by reverse transcriptase-P, 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 HEV-positive subjects were 9.2 and 12.5 years, respectively, much lower than the population sampled (17.6 and 17.5 years, respectively). A total of 15 different serotypes were identified, with the most common being coxsackievirus B1, coxsackievirus B2, coxsackievirus A16, and enterovirus 71. This study is the first to report HEV isolation from respiratory specimens in Peru. Compared with other countries in South America, our HEV prevalence in ILI was similar to those from Ecuador and Brazil, and our HEV-group breakdown was similar to that found in Brazil.
uncomplicated mild nasal discharge 1 to 3 days post vaccination confirmed as bacterial infections; all recovered. One cow was treated for placenta 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 offsprings. 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 (MELANOCONIUM) PORTESI MOSQUITOES FROM IQUITOS, PERU

Julio Evangelista1, Jun Hang2, Robert A. Kuschner2, Helvio Astete3, Cristiam Carey4, Tadeusz J. Kochel5, Amy C. Morrison6, Eric S. Halsey1, Brett M. Forshey1

1U.S. Naval Medical Research Unit - 6, Lima, Peru, 2Viral Diseases Branch, Walter Reed Army Institute of Research, Silver Spring, MD, United States, 3U.S. Naval Medical Research Unit - 6, Iquitos, Peru, 4Health Directorate, Loreto - Iquitos, Peru, 5Naval Medical Research Center, Silver Spring, MD, United States, 6U.S. Naval Medical Research Unit – 6, Iquitos, Peru, University of California, Davis, Davis, California, United States

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 (Mel) 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, Iataqui, Murutucu, Marituba, Oriboca, and Caraparu viruses, were unable to neutralize the virus isolate in microneutralization assays. Full genome sequencing 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

Kahsay Huruy H. Ghezehegn1, Afework Kassu K. Gizaw2, Andargachew Mulu M. Mehane2, Yemataw Wondie Wondie2

1Institute of Virology, Leipzig, Germany, 2University of Gondar, Ethiopia, University of Gondar, Gondar, Ethiopia

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 tuberculoses (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

Sandra S. Morales Ruiz, Daniel D. Clark, Elsa E. González, Eduardo E. Gotuzzo, Michael M. Talledo

Instituto de Medicina Tropical Alexander von Humboldt - Universidad Peruana Cayetano Heredia, Lima, Peru

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 KIR3DL1 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 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 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.
SEROPREVALENCE OF SELECTED ARBOVIRUSES IN IJARA AND MARIGAT DISTRICTS, KENYA

Victor O. Ofula1, Joseph Oundo2, Zephaniah Irura3, Edith Chepkorir4, Caroline Tigo4, Juliette Ongus3, Randall Schoepf3, Cindy Ross4, Eyako K. Wrupapa5, Hesbon Sonde6, Rosemary Sang8, 1United States Army Medical Research Unit-Kenya, Nairobi, Kenya, 2Centers for Disease Control and Prevention-Kenya, Nairobi, Kenya, 3Ministry of Public Health and Sanitation, Nairobi, Kenya, 4International Center for Insect Physiology and Ecology, Nairobi, Kenya, 5Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya, 6U.S. Army Medical Research Institute for Infectious Diseases, Frederick, MD, United States, 7Ministry of Medical Services, Ijara, Kenya, 8Kenya Medical Research Institute, Nairobi, Kenya

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, Anophèles 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 5iuL 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 MAYAROVIRUS

Eric S. Halsey1, Carolina Guevara1, Crystyan Siles2, Stalin Vilcarromero2, Erik J. Jhonston3, Victor Fiestas4, Patricia Aguilar5, Julia S. Ampuero1

1U.S. Naval Medical Research Unit No. 6, Lima, Peru, 2U.S. Naval Medical Research Unit No. 6, Iquitos, Peru, 3Universidad Nacional de la Amazonia Peruana, Iquitos, Peru, 4Instituto Nacional de Salud, Lima, Peru, 5University of Texas Medical Branch, Galveston, TX, United States

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 Mayarovirus (MAYV), 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 MAYV 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-EPIDEMIC SEROPREVALENCE OF RIFT VALLEY FEVER VIRUS AMONG SOMALI VILLAGES IN NORTHEASTERN KENYA

A. Desiree LaBeaud1, Laura J. Sutherland2, Samuel Muiruri3, Saidi Dahir4, Zach Traylor5, Eric Muchini6, Amy G. Hise2, James W. Kazura7, Charles H. King2

1Children’s Hospital Oakland Research Institute, Oakland, CA, United States, 2Case Western Reserve University, Cleveland, OH, United States, 3Division of Vector-borne and Neglected Tropical Diseases, Ministry of Health, Nairobi, Kenya

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=242, 70 households), Korahindi (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 and 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), Korahindi (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% 7.76-26.86). 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

Grace E. Snow1, Benjamin Haaland2, Eng Eong Ooi2, Duane J. Gubler3

1Duke University, Durham, NC, United States, 2Duke-NUS Graduate Medical School, Singapore, Singapore

There is no good animal model for dengue, one of the most important emerging tropical infectious diseases. As a result, we have relied on...
human studies done by the US Army in the 1920s and 1940s for basic information on incubation period, cross protective immunity and early clinical presentation. One study was conducted by Albert Sabin, who infected 118 volunteers with DENV-1 and -2 isolated in Hawaii, New Guinea and India. Sabin presented a broad overview of these studies in his oft-cited 1952 paper, but the details were never published. His original laboratory records, which were bequeathed to one of us (DJB), provided the opportunity to re-visit these seminal studies. Of 118 primary infections (104 DENV-1 and 14 DENV-2), 20 subjects were reinfected with the heterologous serotype at intervals of 1.5 - 9 months to evaluate the duration of cross-protective immunity between serotypes. Median fever duration for primary DENV-2 infections was 2.2 days compared to 3.3 days for primary DENV-1 (p < 0.05), although maximum temperature and severity of illness were comparable. Of 7 secondary infections at 6-8 weeks, all but one had temperature <38°C, one with a maximum temperature of 38°C on day 8, was viremic. At 8-10 weeks post primary infection, 4 out of 5 (80%) subjects became ill with maximum temperature between 38.1 and 38.8°C. At 4-9 months post primary infection, 7 out of 8 (88%) subjects with secondary infection developed fever and 3 (38%) had a maximum temperature >39°C. There was a trend towards a shorter incubation period in secondary infections at 4-9 months (median 4.2 days) compared to primary infections (median 6.9 days), and illness was generally milder in secondary infections. The data suggest that the DENV serotype and/or strain may influence the duration of fever in primary infection, and the time elapsed from the primary infection influences the symptoms, duration and severity of fever and leukopenia in secondary infections. A detailed analysis of the Sabin experiments will be presented.

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**DENGUE VIRUS INFECTION IN THE SKIN: DENDRITIC CELLS AND THE ROLE OF MOSQUITO SALIVA**

Michael A. Schmid1, Karla N. Gonzalez2, Matthew T. Aliota3, Laura Kramer3, Eva Harris1

1Division of Infectious Diseases and Vaccineology, School of Public Health, University of California, Berkeley, Berkeley, CA, United States, 2Laboratorio Nacional de Virologia, Centro Nacional de Diagnostico y Referencia, Ministerio de Salud, Managua, Nicaragua, 3Wadsworth Center, New York State Department of Health, Slingerlands, NY, United States

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 when infected Aedes aegypti or Ae. albopictus mosquitoes bite humans, and may result in dengue, the most prevalent arthropod-borne human viral disease. Primary DENV infection confers life-long protection to the same serotype, while secondary infection with a distinct DENV serotype is a major risk factor for severe disease via serotype cross-reactive enhancing antibodies (ADE) or T cells. DCs, monocytes, and macrophages are targets of DENV in humans, yet little is known about initial DENV infection in the skin after the bite of an infected mosquito or about subsequent systemic viral spread. Here, we report a novel intradermal (i.d.) infection model with DENV2 strain D220 in the ears of C57BL/6 mice deficient in the IFN-α/β receptor (IFNAR−/−), under primary and ADE infection conditions. Ear skin is digested to separate epidermal from dermal sheets and to prepare single cell suspensions, which are stained with monoclonal antibodies (MAbs) against surface markers to distinguish cell populations and MAbs against intracellular DENV nonstructural protein 3 and envelope to identify DENV-infected cells by flow cytometry. 24 and 48 hours after infection, replicating DENV was detected in epidermal Langerhans cells, dermal CD11b+ DC and CD103+ DC subsets, and CD45− non-hematopoietic cells in both the epidermis and dermis. Inoculation of sub-neutralizing levels of DENV-specific MAbs resulted in increased morbidity and mortality of IFNAR−/− mice after i.d. DENV infection; we are now analyzing DENV-infected skin cell populations under ADE conditions. While probing for blood vessels, mosquitoes eject saliva, which contains pharmacologically active substances, into the skin. When mice were pre-sensitized to salivary gland extracts (SGE) from female Ae. aegypti mosquitoes by repeated i.d.

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**ANALYSIS OF EARLY DENGUE VIRAL INFECTION IN MICE AS MODULATED BY AEDES AEGYPTI PROBING**

Michael K. McCracken, Rebecca C. Christofferson, Christopher N. Moren

Louisiana State University, Baton Rouge, LA, United States

Mosquito saliva contains proteins with anti-inflammatory, anti-hemostatic, and immuno-modulatory capabilities. Arbovirus-infected mosquitoes expectorate saliva and virus immediately prior to blood feeding and this saliva has been shown to aid the establishment of arboviruses such as Sindbis and West Nile within the vertebrate host. Limited work exists on the functional relationship between Aedes aegypti salivary proteins and vertebrate infection with dengue virus. Most studies have focused on associations between disease severity and changes observed in the early days post exposure rather than interactions that could affect establishment of dengue virus within a vertebrate host. Here, we examine in vivo transcriptional changes of critical innate immune pathways three hours post exposure at inoculation sites of IRF3/7−/− (C57BL/6) mice. Mice received dengue serotype 2, strain 1232 intradermal needle-inoculation where field collected Ae. aegypti mosquitoes 1) had and 2) had not recently probod. At inoculation sites where mosquitoes had probod, we observed a generalized up-regulation of the transcripts for transcription factors, such as relA, and transcription factor associated proteins. We found substantial down-regulation of various cytokines including the interferon γ transcript and the transcript of a monocyte chemotactic molecule, IP-10. Additionally, we found an approximately 45-fold down-regulation of the transcript for toll-like receptor 7, an endosomal pattern recognition receptor for single-stranded RNA. The down-regulation of these transcripts early in the infection process could indicate important mechanisms by which mosquito salivary proteins serve to enhance the establishment of dengue viral infections in the vertebrate host.

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

Smita Jaiswal1, Marcia Woda1, Pamela Pazoles1, Leonard Shultz2, Dale Greiner1, Michael Brehm1, Anuja Mathew1

1University of Massachusetts Medical School, Worcester, MA, United States, 2Jackson Labs, Bar Harbor, ME, United States

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 IL2γt−/− 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 generated and 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.
and predicted protein function, determined whether intra-host variants and viral clade identity. Additionally, the abundance of viral variants within 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 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.

TRANSMISSION OF DENGUE TO MOSQUITOES DURING PERIODS OF LOW VIREMIA

Rebecca C. Christofferson, Christopher N. Mores
Louisiana State University, Baton Rouge, LA, United States

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 locally 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 refeed on naïve mice, and were then tested for virus infection and dissemination. Critically, at viremias as low as 1x10^1 and 1x10^2 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 PHYLOGENIC CLADE AND INTER-HOST DIVERSITY

Poornima Parameswasan1, Patrick Charlebois2, Yolanda Tellez3, Andrea Nuñez2, Elizaneth M. Ryan2, Christine M. Malboeuf2, Joshua Z. Levin2, Niall J. Lennon2, Angel Balmaseda4, Matthew R. Henn2, Eva Harris1
1Division of Infectious Diseases and Vaccinology, School of Public Health, University of California, Berkeley, Berkeley, CA, United States, 2Broad Institute of Massachusetts Institute of Technology and Harvard, Cambridge, MA, United States, 3Laboratory Nacional de Virologia, Centro Nacional de Diagnostico y Referencia, Ministerio de Salud, Managua, Nicaragua

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

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

Meta Michels1, Quirijn de Mast1, Uun Sumardi2, André J. van der Ven1, Bachti Alisjahbana2
1Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands, 2Faculty of Medicine, University of Padjadajaran, Hasan Sadikin Hospital, Bandung, Indonesia

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.
Potential Efficacy of Citicoline as Adjunct Therapy for Cerebral Malaria

Fatima El-Assaad1, Valery Combes1, Georges E. Grau1, Ronan Jambou2

1Vascular Immunology Unit, Faculty of Medicine, University of Sydney, Sydney, Australia, 2Institut Pasteur de Madagascar, Antananarivo, Madagascar

During cerebral malaria (CM), sequestration of parasitised erythrocytes, platelets and leukocytes 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 citicoline (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 (1mg/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 Glucose-6-Phosphate Dehydrogenase Deficient Human Erythrocytes

Narayan D. Chaurasia1, Rajnish Sahu1, Vijender Adelli1, N.P. Dhammika Nanayakkara1, Colin Ohrt2, Larry A. Walker1, Babu L. Tekwani1

1School of Pharmacy, University of Mississippi, University, MS, United States, 2Walter Reed Army Institute of Research, Silver Spring, MD, United States

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-HPO), 8-N hydroxy 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-HPO 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-HPO. 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 hemolytic response of aminophenols may be useful for designing PQ analogs with better therapeutic index.

In Vivo Glucose-6-Phosphate Dehydrogenase (G6PD)-Deficient Mouse Model To Predict Hemolytic Toxicity of Candidate 8-Aminoquinoline (8-AQ) Anti-Malarial Drugs

Prabhati Ray, Peng Zhang, Jack Amnuaysirikul, Max Grogli, Colin Ohrt, Mike O’Neil, Mark Hickman Walter Reed Army Institute of Research, Silver Spring, MD, United States

Many of 8-aminoquinoline (8-AQ) compounds investigated to treat relapsing malaria are hemolytic in subjects with glucose-6-phosphate dehydrogenase deficiency (G6PD). 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 G6PD-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/d) 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 1/2 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 induce 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)

Ronnatrai Rueangweerayut1, Germana Barcone2, Andrew P. Beelen3, Nick Carter4, Stephan Duparc5, Justin A. Green4, Emma J. Harrell6, Jörg-Peter Kleim4, Ann K. Miller6, Jörg Möhrle5, Ammar Qureshi2, Nushara Yubon4, Luca Luzzatto7, Francois H. Nosten2, Supornchai Kongpatakanukul3

1Mae Soe General Hospital, Mae Soe, Thailand, 2Shkolko Malaria Research Unit, Mae Soe, Thailand, 3formerly GlaxoSmithKline; currently with Myrexis Inc., Salt Lake City, UT, United States, 4GlaxoSmithKline, Uxbridge, United Kingdom, 5Medicines for Malaria Venture, Geneva, Switzerland, 6GlaxoSmithKline, King of Prussia, PA, United States, 7GlaxoSmithKline, Bangkok, Thailand, 8Istituto Toscano Tumori (ITT), Firenze, Italy, 9Department of Pharmacology, Faculty of Medicine, Siraj Hospital, Mahidol University, Bangkok, Thailand

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 malarious 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, as reported previously. G6PD normal 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 decline of ≥2.5 g/dL of 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

James S. McCarthy1, Silvana Sekulski2, Katharine Trenholme1, Suzanne Elliott2, Paul Griffin1, Jane Gaydon1, Louise Marquart1, Peter O’Rourke1, Andrew Honeycombe3, Joerg Moehrle1, Theo Sloots1, Stephan DuParc1, Mark Baker4

1Queensland Institute of Medical Research, University of Queensland, Herston, Australia, 2Queensland Institute of Medical Research, Herston, Australia, 3QPID, University of Queensland, Herston, Australia, 4Medicines for Malaria, Geneva, Switzerland

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 parasitemia 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 arteether-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

Mark B. Baker, Fiona Macintyre, Joerg Moehrle
Medicines for Malaria Venture, Geneva, Switzerland

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 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 SCHIZOGONY

Bareza A. Rasoul¹, Marcus Skaflen², Steven P. Maher³, Anatoli Naumov⁴, Chris Lantz⁵, John H. Adams⁶, Bharath Balu¹
¹SRI International, Harrisonburg, VA, United States, ²James Madison University, Harrisonburg, VA, United States, ³University of South Florida, Tampa, FL, United States

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

Judith Straimer¹, Marcus C. Lee¹, Bryan Zeitler², Andrew H. Lee³, Jocelynn R. Pearl², Lei Zhang², Edward J. Rebar², April Williams³, Manuel Llinás⁴, Philip D. Gregory⁵, Fyodor D. Urnov⁶, David A. Fidock¹
¹Columbia University, New York, NY, United States, ²Sangamo BioSciences, Inc., Richmond, CA, United States, ³Princeton University, Princeton, NJ, United States

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.
TOWARD RNA-BASED APPROACHES FOR STUDYING MALARIA PARASITE BIOLOGY

Jeffrey C. Wagner, Jacquin C. Niles

Massachusetts Institute of Technology, Cambridge, MA, United States

Malaria caused an estimated 655,000 deaths in 2010, mostly in children under the age of five years. Plasmodium falciparum is responsible for the majority of malaria morbidity and mortality. Research efforts aimed at gaining biological insights into this parasite are hampered by a lack of gene regulatory technologies that permit elucidation of genetic pathways in situ. Inducible expression is an important strategy for establishing how genes function, and for determining their interactions in biological pathways. Pathways that are both essential and unique to the parasite can then be targeted in drug development efforts. Based on this need, we have reconstituted an inducible T7 RNA polymerase (T7 RNAP) transcriptional system in P. falciparum. We have demonstrated T7 RNAP-directed, IPTG-inducible target transcript production in situ at levels similar to or greater than those generated by characterized native polymerase II promoters. In the repressed state, target transcript levels are reduced by more than ten-fold. With this new tool, we envision using RNA-based approaches, such as conditionally expressing aptamers that can disrupt protein function, to complement the characterization and validation of potential parasite drug targets.

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

Sheila Castro1, Isidro Gonzales1, Silvia Rodriguez1, Martha Flores1, Sarah Gabriél1, Yesenia Castillo1, Pierre Dorny2, Hector H. Garcia3

1Instituto Nacional de Ciencias Neurologicas, Lima, Peru, 2Prince Leopold Institute for Tropical Medicine, Antwerp, Belgium, 3Universidad Peruana Cayetano Heredia, Lima, Peru

Circulating antigen levels may provide a monitoring tool for human neurocysticercosis (NCC). Experience with serum levels shows that they correlate with parasite burden and evolution, and that they drop in a few months after successful antiparasitic treatment. Also, under clinical circumstances, urine levels have been shown to correlate with serum 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 ELISA, 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 decreasing. 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.

IMMUNE RESPONSE DURING NEUROCYSTICERCOSIS IN MADAGASCAR

Prisca Ramandrainiainy1, Remy Guebey2, Romy Razakandrainibe2, Mathilde Bousnard2, Julien Razafimaheta3, Mahenina Rakotondrazaka2, Emma Rakotomalala2, Zara Razafiarimanga1, Ramaroson Vololoniaina1, Ronan Jambou2

1Immunology Unit Faculty of Sciences, Antananarivo, Madagascar, 2Institut Pasteur de Madagascar, Antananarivo, Madagascar, 3Clinic of Neurology, Befelatanana Hospital, Antananarivo, Madagascar

Neurocysticercosis (NCC) is the most important cause of seizure in tropical 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-helmhnts 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-Taolum 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 enlightened 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

For the Cysticercosis Working Group in Peru6

1National Institutes of Health, Bethesda, MD, United States, 2Naval Research Unit 6, Lima, Peru, 3Instituto Nacional de Ciencias Neurologicas, Lima, Peru, 4Universidad Peruana Cayetano Heredia, Lima, Peru, 5Hospital Albert Sabogal, ESSALUD, Callao, Peru, 6For the Cysticercosis Working Group in Peru, Lima, Peru

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 undo 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

Luz M. Moyano1, Mayuko Saito2, Silvia Montano3, Guillermo E. Gonzalez2, Sandra Olaya3, Viterbo, Ayvar3, Isidro Gonzales4, Luis Larrauri4, Victor C. Tsang1, Fernando Llanos5, Silvia Rodriguez2, Armando E. Gonzalez4, Robert H. Gilman2, Hector H. Garcia1, For The Cysticercosis Working Group for Peru1

1Cysticercosis Elimination Program and Center for Global Health- Tumbes, Universidad Peruana Cayetano Heredia, Tumbes, Peru, 2Department of International Health, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD, United States, 3U.S. Naval Medical Research Unit 6, Lima, Peru, 4Instituto Nacional de Ciencia Neurologicas, Lima, Peru, 5Georgia State University, Atlanta, GA, United States, 6School of Public Health and Department of Microbiology, School of Sciences, Universidad Peruana Cayetano Heredia, Peru, 7Instituto Nacional de Ciencia Neurologicas, Lima, Peru, 8School of Veterinary Medicine, Universidad Nacional Mayor de San Marcos, Lima, Peru

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 that 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

Hélène Carabin1, Athanase Millogo2, Rasmané Ganaba3, Nicolas Praet4, Jean-Bosco Ouédraogo4, Linda D. Cowan1, Pierre Dorny4

1University of Oklahoma Health Sciences Center, Oklahoma City, OK, United States, 2Centre Universitaire Sourou Sanou, Bobo Dioulasso, Burkina Faso, 3AFRICSanet, Bobo Dioulasso, Burkina Faso, 4Institute of Tropical Medicine, Antwerp, Belgium, 5Centre MURAZ, Bobo Dioulasso, Burkina Faso

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), Boulikiemédé (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 Boulikiemédé, 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

Enrico Brunetti1, Raffaella Lissandrin2, Luca Piccoli2, Carmine Tinelli3, Claudia Cevini4, Roberta Maserati2, Antonella Bruno2, Sam R. Gobbrich3, Simona Gatti2

1University of Pavia, Pavia, Italy, 2IRCCS Policlinico San Matteo, Pavia, Italy, 3University of Minnesota, Minneapolis, MN, United States

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 retrospectively 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 (IHA and ELISA) results. Cysts were stratified by stages (active, transitional and inactive cysts, WHO IWGE) and by dimension (5-10 cm and ≤ 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 ECHINOCOCCOSIS

Tuerhongjiang Tuxun1, Jun-hua Wang2, Ren-Yong Lin2, Jiao-Yu Shan2, Qin-Wen Tai3, Tao Li1, Jin-Hui Zhang1, Jin-Ming Zhao3, Hao Wen2

1 Department of Liver and Laparoscopic Surgery, Digestive State Key Laboratory Incubation Base of Xinjiang Major Diseases Research and Xinjiang Key Laboratory of Echinococcosis, 1st Affiliated Hospital of Xinjiang Medical University, Urumqi, Xinjiang, China, 2State Key Laboratory Incubation Base of Xinjiang Major Diseases Research and Xinjiang Key Laboratory of Echinococcosis, 1st Affiliated Hospital of Xinjiang Medical University, Urumqi, Xinjiang, China, 3Department of Liver and Laparoscopic Surgery, Digestive and Vascular Centre, 1st Affiliated Hospital of Xinjiang Medical University, Urumqi, Xinjiang, China

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-β1) and transcription factor (Foxp3) levels and moderate (IL-10 and TGF-β1) and transcription factor (Foxp3) levels and moderate decrease in Th17 number, related cytokines (IL-17 and IL-23) and transcription factor (ROTY) 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

Aurelio Di Pasquale1, Bruce MacLeod2, Willem Takken3, Alexandra Hiscox1, Richard W. Mukabana4, Henry Mwanyika1, Nicolas Maire1

1 Swiss Tropical and Public Health Institute, Basel, Switzerland, 2University of Southern Maine, Portland, ME, United States, 3Wageningen University and Research Centre, Wageningen, The Netherlands, 4ICipe—African Insect Science for Food and Health, Mbita, Kenya

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 Mbita, Nyanza (Kenya).

ROLE OF POOR ENVIRONMENT AND SOCIAL STATUS ON LEPTOSPIRA TRANSMISSION IN THE URBAN SLUM SETTING: A PROSPECTIVE PROPENSITY SCORE-MATCHED COHORT STUDY

Jose E. Hagan1, Ridalwa D. Felzemburgh2, Guilherme S. Ribeiro3, Renato B. Res2, Astrid X. Melendez2, Francisco S. Santana4, Sharif Mohr5, Federico Costa6, Deborah Fraga7, Balbino L. dos Santos2, Adriano Q. Silva2, Andreia C. Santos7, Romy R. Ravines8, Wagner S. Tassinari9, Marília S. Carvalho9, Mitermayer G. Reis8, Albert I. Ko1

1Yale School of Public Health, New Haven, CT, United States, 2Instituto Gonçalo Moniz, Fundação Oswaldo Cruz, Salvador, Brazil, 3Instituto de Saúde Coletiva, Universidade Federal da Bahia, Salvador, Brazil, 4Secretaria Estadual de Saúde da Bahia, Salvador, Brazil, 5Escola Nacional da Saúde Pública, Rio de Janeiro, Brazil, 6Universidade Federal Rural do Rio de Janeiro, Rio de Janeiro, Brazil

Leptospirosis is an important health problem in slum settlements worldwide, which have environmental and social conditions for rat-borne transmission. We performed a propensity score-matched analysis of longitudinal data from a cohort of urban slum residents to delineate the role of poor environment and social status on Leptospira infection risk. A prospective study identified Leptospira infections, potential environmental transmission sources, socioeconomic factors, and risk behaviors among slum residents during four annual serosurveys. We calculated a propensity score to estimate the probability of an individual residing in proximity to environmental transmission sources, based on 13 socioeconomic variables, and a second score to estimate the probability of having a per capita daily household income below the median of $1.32, based on 16 environmental variables. We then created two matched cohorts based on these propensity scores in order to evaluate the isolated infection risk due to slum environment and social status, respectively, in Generalized Estimation Equation models. Among 1224 pairs of person-years of follow-up, matched according to propensity for environmental exposures, we found the 15-24 year (RR, 2.60, 95% CI, 1.29 - 5.23) and 25-34 year (RR, 3.29, 95% CI, 1.61 - 6.72) age groups, male gender (RR, 2.15, 95% CI, 1.31 - 3.52), illiteracy (RR, 2.94, 95% CI, 1.68 - 5.17), and household proximity to open waste sewers and flood risk areas (RR, 1.90; 95% CI, 1.03 - 3.50) to be independent risk factors. Among 1315 pairs of person-years of follow-up, matched according to propensity for low income, we found belonging to 25-34 year age group (RR, 3.13; 95% CI, 1.03 - 3.50) to be independent risk factors. Among 1315 pairs of person-years of follow-up, matched according to propensity for low income, we found belonging to 25-34 year age group (RR, 3.13; 95% CI, 1.03 - 3.50) to be independent risk factors. Among 1315 pairs of person-years of follow-up, matched according to propensity for low income, we found belonging to 25-34 year age group (RR, 3.13; 95% CI, 1.03 - 3.50) to be independent risk factors. Among 1315 pairs of person-years of follow-up, matched according to propensity for low income, we found belonging to 25-34 year age group (RR, 3.13; 95% CI, 1.03 - 3.50) to be independent risk factors.
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

William Avilés, Heather Zornetzer
Sustainable Sciences Institute, Managua, Nicaragua

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

Kayla F. Laserson1, Penelope Phillips-Howard2, Frank Odhiambo1, Mary Hamel3, Kubaje Adazu1, Marta Ackers2, Anne van Eijk2, Vincent Orimba3, Anja van’t Hoog1, Caryl Beynon4, John Vulule3, Mark Bellis4, Laurence Slutsker3, Kevin deCock3, Robert Breiman6
1KEMRI/Centers for Disease Control and Prevention Research and Public Health Collaboration, Kisumu, Kenya, 2Liverpool School of Tropical Medicine, Liverpool, United Kingdom, 3Centers for Disease Control and Prevention, Atlanta, GA, United States, 4Centre for Public Health, Liverpool John Moores University, Liverpool, United Kingdom, 5KEMRI Centre for Global Health Research, Kisumu, Kenya, 6KEMRI/Centers for Disease Control and Prevention Research and Public Health Collaboration, Nairobi, Kenya

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 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 equaled 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

Shunmay Yeung, Toby Leslie, Clare Chandler, RDTs in Context Working Group
London School of Hygiene and Tropical Medicine, London, United Kingdom

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.

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

Joshua M. Wong1, Dhillon Nyachio2, Noelle Benzakri3, Leonard Cosmas4, Daniel Ondari2, John Neatherlin4, John W. Williamson3, Joel M. Montgomery2, Robert F. Breiman1

1Centers for Disease Control and Prevention, Nairobi, Kenya, 2Kenyan Medical Research Institute, Centers for Disease Control and Prevention, International Emerging Infections Program, Nairobi, Kenya, 3University of California, Los Angeles, Los Angeles, CA, United States, 4Centers for Disease Control and Prevention, International Emerging Infections Program, Nairobi, Kenya

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 morbidity in the developing world than previously thought and are potentially impacted by urbanization where dense population and unsafe cooking environments may increase the risk.
VALIDATION OF AN AUTOMATED RDT READER AND DATA MANAGEMENT DEVICE IN TANZANIA

Seif Shekalaghe1, Salim Abdulla1, Marcela Cancino2, Santiago Ferro2
1Ifakara Health Institute, Dar es Salaam, United Republic of Tanzania, 2Fio Corporation, Toronto, ON, Canada

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 optimal 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, NPV: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

Joel J. Jones1, Yatta Walker2, Fahn Taweh2, Tobias Johnson1, Hannan Bestman1, Luis Benavente6
1National Malaria Control Program, Monrovia, Liberia, 2National Public Health Reference Lab, Monrovia, Liberia, 3Improving Malaria Diagnosis Project, Monrovia, Liberia, 4Medical Care Development Inc., Silver Spring, MD, United States

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 bloated 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 microscopists (Level 1). MIS 2011 collected blood from 3841 children. A random subsample of 476 slides (15%) were selected for MMQA 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 parasites. 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

Happy B. Nchimbi1, Katia Bruxvoort2, Matthew Cairns3, Admirabilis Kalolella4, Rebecca Thomson2, Charles Festo1, Julie Thwing1, Mark Taylor2, Catherine Goodman2, Patrick Kachur3
1Ifakara Health Institute, Dar es Salaam, United Republic of Tanzania, 2London School of Hygiene and Tropical Medicine, London, United Kingdom, 3Centers for Disease Control and Prevention, Atlanta, GA, United States

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

Toby Leslie1, Amy Mikhail2, Ismail Mayan2, Asif Alokozai2, Nader Mohammed2, Anwar Hasanzai3, Habib Bakhtash4, Bonnie Cundill1, Christopher J. Whitty1, Mark Rowland1
1London School of Hygiene and Tropical Medicine, London, United Kingdom, 2Health Protection and Research Organisation, Kabul, Afghanistan, 3HealthNet TPO, Jalalabad, Afghanistan, 4MERLIN, Kunduz, Afghanistan

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 clinic 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.

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

Miriam Nakalemba1, Daniel Kyabazinga1, Yves-Daniel Compaoré3, Michelle Gatton1, Heidi Hopkins1, Sandra Incardona5, Jerry Mulundo4, Aminata Ouattara1, Fabrice Some1, Atis Muehlenbachs4, Issaka Zongo5, Jean-Bosco Ouedraogo3, David Bell1, Jane Cunningham1
1Department of Obstetrics and Gynaecology, School of Medicine, Makerere University, Kampala, Uganda, 2Foundation for Innovative New Diagnostics, Kampala, Uganda, 3Institut de Recherche en Sciences de la Santé (IRSS), Bobo-Dioulasso, Burkina Faso, 4Queensland Institute for Medical Research, Brisbane, Australia, 5Foundation for Innovative New Diagnostics, Geneva, Switzerland, 6Department of Pathology, University of Washington, Seattle, WA, United States, 7UNICEF/UNDP/World Bank/ World Health Organization Special Programme for Research and Training in Tropical Diseases (TDR), Geneva, Switzerland

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

Margaret Pinder1, Musa S. Jawara1, Lamin B. Jarju2, Ballah Kande3, David Jeffries1, Kalifa A. Bojang1, Umberto D’Alessandro1, David J. Conway1, Steve W. Lindsay4

1MRC Unit, The Gambia, Banjul, Gambia, 2National Malaria Control Programme, Banjul, Gambia, 3London School of Hygiene and Tropical Medicine, London, United Kingdom, 4Durham University, Durham, United Kingdom

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 IRS 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

Stella Chenet1, Leopoldo Villegas2, Ananias A. Escalante1

1Center for Evolutionary Medicine and Informatics, Arizona State University, Tempe, AZ, United States, 2Centro de Investigacion de Campo Francesco Vitanza, Venezuela and ICF International, International Health and Development Division, Calverton, MD, United States

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 sympathy 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 undergo 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.
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 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.

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

Hugh J. Sturrock\(^1\), Joe M. Novotny\(^2\), Simon Kunene\(^3\), Sabelo Dlamini\(^4\), Zuli Zulu\(^5\), Justin M. Cohen\(^1\), Michelle S. Hsiang\(^6\), Bryan Greenhouse\(^7\), Roly D. Gosling\(^8\)

\(^1\)University of California San Francisco, San Francisco, CA, United States, \(^2\)Clinton Health Access Initiative, Boston, MA, United States, \(^3\)National Malaria Control Programme, Manzini, Swaziland

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

David A. Larsen\(^1\), John M. Miller\(^2\), Joseph Keating\(^1\), Joshua Yuki\(^1\), Busiku Hamainza\(^3\), Hawela Moonga\(^3\), Katula Silumbe\(^3\), Chris Lungu\(^4\), Jacob Chirwa\(^1\), Thomas P. Eiselle\(^1\)

\(^1\)Tulane University School of Public Health, New Orleans, LA, United States, \(^2\)PATH Malaria Control and Evaluation Partnership in Africa (MACEPA), Lusaka, Zambia, \(^3\)National Malaria Control Center, Ministry of Health, Lusaka, Zambia

In Zambia the current interventions of insecticide-treated mosquito nets, indoor residual spraying and case management with artemisinin 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 PF 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 comparisons, and known biases in health facility routine data. Preliminary results will be available in September 2012.
THE ROLE OF LUTZOMYIA INTERMEDIA SANDFLY SALIVA ON THE EARLY EVENTS OF LEISHMANIA BRAZILIENSIS INFECTION

Tiffany S. Weinkopf1, Yazmin Hauyon-La Torre1, Camila de Oliveira2, Aldina Barrai2, Fabienne Tacchini-Cottier1

1University of Lausanne, Epalinges, Switzerland, 2IPICRUZ, Salvador, Brazil

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.

DEMONSTRATION OF REPRODUCIBLE VISCERALIZATION OF LEISHMANIA DONOVANI FOLLOWING TRANSMISSION BY SAND FLY BITES IN BALB/C MICE AND HAMSTERS

Ranadhir Dey1, Hamide Aslan2, Claudio Meneses2, Pradeep K. Dagur3, John Philip McCoy3, Robert Duncan1, Jesus G. Valenzuela2, Hira L. Nakhasi1, Shaden Kamhawi2

1Division of Emerging and Transmitted Diseases, Center for Biologies Evaluation and Research, Food and Drug Administration, Bethesda, MD, United States, 2Vector Molecular Biology Unit, Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States, 3Flow Cytometry Core, National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, MD, United States

Visceral leishmaniasis (VL) caused by Leishmania donovani is a vector-borne anthroponotic disease transmitted by sand fly bite with no available human vaccines. Following vaccination, animals protected against cutaneous leishmaniasis upon needle challenge failed against the virulence of a sand fly-initiated infection. This highlights the significance of developing models of vector-transmission for VL, particularly in vaccine evaluation. Here, we present models of visceral leishmaniasis in BALB/c mice and Golden Syrian hamsters using Lutzomyia longipalpis sand flies infected with L. donovani. Sand flies with transmissible infections were allowed to feed on animal ears for 2 hours. In BALB/c mice, most animals developed Leishmania-specific IgG antibodies around 5 weeks post-infection. Ten weeks post-infection, the parasites had disseminated into the spleen and liver reaching a maximum burden of 1x10^6 and 2x10^5 in the spleen and liver, respectively, at 20-25 weeks. Thirty weeks post-infection, the mice had not cleared the infection displaying a significant number of parasites (6.5x10^5) in the spleen although none were detectable in the liver. The progressive growth of parasites in the spleen and liver of infected mice following vector-initiated infection demonstrates the utility of this model to study VL. In hamsters, the animals succumbed to disease within 3-9 months post-sand fly transmission showing parasite visceralization accompanied by clinical manifestations of VL including enlarged spleens and livers whose mean weight was 5.8- and 1.3-fold higher, respectively, than those of naïve hamsters. Currently, studies in BALB/c mice and hamsters are focusing on the comparative evaluation of the immune response following infection with either sand fly bite or needle injection and also are oriented towards testing promising vaccines that protected against needle-challenge. Overall, these models facilitate our understanding of the host immune response to vector-initiated VL and represent an improved tool for the assessment of potential drugs and vaccine candidates.
Protozoan pathogen, Trypanosoma cruzi, is the causative agent of 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 debilitating 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 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 impact 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.

Towards Trypanosoma Cruzi Lineage-Specific Serology for Chagas Disease

Tapan Bhattacharyya, Michael A. Miles

London School of Hygiene & Tropical Medicine, London, United Kingdom

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 debilitating 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 demonstrated here the capacity of synthetic peptides based on the TcII/V/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 TcI-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.

Comparative Genomics and Phylogenomics of the Protozoan Pathogen, Trypanosoma Brucei

Mark Sistrom¹, Benjamin Evans¹, Robert Bjornson¹, Wendy Gibson², Oliver Balmer³, Pascal Maser³, Serap Aksoy², Richard Echodu¹, Barbara Nerima³, John Enyaru², Adalgisa Caccone¹

¹Yale University, New Haven, CT, United States, ²University of Bristol, Bristol, United Kingdom, ³Swiss Tropical and Public Health Institute, Basel, Switzerland, ⁴Gulu University, Gulu, Uganda, ⁵Uganda Virus Institute, Entebbe, Uganda, ⁶Makerere University, Kampala, Uganda

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 repetition 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.

Role of the Chromatin Remodeling Enzyme HDAC1 in Leishmania Amazonensis Infection: Implications for Host Transcription Repression

Teresa Cristina Calegari-Silva, Aslán Carvalho Vivarini, Gisele M. Silva, Gilherme Rodrigo R. dos Santos, Ulisses Gazos Lopes

Federal University of Rio de Janeiro, Rio de Janeiro, Brazil

Leishmania parasites subvert important host cell signaling pathways involved in the control of the infection. NF-κB 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-κB 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 deacetylases 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-κB 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 HDAC1 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-κB 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-κB homodimer are taking place in infected macrophages.
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 biopsies study revealed that non-classical monocytes were the main MMP-9 producing cells. These results show that monocyte subpopulations contribute differently to the immunopathology observed in CL patients.

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**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**

**Joseph Kubofck**¹, **Yaya I. Coulibaly**², **Salif S. Doumbia**², **Sory I. Keita**², **Zana L. Sanogo**¹, **Massitan Dembele**¹, **Thomas B. Nutman**¹

¹National Institutes of Health, Bethesda, MD, United States, ²University of Bamako, Bamako, Mali, ³National Program for the Elimination of Lymphatic Filariasis, Bamako, Mali.

Significant progress has been made toward the global 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 immunoassays 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.

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

**Poppy H. Lamberton**¹, **Robert A. Cheke**², **Mike Y. Osei-Atweneboana**³, **Peter Winskill**¹, **Kelly J. Shew**², **Michael D. Wilson**⁴, **Rory J. Post**⁴, **María-Gloria Basáñez**³

¹Imperial College London, London, United Kingdom, ²University of Greenwich, Chatham, United Kingdom, ³Council for Scientific and Industrial Research, Accra, Ghana, ⁴University of Ghana, Legon, Ghana, ⁵London School of Hygiene and Tropical Medicine, London, United Kingdom

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

Lisa Reimer1, Sara Erickson2, Edward Thomsen1, John Keven1, Naomi Vincent1, Moses Bockarie3, Peter Siba4, James Kazura5, Bruce Christensen6

1Papua New Guinea Institute of Medical Research, Madang, Papua New Guinea, 2Walter and Eliza Hall Institute, Parkville, Australia, 3Liverpool School of Tropical Medicine, Liverpool, United Kingdom, 4Papua New Guinea Institute of Medical Research, Goroka, Papua New Guinea, 5Case Western Reserve University, Cleveland, OH, United States, 6University of Wisconsin, Madison, WI, United States

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 microfilaremic 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/20 ml) 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 1000h, 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. Filaria 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.
OPTIMISM FOR LYMPHATIC FILARIASIS ELIMINATION:
A CASE STUDY OF TANDAHIMBA DISTRICT, SOUTHERN TANZANIA

Upendo J. Mwingira1, Paul Simonsen2, Akili Kalinga3, Maria J. Chikawe1, Irene Mremi1, Andreas M. Nshala4, Brian Chu1, A. Pavlović2, D. Kyelêm3, E. Ottesen5, M. N. Malecela1

1National Institute for Medical Research, Dar Es Salaam, United Republic of Tanzania, 2University of Copenhagen, Copenhagen, Denmark, 3National Institute for Medical Research, Mbeya, United Republic of Tanzania, 4Neglected Tropical Diseases Control Program, Dar Es Salaam, United Republic of Tanzania, 5Taskforce for Global Health, Atlanta, GA, United States

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 Microfilaremia (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

Israt Hafiz1, Rousole Haq1, Meerjady Sabrina Flora2, Moses J. Bockarie1, Louise A. Kelly-Hope1

1Filariasis Elimination Program, Directorate General of Health Services, Dhaka, Bangladesh, 2Centre for Neglected Tropical Diseases-Liverpool School of Tropical Medicine, Liverpool, United Kingdom

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. This result indicates 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

Kristen M. Little1, Beatriz Lopez2, Patricia Julia3, Fredy Muñoz2, John McCracken2, Gordana Derado1, Victoria Cuellà1, Andy Thornton1, Jaymin C. Patel1, Gerard Lopez1, Lissette Reyes3, Kim Lindblade3, Sharon Roy1

1Centers for Disease Control and Prevention, Atlanta, GA, United States, 2Universidad del Valle de Guatemala, Guatemala City, Guatemala, 3Global Disease Detection Program, Centers for Disease Control and Prevention Regional Office for Central America and Panama, Guatemala City, Guatemala, 4Health Area of Santa Rosa, Ministry of Public Health and Social Welfare, Guatemala City, Guatemala

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., Trichuris 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% were positive for STH. Facility-level prevalence was highest among SAC (6%, 6/98), 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 underestimate community-level STH burden and skew 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.

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SOIL-TRANSMITTED HELMINTHS IN URBAN SCHOOL- AND PRE-SCHOOL-AGED CHILDREN: PREVALENCE AND MORBIDITY IN KIBERA, NAIROBI

Stephanie M. Davis1, Caitlin M. Worrell1, Parminder Suchdev2, Laird Ruth2, Gerard Lopez3, Ryan Wiegand4, Kennedy OderoB, Leonard Cosmas1, John Neatherlin3, Sammy Njenga1, Joel M. Montgomery4, LeAnne Fox1

1Parasitic Diseases Branch, Centers for Disease Control and Prevention, Atlanta, GA, United States, 2Division of Nutrition, Physical Activity and Obesity, Centers for Disease Control and Prevention, Atlanta, GA, United States, 3Division of Parasitic Diseases and Malaria, Centers for Disease Control and Prevention, Atlanta, GA, United States, 4Global Disease Detection Branch, Centers for Disease Control and Prevention, Nairobi, Kenya.

The study aimed to define the burden of soil-transmitted helminth infections in pre-school-aged children in Kibera, Nairobi, Kenya. A total of 631 children were screened for soil-transmitted helminth infections (STH). The prevalence of any STH infection was 42.3% (41.4% for pre-school-aged children and 43.4% for school-aged children). The most prevalent STH infection was Trichuris, followed by Ascaris and hookworm. The study also assessed the impact of STH infections on morbidity, including anemia and malnutrition, with a prevalence of 20.9% and 21.7%, respectively. The findings suggest that routine screening and targeted treatment programs are needed to address the high prevalence of STH infections in this population.

Screening for Strongyloides infection in an immigrant population in Bronx, New York

Fabiola Espinoza1, Herbert Tanowitz1, Phyllis Andrews2, Inessa Gendlin2, Jacinth S. Ruddock2, Christina Coyle1

1Albert Einstein College of Medicine, Bronx, NY, United States, 2Jacobi Medical Center, Bronx, NY, United States

The study aimed to screen immigrant children for Strongyloides infection. Of the 540 children screened, 23 had a positive stool ova/parasite examination. Sibling stools were also tested in 126 children, of whom 12 had positive results. Additionally, 126 children had positive serology for Strongyloides. The study found that children with positive serology were more likely to have eosinophilia and had elevated IgE levels. The findings suggest that routine screening for Strongyloides is necessary in this population to prevent severe infections and associated morbidity.

A novel multi-parallel real-time PCR approach for eight gastrointestinal parasites provides improved diagnostic capabilities to resource-limited “at risk” populations

Rojelio Mejia1, Yosselin Vicuña2, Nely Broncano2, Carlos Sandoval2, Marthia Chico3, Phil J. Cooper3, Thomas B. Nutman1

1National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States, 2FEPIS, Quito, Ecuador

The study aimed to develop a novel multi-parallel real-time PCR (qPCR) approach for the detection of eight gastrointestinal parasites. The qPCR assay was able to detect infections for the 8 most common gastrointestinal parasite pathogens -- Ascaris lumbricoides, Necator americanus, Ancylostoma duodenale, Giardia lamblia, Cryptosporidium parvum, Entamoeba histolytica, Trichuris trichuria and Strongyloides stercoralis -- in a standardized and quantitative manner. The qPCR improved the sensitivity for Ascaris by 21.9% and had 97.8% specificity and 96.8% negative predictive value. The findings suggest that the qPCR approach could be used to improve the diagnosis of gastrointestinal parasites in resource-limited settings.

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BZ resistance under isothermal conditions without PCR amplification. \(^\beta\) to detect mutations of the \(-tubulin isotype 1 gene associated with genotyping assay based on the Smart Amplification Process (SmartAmp2) detecting BZ resistance, that could be adaptable to field conditions, would development of simple, rapid and cost-effective molecular tools for sequencing is a time consuming and complicated procedure, not suitable of low levels of drug resistance. Previous molecular assays for putative of resistance in the field. This assay is very insensitive for the detection of STHs. A growing concern is that extensive use of anthelmintics to control tropical areas of the world. Benzimidazole (BZ) drugs, albendazole and mebendazole have been used extensively for large-scale treatment of tropical nematodes causing human morbidity in Ascaris lumbricoides, Necator americanus, N. americanus, 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 strongyloidiasis 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 strongyloidiasis 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

Nour Rashwan
Institute of Parasitology, McGill University, Montreal, QC, Canada

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 \(\beta\)-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 \(\beta\)-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

Sara A. Nguyen1, Debbie Humphries1, Joseph Otchere2, Sunny Kumar3, Jon J. Vermeire4, Rebecca Treger3, Martin Keil3, Josephine E. Quagraine2, Lisa M. Harrison4, Daniel A. Boakye2, Michael Wilson2, Michael Cappello4
1Yale School of Public Health, New Haven, CT, United States, 2Noguchi Memorial Institute for Medical Research, Accra, Ghana, 3Yale University, New Haven, CT, United States, 4Yale School of Medicine, New Haven, CT, United States

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) (\(\chi^2; p < 0.01\)). 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.
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.

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

Juan M. Bustamante1, Ashley N. Hartley1, Ellen M. Dotson2, Cecilia Perez Brandan3, Rick L. Tarleton4

1Center for Tropical and Emerging Global Diseases, University of Georgia, Athens, GA, United States, 2Centers for Disease Control and Prevention, Atlanta, GA, United States, 3Instituto de Patologia Experimental - CONICET, Universidad Nacional de Salta, Salta, Argentina, 4Center for Tropical and Emerging Global Diseases and Department of Cellular Biology, University of Georgia, Athens, GA, United States

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: Enol-CoA hydratase/somerase 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 cells 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.
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 Plasmodium 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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**THE TRANSCRIPTION FACTOR T-BET REGULATES PARASITEMIA AND PROMOTES PATHOGENESIS DURING MURINE MALARIA**

Miranda S. Oakley, Leda Lotspeich Cole, Bikash Sahu, Nehal R. Solanki, Victoria Majam, Steven C. Derrick, Sheldon L. Morris, Sanjai Kumar

*Food and Drug Administration, Bethesda, MD, United States*

We investigated the role of the transcription factor T-bet (the master regulator of Th1) cells during murine malaria infections. Using mice deficient for T-bet, we report that T-bet interferes with the formation of a protective immune response during Plasmodium yoelii 17XNL murine malaria. On day 12 post-infection, T-bet deficient mice (5.7 ± 1.6%) had 2.9 fold lower parasitemia (p=0.0013) than wildtype controls (16.8 ± 1.7%). Although T-bet deficient mice had diminished levels of total IgG antibody, these mice had significantly higher levels of IgG1 (81,920 ± 12,540) compared to wildtype controls (16,640 ± 3840) indicating that this antibody isotype may be important for malaria protective immunity (p=0.0011, Student T test). In the Plasmodium berghei ANKA murine model of experimental cerebral malaria (ECM), we demonstrate that while T-bet regulates parasite burden, it also promotes the pathogenesis of ECM possibly by impeding the formation of an anti-inflammatory Th2 immune response. T-bet deficient mice had higher parasitemia than wildtype controls during the ECM phase of disease (17.7 ± 3.1% verses 10.9 ± 1.5%). In addition, while 100% (10/10) of wildtype mice developed ECM by day 9 post-infection, only 30% (3/10) of T-bet deficient mice succumbed to disease during the cerebral phase of infection (p=0.000029, Log rank). Resistance to ECM in T-bet deficient mice was associated with a Th2 immune response characterized by enhanced production of GATA-3+ CD4+ T cells and elevated levels of the eotaxin, MCP-1, and G-CSF cytokines. Our results suggest that in the mouse models studied, Th1-type immune responses render deleterious outcomes as they interfere with the acquisition of immunity and mediate the pathogenic features of cerebral malaria.

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**ANTIBODY DYNAMICS AFTER ACUTE MALARIA INFECTION IN CHILDREN**

Arlene E. Dent1, Rhonda Kimmel1, John Vulule2, Ann Moormann3

1Case Western Reserve University, Cleveland, OH, United States, 2Kenya Medical Research Institute, Kisumu, Kenya, 3University of Massachusetts, Worcester, MA, United States

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) luminescent multiplex serology to 13 malaria antigens and b) functional antibody-mediated growth inhibition of cultured parasites. We found that total IgG to MSP-142 (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. MSP142 invasion inhibitory antibodies and global growth inhibition antibodies against PDid10 remained unchanged after infection. In addition, we found that IgG to merozoites 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 OOCYST-DERIVED SPOROZOITES AND REQUIRED FOR SALIVARY GLAND INVASION**

Tomoko Ishino1, Eri Murata2, Naohito Tokunaga2, Mayumi Tachibana1, Takafumi Tsuboi1, Motomi Torii1

1Department of Molecular Parasitology, Graduated School of Medicine, Ehime University, Toon, Japan, 2Venture Business Laboratory, Ehime University, Matsuyama, Japan, 3Malaria Research Unit, Cell-Free Science and Technology Research Center, Ehime University, Matsuyama, Japan

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
salivary glands was greatly reduced by ron2 silencing, despite sporogony, sporozoite release into hemocoel and their motility were normal. These results showed that RON2 is required for salivary gland invasion. This is the first genetic approach to show that RON2 has an important role in target cell invasion.

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**IDENTIFICATION AND CHARACTERIZATION OF A PLASMODIUM FALCIPARUM ORTHOLOGUE OF THE YEAST UBIQUINONE-BINDING PROTEIN, COQ10P**

Bethany J. Jenkins, Joanne M. Morrissey, Thomas M. Daly, Michael W. Mather, Akhil B. Vaidya, Lawrence W. Bergman

Drexel University College of Medicine, Philadelphia, PA, United States

Coenzyme Q (CoQ, ubiquinone) is a central electron carrier in mitochondrial respiration. CoQ is synthesized through multiple steps involving a number of different proteins. The prevailing view that the CoQ used in respiration exists as a free pool that diffuses throughout the mitochondrial inner membrane bilayer has recently been challenged. In the yeast Saccharomyces cerevisiae, deletion of the gene encoding Coq10p results in respiration deficiency without altering total size of the available CoQ pool, suggesting that the Coq10p is critical for the delivery of CoQ to the site(s) of respiration. The precise mechanism by which this is achieved remains unknown at present. Because mitochondrial respiration is a validated target for antimalarial drugs such as atovaquone, we are interested in examining its regulation in malaria parasites. We have identified an orthologue of Coq10p, PfCoq10, in P. falciparum, the most virulent species of malaria parasite, and demonstrated that a GFP-tagged version of Pfcoq10 localized to the parasite mitochondrion. Expression of PfCoq10 in the S. cerevisiae coq10 deletion strain restored the capability of the yeast to grow on respiratory substrates, suggesting a remarkable functional conservation of this protein over a vast evolutionary distance, and despite a relatively low level of amino acid sequence identity. We are currently assessing effects of PfCoq10 overexpression on the atovaquone sensitivity of P. falciparum. We are also examining the possibility of altered response to atovaquone in yeast mitochondria expressing the parasite Coq10. These studies may provide insights into respiration regulation in general, as well as in malaria parasites.

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**ROLE OF PFRAD54 AND REPPLICATION PROTEIN A IN RAD51-MEDIATED DNA STRAND EXCHANGE AND REPAIR OF DNA DAMAGE INDUCED BY MMS IN PLASMODIUM FALCIPARUM**

Anusha M. Gopalakrishnan, Nirbhay Kumar

Tulane University School of Public Health, New Orleans, LA, United States

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 P. falciparum, as reported previously. Here, we evaluated the catalytic functions of PfRad51, exhibited ATPase activity and promoted DNA strand exchange homologue Rad51 play a central role in homologous DNA strand switching, a major impediment in developing an effective vaccine against P. falciparum, as reported previously. Here, we evaluated the catalytic functions of PfRad51 and its putative interacting partners. Besides understanding molecular machinery involved in DNA repair and recombination, we wish to extend our studies to understand the biochemical and genetic basis of gene rearrangements at the var gene locus associated with phenomenon like antigenic variation.

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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**

Grace Okello1, Zachary Karim1, Prakash Kemapia1, Eric Otieno2, James Hittner1, John Vulve2, John Ong’e’cha2, Douglas Perkins1, Tom Were4

1Center for Global Health - University of New Mexico, Albuquerque, NM, United States, 2Laboratories of Parasitic and Viral Diseases, Centre for Global Health Research, Kisumu, Kenya, 3Department of Psychology, College of Charleston, Charleston, SC, United States, 4Centre for Global Health Research, Kenya Medical Research Institute, Kisumu, Kenya

Stromal cell-derived factor (SDF)-1a (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-1a 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, rs2839686) 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 (Hb5.0-9g/dL; n=425), high-density parasitemia (HDP; >10,000 parasites/µL; n=477) vs. low-density parasitemia (LDP; ≤10,000 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-thalassemia, 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 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.

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**GENOMIC DIVERSITY AND EVOLUTIONARY HISTORY OF PLASMODIUM VIVAX**

Ernest R. Chan1, Didier Menard2, Odile Mercereau-Puijalon3, Peter Zimmerman4, David Serre2

1Cleveland Clinic Foundation, Cleveland, OH, United States, 2Institut Pasteur du Cambodge, Phnom Penh, Cambodia, 3Institut Pasteur, Paris, France, 4Case Western Reserve University, Cleveland, OH, United States

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...