The association between malaria parasitemia, erythrocyte polymorphisms, malnutrition and anaemia in children less than 10 years in Senegal

Tine, Roger C.; Hansson, Helle Smedegaard; Ndiaye, Magatte; Alifrangis, Michael; Faye, Babacar; Ndour, Cheikh T.; Ndiaye, Jean L.; Magnussen, Pascal; Bygbjerg, Ib Christian; Gaye, Oumar

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HOST SELECTION, DEFENSIVE BEHAVIORS AND FEEDING SUCCESS OF CULEX QUINQUEFASCIATUS IN EXPERIMENTAL TRIALS

Joseph R. McMillan1, Paula L. Marcet2, Uriel Kitron1, Gonzalo M. Vazquez-Prokopec1

1Emory University, Atlanta, GA, United States, 2Centers for Disease Control and Prevention, Atlanta, GA, United States

Studies describing common blood sources of field collected mosquitoes are inconsistent in their description of the host selection behavior of Culex quinquefasciatus. Host selection is an important determinant of pathogen transmission, and this knowledge gap in mosquito behavior is limiting our understanding of vector-host contacts and the importance of reservoir hosts in West Nile virus (WNV) transmission. We conducted host-choice experiments under semi-natural conditions to quantitatively host feeding preference by Cx. quinquefasciatus mosquitoes when presented with an array of common passerine hosts: Northern Cardinals, American Robins, Blue Jays, Brown Thrashers, and Gray Catbirds. The experimental design consisted of: 1) a 1.5m x 0.75m x 0.75m enclosure inside of which two bird cages were placed, 2) 30 recently emerged female Cx. quinquefasciatus originating from wild eggs, and 3) an infra-red camera recording system. We performed 12 two-bird choice experiments in which we calculated the feeding index for each potential host and tested the null hypothesis of random host selection. We also quantified the number of defensive behaviors exerted by each bird. The blood sources for the 168 mosquitoes that successfully obtained a bloodmeal were assessed by amplifying a fragment of the 16s ribosomal gene using generalist avian primers, sequencing each amplified fragment, and comparing the fragment to reference sequences. Host selection differed significantly from random, exhibiting the following preference structure: American Robins preferred over Blue Jays and over Northern Cardinals, and Northern Cardinals preferred over Brown Thrashers. The most common types of defensive behaviors were those protecting the feet and head, but the number of defensive behaviors did not differ significantly between hosts. Further experiments are needed to determine the role of these defensive behaviors in host selection and feeding success by vectors. Our results indicate a non-random pattern of host selection by vectors that needs to be considered when modeling WNV transmission.

INTERACTIVE TOOLS FOR IDENTIFICATION OF MOSQUITO AND SAND FLY VECTORS OF INFECTIOUS DISEASES

Leopoldo M. Rueda, James E. Pecor, Richard C. Wilkerson, Lewis S. Long, Jason H. Richardson

Walter Reed Army Institute of Research, Silver Spring, MD, United States

Computerized interactive tools to identify mosquito and sand fly vectors of infectious human diseases were developed for various regions of the world (see Walter Reed Biosystematics Unit/WRBU website, www.wrbu.org). Using LUCID programs, WRBU identification keys for mosquito and sand fly vectors and their associated groups included morphological diagnostic characters primarily of the head, thorax, abdomen, legs and wings. Automontage images of diagnostic characters of various insect body parts were attached to each key. Genus and species pages for selected vectors and related groups were developed, including basic taxonomy, distribution, bionomics, medical importance, selected references, and detailed photos of habitus and other morphological parts. World catalogs of mosquitoes and sand flies, with updated taxonomy and hierarchic classification were linked to each key. In addition, comprehensive lists of known and potential vectors, and their associated taxonomic information, were included in the WRBU website. New LUCID identification keys were recently developed, namely: African Anopheles adult and larval keys (include 140+ species and groups for adult key, 120+ for larval key); South American Culicine mosquitoes (include vector adult and larval keys of Aedes, Culex, Coquiellitidia, Haemagogus, Mansonia, Psorophora, Trichprosophon); South American Phlebotomine Sand flies (include male and female keys of genera, subgenera, and vector species of Dampomyia, Evandromyia, Helocrotyria, Lutzomyia, Nyssomyia, Psathyromyia, Psychodopygus, Scopemyia, Trichophoromyia, Verrucarum Group). Diagnostic characters, updated taxonomy and related information of new vector identification tools are noted and discussed.

SRPN2 DEPLETION REDUCES MOSQUITO FITNESS AND BITING FREQUENCY

Karaloo Sprigg1, Andrew F. Read2, Kristin Michel1

1Kansas State University, Manhattan, KS, United States, 2Pennsylvania State University, University Park, PA, United States

The mosquito’s immune system is at the vector-pathogen interface and largely determines susceptibility. One consequence of its manipulation can be the reduction in vectorial capacity. Therefore, the mosquito immune system provides potential targets for novel intervention strategies aimed to reduce vector-borne disease burden. Melanization is a powerful immune response in arthropods that leads to encapsulation and killing of invading pathogens. This process renders some mosquito species partially or completely resistant to infection with pathogens of global public health significance. One of its rate-limiting steps of melanization is the activation of prophenoloxidase (PPO), which is controlled by an extracellular protease cascade and serpin inhibitors. The molecular composition of this system is largely unknown in mosquitoes with the exception of Anopheles gambiae SRPN2 and CLIP9B, which constitute the first known regulatory unit that controls melanization. If uncontrolled, e.g. by the depletion of the inhibitor SRPN2, melanization can kill adult females late in life, and thus potentially reduce the vectorial capacity of An. gambiae. This feature makes PPO activation, which is a rate-limiting step in melanin production, a potential target for novel malaria control strategies. Using life table analyses, we determined the consequences of SRPN2 depletion by RNAi on several demographic growth parameters under standard laboratory settings. Net reproductive rate (Ro) was decreased by 29%, while mean generation time was unaffected. As a consequence, doubling time (Td) was moderately increased by 9%. The negative effect on net reproductive rate is largely attributable to a significant decrease in bloodfeeding propensity. Bloodfeeding propensity and survival were disproportionally reduced in older mosquitoes after the first two gonotrophic cycles. As a consequence, the number of potentially infectious bites is at least reduced by 83%. Taken together, these data suggest that SRPN2 constitutes a viable target for novel malaria intervention strategies.

SPATIAL DISTRIBUTION, SEASONALITY AND BEHAVIOR OF NOVEL MALARIA VECTORS IN THE WESTERN KENyan HIGHLANDS

Jennifer C. Stevenson1, Brandy St. Laurent2, Neil Lobo3, Lorna Culverwell1, Mary Cooke1, Samuel Kahindi4, Chrispin Owaga4, Elizabeth Ayoma4, Robin Oriango5, Ralph Harbach3, Chris Drakeley1, Jonathan Cox1

1London School of Hygiene and Tropical Medicine, London, United Kingdom, 2Eck Institute for Global Health, University of Notre Dame, South Bend, IN, United States, 3Natural History Museum, London, United Kingdom, 4Centre for Global Health Research, Kenya Medical Research Institute/Centers for Disease Control and Prevention, Kisumu, Kenya

Results from a light trap study carried out in 2010, presented previously, revealed the presence of previously unidentified mosquito species carrying Plasmodium falciparum sporozoites in Kisii district in the western Kenya highlands, an area prone to epidemics of malaria. The majority of these specimens could not be definitively identified to the species level using the commonly used morphological keys, and sequencing revealed that there were no matching published sequences available at ribosomal ITS2 and...
mitochondrial CO1 loci. To further morphologically describe these species, study their behavior and investigate their temporal and spatial distribution, mosquitoes collected during both longitudinal and transect studies were identified morphologically and sequenced at rDNA ITS2 and mtCO1 loci. To study spatial and temporal distribution of any novel vectors, monthly pyrethrum spray catches were made to sample indoor resting mosquitoes in 10 villages across Kisii Central, Kisii South and Rachuonyo South districts over an 18-month period between 2009 and 2011. To determine host-seeking activity and peak biting times, mosquitoes were collected from light traps set up indoors and outdoors over a 12-month period from June 2011. To fully describe larval and adult stages, a wide range of larval breeding sites were sampled across Rachuonyo South in November 2011 and 400 *Anopheles* larvae were reared to obtain adults with associated larval and pupal exuviae. For specimens found to have novel unpublished sequences, taxonomic studies were conducted to establish the identity of the species. Results on the presence of unusual species, their associated behaviors, spatial distributions and seasonality are presented.

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**INVESTIGATING THE ROLES OF ANOPHELES GAMBAE G PROTEIN-COUPLED RECEPTORS IN GUSTATION**

*Kimberly Regna, Marc A.T. Muskavitch*

*Boston College, Chestnut Hill, MA, United States*

Vector-targeted control strategies remain our most effective tools for reduction of malaria transmission and incidence. However, the threat and continuing increase in insecticide-resistance motivate discovery of novel insecticides. G protein-coupled receptors (GPCRs) are well known as one of the most “druggable” targets in many organisms. Numerous GPCRs mediate developmental, sensory or other physiological pathways that can greatly impact vectorial capacities of *Anopheles gambiae* and other malaria vectors. Gustatory GPCRs are central to the ability of insects to identify foods, including sugars, and detect noxious compounds in the environment. We are investigating the abilities of *An. gambiae* to detect various sugars and noxious compounds when given a choice between sugar meals, or between a sugar meal and a sugar/compound meal. Dye-labeling of meals enables colorimetric detection of intake. By using RNA interference to independently knockdown individual gustatory receptors, we will identify specific GPCRs required for sugar and noxious compound gustation. We have begun by analyzing sugar preferences among glucose, sucrose, fructose, galactose and mannose. Among these sugars, preliminary results imply mosquitoes prefer glucose and sucrose. Initial data suggests that mosquitoes exhibit strong aversion to berberine, a canonical preliminary results imply mosquitos prefer glucose and sucrose. Initial data glucose, sucrose, fructose, galactose and mannose. Among these sugars, sequences, taxonomic studies were conducted to establish the identity of the species. Results on the presence of unusual species, their associated behaviors, spatial distributions and seasonality are presented.

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**SCHISTOSOMIASIS AND SOIL TRANSMITTED HELMINTHIASIS IN KAÉDI, SOUTHERN MAURITANIA**

*Sunkaru Touray*

*Swiss Tropical and Public Health Institute, Basel, Switzerland*

We report findings of a cross-sectional parasitological study on schistosomiasis and soil transmitted helminthiasis among school aged children since the establishment of irrigation schemes in Kaédi, located at the confluence of the Gorgol and Senegal Rivers in Southern Mauritania. Stool and urine samples were obtained from 246 children between the ages of 5 to 15 years recruited from randomly selected households followed by administration of a parental questionnaire. Urine samples were analysed the same day using the centrifugation method, while stool samples were collected early the next morning and analyzed by the Kato-Katz technique. We found a low prevalence of *Schistosoma haematobium* (3.7 %) with a mean infection intensity of 38 eggs per 10 mls of urine, while no cases of *S. mansoni* were detected in all participants. Only one participant was infected with a soil transmitted helminth (*A. lumbricoides*). Working in the rice paddies during the annual flood recession (Oualo farming) was the strongest predictor of infection intensity among participants when we fitted a zero-inflated negative binomial (ZINB) model for egg counts with potential confounding factors controlled for in multivariate analyses. Prevalence of *S. haematobium* seems to have decreased compared to last estimates before the establishment of the irrigation schemes (14 %). These findings highlight a need for an integrated surveillance system aimed at transmission control targeting various aspects of the transmission cycle *S. haematobium*. infections, outbreaks of acute fasciolasis can occur in endemic areas related to ingestion of a contaminated food item. Despite the high prevalence of *F. hepatica* in many areas, its importance has been largely neglected. We investigated clusters of *F. hepatica* which occurred in four family groups in rural Argentina. A total of 34 confirmed cases of acute fasciolasis from 4 different families (15, 6, 8, and 5 affected members) were investigated. Three of the four families were residents of endemic areas, while a fourth family vacationed in an endemic area. All members from the 4 families reported ingestion of watercress. The most common clinical symptoms were right upper quadrant pain and fever, present in all subjects. All cases had elevated liver transaminase tests as well as absolute eosinophilia. *Fasciola* ova were detected in stool samples in all 34 subjects. Serology was performed in 16 subjects and was positive in 13 (81%). Abdominal ultrasound (performed in 22 subjects) showed diffuse hepatomegaly in 19 cases (86%). Twenty patients were treated with intravenous emetin and 14 patients with triclabendazole. Of 29 subjects who presented for post-treatment stool ova and parasite examination at 60-days, 26 (90%) became negative, with the remaining 3 patients becoming negative by 90-days post-treatment. In the triclabendazole group, 2 subjects had relapse with one subject needing repeat treatment and another subject needing two additional treatments. In the emetin group one patient needed repeat treatment, however three individuals developed hypotension as a treatment complication requiring hospitalization. *F. hepatica* remains an under-recognized infection. In fact, most subjects reported here did not seek medical care since they had only mild clinical symptoms and were only detected through the outbreak investigation. There is a need for increased education and awareness of fasciolasis in patients living and traveling to endemic areas.
DEVELOPMENT OF A RECOMBINANT PROTEIN VACCINE AGAINST SCHISTOSOMA MANSONI INFECTION USING CATHEPSIN B AND PEROXIREDOXIN 1 ANTIGEN

Alessandra Ricciardi, Bibiana G. Santana, John Dalton, Momar Ndao
McGill University, Montreal, QC, Canada

Schistosomiasis is a fresh-water-borne parasitic disease caused by trematode worms of the genus Schistosoma. Due to its morbidity and mortality, Schistosomiasis is the most important helminth infection. The pathology of the disease is due to egg deposition, by the female worm, which will trigger an immune reaction and consequently cause progressive damage to the organs. The lack of therapeutic drugs and preventative measures, as well as the high disease burden caused by the infection are justifications for developing a vaccine against schistosomiasis. The development of a recombinant protein vaccine against this parasitic disease has the potential to contribute a long-lasting decrease in disease spectrum and transmission. Furthermore, it would relieve some of the concern surrounding the potential emerging resistance to praziquantel; the drug which is solely being used to treat the infection. Our group has chosen to focus on the S. mansoni antigens Cathepsin B and Peroxiredoxin 1 (Prx1) as vaccine candidates. It is hypothesized that immunization with either recombinant Cathepsin B or recombinant Prx1 in the presence of an adjuvant can elicit protective immunity against Schistosoma infection. The objective of this research project is to develop a safe recombinant protein vaccine against schistosomiasis that will stimulate an optimal immune response which will prevent pathology. Upon cloning, expressing, and purifying the proteins of interest, mice were firstly immunized with recombinant Cathepsin B in the presence of either synthetic oligodeoxynucleotides (ODN) containing unmethylated CpG dinucleotides or Montanide ISA 720 VG. The mice received two booster injections following the first immunization. The vaccine formulations were not toxic, and all of the mice survived until the end of the study. The vaccine elicited a pronounced production of S. mansoni Cathepsin B specific antibodies whereas no antigen-specific antibodies were found in the control animals. Splenocytes proliferated in response to Cathepsin B and produced elevated levels of Th1, Th17, and inflammatory cytokines. These results highlight the potential of S. mansoni Cathepsin B as a promising vaccine candidate for schistosomiasis. The investigation concerning Prx1 is ongoing.

PROJET-CREVETTE: AN INTERNATIONAL COLLABORATION TO REDUCE PARASITIC DISEASE, RESTORE THE ENVIRONMENT AND IMPROVE LIVELIHOODS IN WEST AFRICA THROUGH AQUACULTURE

Susanne H. Sokolow1, Elisabeth Hutttinger2, Shawn Coyle3, James Tidwell1, Kyle Schneider1, Oumar Tall Dia2, Mouhamadane Mbacke Seye1, Djibril S. Faye1, Kevin Lafferty1, Armand Kuris1
1University of California Santa Barbara, Santa Barbara, CA, United States, 220/20 Initiative, California, CA, United States, 3Kentucky State University, Frankfort, KY, United States, 4Ecole Doctorale de la Vie, de la Sante et de l’Environnement, Dakar, Senegal, 5Western Ecological Research Center, Santa Barbara, CA, United States

“Projet-Crevette” is a collaborative research project to investigate novel ways to combat the spread of schistosomiasis in rural Africa. In 1986, the Diama Dam was built on the Senegal River in West Africa in order to stabilize river flow, reduce drought conditions, and support a growing agriculture industry. Within 5 years after dam construction, schistosomiasis spread rapidly, leading to an epidemic that has persisted until today, with > 90% prevalence among some rural villages along the river. River prawns, which at one time were voracious predators of snails, have recently been decimated in the Senegal River due to habitat loss above the Diama Dam. Thus, Projet-Crevette aims to develop an aquaculture program to supplement prawn reproduction and re-introduce native river prawns to the Senegal River basin as predators of snails that carry schistosome parasites. Projet-Crevette has monitored the distribution and abundance of prawns, snails, and schistosome parasites at 11 sites throughout the Senegal River Basin over the course of one full year during 2011. This baseline data will pave the way for development of an innovative parasite-control strategy that promises to simultaneously combat disease transmission, restore the environment, and improve livelihoods by restoration of an artisanal prawn fishery.

IN VITRO, HUMAN EOSINOPHILS DOWN MODULATE PERIPHERAL BLOOD MONONUCLEAR CELLS RESPONSES TO SCHISTOSOMA MANSONI ADULT WORM ANTIGENS

Robert Twewongyere1, Shona Wilson2, Peter Naniima3, Stephen Case1, Edridah M. Tukahebwa4, Alison M. Elliott3, David W. Dunne2
1Department Vet. Pharmacy, Clinical and Comparative Medicine, Makerere University, Kampala, Uganda, 2Department Pathology, Cambridge University, Cambridge, United Kingdom, 3Uganda Virus Research Institute, Kampala, Uganda, 4Vector Control Division - Ministry of Health, Kampala, Uganda

Eosinophils have been regarded as terminally differentiated non-replicating effector cells observed in a number of health disorders including parasitic infections and allergic diseases where they play a beneficial role in the host defence against helminth infections or cause a harmful inflammatory response respectively. In schistosomiasis eosinophils have been associated with direct or indirect killing of schistosomula. However, there is growing evidence that eosinophils can play an additional immunoregulatory role in both adaptive and innate immunity to parasitic infections. Here we report results of a study, using samples from Schistosoma mansoni infected individuals, in which we investigated the effects of co-culturing human eosinophils with peripheral blood mononuclear cells (PBMC)
on in vitro cytokine production in response to S. mansoni adult worm antigen. PBMCs obtained from 26 S. mansoni infected adults were examined for cytokine responses to S. mansoni adult worm antigen (SWA) when stimulated alone or when enriched with autologous eosinophils. Production of IL-4, IL-5 and IL-13 was lower (p=0.017, 0.018 and <0.001 respectively) in PBMC-eosinophil cultures than in PBMC-only cultures stimulated with SWA. IL-13, IL-10, IFN and TNF were released in eosinophil-only cultures but none of these cytokines produced by the eosinophils showed a significant association with the observed eosinophil-induced drop in cytokine responses of PBMCs. This preliminary study shows that eosinophils can exert a down-modulatory effect on schistosome specific responses. The mechanism of this immune-modulation remains to be elucidated.

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**SCHISTOSOMA HAEMATOBIIUM RECOMBINANT PROTEINS AS A VACCINE CANDIDATE FOR HUMAN SCHISTOSOMIASIS**

Kwaku B. Ahmed1, Iain W. Chalmers2, Martha Turcott2, Maria Yazdanbakhsh3, Cornelis H. Hokke1, Daniel A. Boakye1, Karl F. Hoffmann2

1Noguchi Memorial Institute for Medical Research, University of Ghana, Accra, Ghana, 2Institute of Biological Environmental Rural Sciences, Aberystwyth University, Aberystwyth, United Kingdom, 3Leiden University Medical Center, Leiden, The Netherlands

In Sub Saharan Africa human schistosomiasis is largely caused by *Schistosoma mansoni* and *S. haematobium*. The current strategy for controlling morbidity of the disease is through mass drug administration using praziquantel, the drug of choice. This dependency of using only one drug can possibly induce praziquantel resistance of the parasite and could render this method of intervention ineffective. This has therefore necessitated the urgency for the development of a vaccine to combat the disease. With the focus of most schistosomiasis vaccine developments on *S. mansoni* parasite, it could be challenging for identified putative vaccine candidates to elicit the required immunological responses in *S. haematobium*-endemic communities in African populations, where the disease is caused by either *S. haematobium* or in regions of co-endemicity. If this condition arises, it could lead to the detriment of full potential of *S. mansoni* vaccine candidates. It will therefore be complementary for vaccine design efforts to strive into proteomic and immunology of *S. haematobium* counterpart as well. Here we discovered 17 orthologs sequences. This bio-informatic lead analysis was performed using tBLASTn search of Wellcome Sanger *S. haematobium* ESTs libraries against predicted proteomes of the parasites in order to identify and classify new vaccine candidates against schistosomiasis. In this work, we have identified and characterized all enzymes involved in histone acetylation and methylation of chromatin modifications. Furthermore, aberrant epigenetic states are often associated with human diseases, leading to great interest in HMEs as therapeutic targets. The availability of the genomic data of *S. mansoni* and *S. haematobium* facilitates the reduction of candidates. In conclusion, if vaccine candidates are identified from *S. haematobium*, it will not only mark a crucial milestone in terms of vaccine development for disease but will drastically facilitate the reduction of schistosomiasis in Africa.

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**VACCINATION WITH RECOMBINANTLY EXPRESSED GLYCAN ANTIGENS FROM SCHISTOSOMA MANSONI INDUCES GLYCAN-SPECIFIC ANTIBODIES AGAINST THE PARASITE**

Nina S. Prasaphanich1, Anthony Luyai2, Megan L. Mickum3, Ziad S. Kawar2, Jamie Heimbarg-Molinaro2, Yi Lasanajak2, Xuezheng Song2, David F. Smith2, Richard D. Cummings3

1Emory University Department of Biochemistry, Emory University Graduate Program in Immunology and Molecular Pathogenesis of the Graduate Division of Biological and Biomedical Sciences, and Emory University Medical Scientist Training Program, Atlanta, GA, United States, 2Emory University Department of Biochemistry, Atlanta, GA, United States, 3Emory University Department of Biochemistry, Emory University Graduate Program in Immunology and Molecular Pathogenesis of the Graduate Division of Biological and Biomedical Sciences, Atlanta, GA, United States

Schistosomiasis caused by infection with the parasitic helminth *Schistosoma mansoni* is a major global health problem due to inadequate diagnosis and treatment, and lack of a vaccine. Vaccine candidates have failed due to the worm’s complex architecture and life cycle, exquisite modulation of host immunity, and our incomplete understanding of antigens targeted during infection. The immune response to schistosomes is primarily directed against glycans, rather than protein antigens, and evidence suggests that glycans could be valuable diagnostic markers and protective vaccine targets. The di- and tri-saccharide motifs LacdiNac (GalNAcβ1,4-GlcNAc; LDN) and fucosylated LacdiNac (GalNAcβ1,4-(Fucα1-3)GlcNAc; LDNF) are expressed throughout the *S. mansoni* life stages and are densely distributed among many glycoconjugates in monomeric form or as repeating units (poly-LDNF). Such determinants are lacking in mammals. LDN and LDNF are antigenic in several *S. mansoni*-infected mammals, yet, how to make such glycans antigenic in the context of a defined vaccine has remained elusive. We have developed a recombinant expression system in which a Chinese Hamster Ovary (CHO) cell mutant termed Lec8 expresses repeating forms of LDN (LecBG7) and LDNF (LecBGFTFT) abundantly on its glycoproteins. Immunizing mice with these cells induced glycan-specific antibodies and a sustained booster response. The LecBGFTFT anti-sera were cross-reactive with *S. mansoni* and displayed exquisite specificity for particular presentations of LDNF antigen on glycan microarrays. We are currently investigating the cellular mechanisms supporting this anti-glycan antibody production, including T-cell dependence and memory B cell compartments, and we are using glycan microarrays to more specifically define the structures that comprise antigenic LDNF in *S. mansoni* infection. Our recombinant expression system has proven to be successful at invoking antibodies to the antigenic glycans of *S. mansoni*, and can be adapted to study many other pathogens and novel glycan antigens for use in vaccines and diagnostics.

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**HISTONE MODIFYING ENZYMES AS PUTATIVE DRUG TARGETS FOR SCHISTOSOMIASIS**

Marina M. Mourão1, Luiza F. Andrade1, Laila A. Nahum1, Adhemar Zerlotini1, Raymond J. Pierce2, Guilherme Oliveira1

1Fundação Oswaldo Cruz, Belo Horizonte, Brazil, 2Institut Pasteur de Lille, Lille, France

Histone modifying enzymes (HMEs) play key roles in the regulation of chromatin modifications. Furthermore, aberrant epigenetic states are often associated with human diseases, leading to great interest in HMEs as therapeutic targets. The availability of the genomic data of three *Schistosoma* species provides an opportunity to identify new drug candidates against schistosomiasis. In this work, we have identified and characterized all enzymes involved in histone acetylation and methylation that include: histone acetyltransferases (HATs), deacetylases (HDACs), methyltransferases (HMTs), and demethylases (HDMs). We analyzed the predicted proteomes of the parasites in order to identify and classify the HMEs through computational approaches, mainly by using Hidden
Markov Model profiles. We were able to identify around 60 HMEs with some variation within the three Schistosoma species. From the identified enzymes, 24 were tested individually as therapeutic targets using RNA interference in cultured larval stages (schistosomula) to invalidate each corresponding gene. Although, gene knockdown of up to 90% could be achieved, no phenotype could be observed after 7 days of dsRNA exposure. Loss of motility could be observed as a phenotype for two HDMs after 30 days of dsRNA exposure. In addition, in order to assess the role of genes in the presence of the host environment under immunological pressure, knockdown parasites for four HMEs (HDAC8, KDM1/ KDM2 and PRMT3) were tested *in vivo*. A significant reduction of worm burden (50%) could be observed in mice infected with knockdown parasites for HDAC8 when compared to unspecific control. Finally, egg count was significantly reduced in mice livers for all tested HMEs. In conclusion, our work improved the functional annotation of over 20% of *S. mansoni* HAT and HDAC proteins. Parasites with reduced levels of HDAC8, KDM1/KDM2 and PRMT3, seem to diminish the oviposition and ability to survive (for HDAC8) in the host milieu, indicating that these enzymes could be good target candidates for drug development.

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### SCHISTOSOMIASIS COLLECTION AT THE NATURAL HISTORY MUSEUM (SCAN)

**Aidan M. Emery, Fiona E. Allan, Muriel E. Rabone, David Rollinson**

*The Natural History Museum, London, United Kingdom*

The Natural History Museum, London, maintains one of the largest biodiversity collections in the world and is a WHO Collaborating Centre for the identification and characterisation of schistosomes and their intermediate snail hosts. SCAN, the Schistosomiasis Collection at the Natural History Museum is a new initiative to make existing schistosome and snail host specimens available to the research community, facilitate new monitoring and research projects by providing a sample repository, and make samples available to the research community. Many of our archived schistosome specimens, representing a legacy of decades of field sampling, are suitable for molecular genetic applications, and new schistosome collections, concentrating on the accessible larval stages, are being archived using ambient DNA storage methods. Monitoring and research projects that accompany schistosomiasis control programmes generate specimens and data used to fulfil the objectives of the project. These specimens can also have a value beyond these immediate requirements as new questions emerge, tools improve, or wider comparisons become possible. To facilitate future use, an infrastructure to consolidate, maintain and distribute them is needed. SCAN aims to provide this infrastructure. At present, working primarily with SCORE, the Schistosomiasis Consortium for Operational Research and Evaluation, SCAN is providing support as follows: provision of a central specimen repository for several SCORE sub-projects; assistance with collection and transportation; data entry and consolidation; methods development. Additional to the benefits of an archive, SCAN's collection management priority has immediate advantages for collection, training and data curation activities within SCORE sub-projects. The success of SCAN and depends on the support and trust of control teams, researchers and funding agencies.

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### REDOX BIOLOGY AND DRUG DEVELOPMENT FOR SCHISTOSOMIASIS

**David Williams**

*Rush University Medical Center, Chicago, IL, United States*

Schistosomiasis remains an important neglected disease with 200 million infected individuals. Individual treatment and large-scale control campaigns rely primarily on the use of praziquantel, the only available drug for schistosomiasis treatment. There is concern that praziquantel resistance will evolve and, in the absence of alternative therapies, control measures will be imperiled. Enzymes in the redox pathways of schistosomes have been found to be suitable targets for schistosomiasis drug development and schistosome antioxidant enzymes have been shown to be essential and druggable proteins. Of particular interest is thioredoxin glutathione reductase (TGR), which plays a central role as a multifunctional protein entirely providing the activity of several distinct enzymes present in the human redox network. Therefore, TGR is a redox bottleneck in schistosomes. Oxadiazole 2-oxides have been identified as TGR inhibitors, acting through both nitric oxide production resulting from TGR activity and TGR inhibition. We will present results defining the role of nitric oxide in the action of oxadiazole 2-oxides and other nitrosating agents both in the local context of TGR S-nitrosylation and global context of other schistosome proteins susceptible to modification by nitric oxide. In addition, a rescreen of the NIH Chemical Genomics Center compound library has identified many new classes of small-molecule TGR inhibitors. Mechanisms of action, activities against *in vivo* parasites, and structure-activity relationships of these compounds will be discussed.

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### SIGNIFICANCE OF THE SO-CALLED “APO-AMEBOCYTE PRODUCING ORGAN” IN BIOMPHALARIA GLABRATA

**Samaly S. Souza, Zilton A. Andrade**

*Oswaldo Cruz Foundation, Salvador, Bahia, Brazil*

Internal defense against microorganisms are performed in mollusks by a single cell type: the hemocyte or amebocyte. Their place of origin in *Biomphalaria glabrata* has nowadays become a matter of controversy. Initially, the hypothesis maintained by several authors was that the amebocytes had a multicentric origin. However, more recently it has been postulated that *B. glabrata* amebocytes are instead formed within a central special organ. The initial argument for the Amebocyte Producing Organ (APO) being considered as the locale of origin for hemocyte production in *B. glabrata* was the finding of hyperplasia and mitoses in its cells during the course of *Schistosoma mansoni* infection. The present investigation was concerned with a morphological analysis, with histological, immuno-histochemical, morphometrical, and ultra-structural findings, from the so-called *B. glabrata* APO. Its structure was identified as a collection of epithelial basophilic cells, disposed on one-cell-thick layer or in small round collections, covering a small area of the pericardial surface in the reno-pericardial region. Sometimes it vaguely resembled the epithelial component of the vertebrate juxta-glomerular apparatus of the kidney. During our studies, mitoses were only occasionally found, either in normal or infected mollusks. Also our quantitative studies failed to demonstrate the presence of APO cellular hyperplasia, either in normal or schistosome-infected *B. glabrata*. Therefore, our findings did not provide evidence in support of the so-called APO being considered the central organ for hemocyte production in *B. glabrata*. Multi-focal proliferation of hemocytes was found in many other areas of the mollusk during *S. mansoni*-infection. By contrast, several structural details from the “APO” region in *B. glabrata* were found to be consistent with the suggestion that it is indeed a filtration organ, more related to the kidney, as evidenced in other species of mollusk, such as *Lymnaea truncatula*, rather than bone marrow.
Despite progress on other MDG targets, sanitation coverage continues to fall behind with 2.6 billion people still lacking access to even basic sanitation. More than one billion people still practice open defecation, including an estimated 636 million in India alone. One possible reason for the slow progress in sanitation is the lack of clear, compelling evidence about the effectiveness and cost-effectiveness of sanitation. To date, there is no randomized controlled trial of sanitation interventions to prevent diarrhoea diseases. We describe the design and execution of a large-scale study that seeks to help close the evidence gap on rural sanitation in low-income settings. Using a cluster-randomized trial design, the study aims to assess the effectiveness of a project by Water Aid India to promote the construction and use of individual household latrines in accordance with the Indian Total Sanitation Campaign. The study population consists of 100 villages (about 12,000 people) in a costal district of Orissa, India. The main objective of the trial is to assess whether improved sanitation reduces diarrhoeal and helminth infection among young children. The presentation will emphasis five additional aspects that we believe necessary in designing evaluations of sanitation interventions: (a) comprehensive process evaluation, carefully documenting the manner in which the intervention is actually implemented rather than intended or reported by the program implementers; (b) documenting uptake, the actual use of the intervention by the target population, since there is widespread evidence that latrine use is sub-optimal in India; (c) assessing whether the intervention has actually reduced exposure, a condition to securing health outcomes; (d) spatial analysis and spill-over effects from sanitation interventions, and (d) longer-term assessments due to (i) the longer time required to implement an effective sanitation intervention, (ii) the potential persistence of excreta-related pathogens in the environment even after the widespread uptake of an effective sanitation intervention, (iii) the need to investigate longer-term changes in uptake, and (iv) the need to follow whether safe and effective pit-emptying is underway.

**STUDIES ON THE PRESENCE OF CRYPTOSPORIDIUM SP AROUND WATER TREATMENT PLANTS THAT SUPPLY WATER TO GREATER ACCRA REGION OF GHANA**

George T. Mensah  
Council for Scientific and Industrial Research - Water Research Institute, Accra, Ghana

Cryptosporidium sp a Protozoan parasite. These parasites of the Apicomplexan family were found in association with diarrhea in calves and are water-borne. The organism is second only to rotavirus as a causative agent of diarrhea in newborn calves and infants. As such, it is a potentially serious contaminant in water where cattle graze. In order to estimate the human health risk in cattle rearing areas around water treatment plants, we measured the prevalence of Cryptosporidium oocysts in the fecal matter from four cattle ranges upstream in Jomma near the Densu Dam at Weija in the Ga South Municipality and Kpong in the Lower Manya District all of Southern Ghana. The Modified Ziehl-Neelsen staining technique (MZN) for Cryptosporidium oocysts was used. Of the 320 fecal samples for each species screened, 63 (19.7%) were positive for Cryptosporidium. Prevalence was higher in calves younger than three months of age, as compared to weaned calves and adults. Oocysts were detected in both diarrheic and non-diarrheic samples, with a significantly higher prevalence (p< 0.05) of oocysts shedding in diarrheic samples.

**LACK OF EDUCATION OF HOUSEWIVES CONCERNING THE TRANSMISSION RISKS FACTORS OF TYPHOID FEVER IN THE DR CONGO**

Michel Mandro  
University of Bunia, Bunia, Democratic Republic of the Congo

DRC is still characterized by a critical socioeconomic situation impacting negatively the quality of health services offered to the population, creating a permanent problem of hygiene and prevention of diseases. Every year the Country faces various epidemic outbreaks of avoidable diseases such as typhoid fever (TF). In addition, more than 50 % of Congolese women are illiterate, whilst it is established that the mother's education level is the most determining factor for the family's health and nutritional status. A proper washing of the hands especially before preparation of food, before the meal, after the toilet, access to healthy source of water; safe elimination of human excreta, are the key factors to help reduce the frequency of diseases with oro-faecal transmission. To assess this assertion, we conducted a Community based survey from June 1st, to August 31st, 2008 to assess the knowledge and practices relative to the prevention of TF transmission by questioning 500 domestic women of the City of Bunia in the northeast of the RDC. The Study used a randomization method for the selection of the Housewives by Quarter of the City of Bunia (40% subset of the Quarter population) Of the 500 women interviewed: 288(56.7%) were 20-24 years old; 325(65%) unemployed; 261(52.2%) have not attended school or only primary school; only 198(39%) have some sufficient knowledge of the transmission of TF; 267(53.40%) have little or no knowledge of the good qualities of drinking water; 265(53.00%) are unaware of the rules of food hygiene; only 137(27.00%) practice correctly the washing of the hands; all households use unsafe sources of drinking water and 380(78.8%) among them do not treat the drinking water (boil or treat with Chloramines); 202(42.60%) of women use non hygienic latrines and 85(17%) of households do not have latrines at all. The promotion of hygiene and specifically education of housewives remain fundamental in the improvement of national educational strategies in the DRC. This finding might be relevant to all Stakeholders involved in the fight against TF.
disinfection (56.9 % versus 0.3 %). No difference was found in the use of water filters (about 7 % in both areas). Among urban households, 50 % reported current use of chlorine tablets, vs. 3 % of rural households. Conversely, 60 % of rural households reported current use of liquid chlorine, vs. 11 % of urban households. This clear preference for different products cannot be explained by any difference in intervention approach, and bears further investigation. An engaged commercial sector was able to reach a substantial new market by partnering with NGOs and micro-finance institutions. NGOs can be trained to become effective product demonstrators and micro-distributors. The long-term viability of NGO POU product distribution should be monitored. Commercial partners are now expanding the model in other states in India.

DO THE CHILDREN GETTING WHAT DO THEY NEED TO WASH HANDS IN SCHOOL? EXPERIENCE FROM BANGLADESH
Sadika N. Akhter
International Research Institute, Mohakhali, Dhaka, Bangladesh

Schools are common sites for the spread of gastro-intestinal and respiratory diseases. There are a variety of hygiene interventions linked to hand washing, respiratory hygiene, sanitation and water quality, which have shown some success in preventing and/or reducing these diseases. However, little research has been done on the feasibility and effectiveness of these water, sanitation and hygiene interventions in school settings in Bangladesh. The objective of this study was to understand the current practice regarding hand washing and facilities needed to wash hands from primary school children in a low income country like Bangladesh. The study used data from in-depth interviews and observations conducted with purposively selected school children in Bangladesh. The interviews were conducted with a topic guide line developed based on existing literature and in consultation with study investigators. Transcripts were processed using a thematic-analysis approach. Major findings indicated that increasing hand washing in low resource setting is a complex process, it included that after giving knowledge, knowledge increased but lack of hand washing facilities in school premises influence the practice of hand washing in school. A greater number of informants stated that availability of resources like soap and water supply is important to keep the practice of hand washing in school. Most of the schools do not have fund and capacity to supply soap and water. Children are motivated to wash hands due to school hygiene program but cannot practice their knowledge as schools are not able to supply those facilities. School Authority suggested for better communication with government before implementing intervention so that government can help to generate fund to continue the program.

PRELIMINARY ASSESSMENT OF THE POTENTIAL EFFECTIVENESS OF WATER FILTERS TO REDUCE DIARRHEAL DISEASE BURDEN IN CHILDREN YOUNGER THAN FIVE YEARS OLD IN A PACIFIC ISLAND NATION
Rebecca Psutka1, Patricia Priest1, Tieren Davies2, Teretia Rakunuea2, Andre Reiffer3, Steven Iddings4
1University of Otago, Dunedin, New Zealand, 2Ministry of Health, Bikenibieu, Kiribati, 3World Health Organization, Bikenibieu, Kiribati, 4World Health Organization, Suva, Kiribati

A novel household water treatment device was proposed for use in the Kiribati, a small, remote pacific island nation. In collaboration with the Kiribati Ministry of Health and the WHO (South Pacific) we conducted a field study to understand the extent to which water-borne diarrheal disease is an important health issue, and whether a domestic water filter used in this setting is likely to reduce the incidence of diarrhea. As far as we are aware, this is the first investigation of its kind in any pacific island nation. In this field study of 97 randomly selected households of 802 individuals we found that 7% of participants, and 25% of children under 5 years old had experienced diarrhea in the past month and 7% of children under 5 had experienced diarrhea in the past week. Participants reported high levels of open defecation (59% children) combined with low knowledge of the danger of children's feces and low levels of handwashing, especially after defecation and the handling of children's feces. It is highly likely that contamination from hands and flies goes on to contaminate food and individuals directly leading to high levels of "water-washed" (rather than water-borne) endemic diarrhea. Most individuals we interviewed (86%) reported that their household normally boils their water for drinking. Water samples were highly contaminated, and there was not a statistically significant difference in fecal coliforms between source water and drinking water. In households that boiled their drinking water, it was less contaminated than the source water in only half the samples, suggesting that significant recontamination occurs following boiling, this would likely happen following filtration. Almost all (91% of households) store drinking water and only 24% use safe storage containers, while the remainder access drinking water by dipping dirty cups, vessels, and hands into the water container. Information on behaviour and water quality indicates that the transmission of endemic diarrhoea is likely to be through many pathways other than drinking water, and even treated water is highly susceptible to recontamination. Filtration as a form of household water treatment is likely to have limited effect in this setting.

DEVELOPING A SOCIAL ECOLOGICAL MODEL FOR VIBRIO CHOLERAE TRANSMISSION DYNAMICS IN HAITI: IMPLICATIONS FOR CONTROL STRATEGIES AND PUBLIC POLICY INTERVENTIONS
Annette M. Ruth
University of Notre Dame, Notre Dame, IN, United States

An evaluation of Catholic Relief Service's (CRS) post-earthquake cholera education programming in Haiti was conducted in June 2011 to evaluate the efficacy of their social marketing efforts for cholera prevention. A Knowledge, Attitude, and Practice (KAP) survey implemented throughout Haiti provided cholera incidence data as well as social and behavioral data that indicate sources of disease transmission and water contamination. Evaluation results indicate that there remain gaps in practices such as hand washing and open defecation. These results highlight the importance of monitoring of incidence data and surveillance in countries where poor infrastructure and a lack of proper sanitation facilities necessitate changes in routine behavior to prevent outbreak. Monitoring and prevention, coupled with mathematical compartmental models of transmission dynamics, would enable prediction of future cholera outbreak risk per commune and also would enable the Haitian Ministry of Health, HSPR, and the population to take preventative measures well in advance. Thus, a novel SIR-type social ecological model for Vibrio cholerae transmission dynamics in resource-poor settings has developed, incorporating behavioral data from the KAP evaluation. Furthermore, the behavioral and ecological factors that have been integrated into the base model ensure greater predictive ability at the commune level when the model is back-fitted to prior incidence data. Such models hold the key to affecting control strategies and public policy interventions in ways that ensure a given population is prepared for a potential outbreak when conditions are ideal.
Cholera Outbreaks in Urban Bangladesh in 2011

Farhana Haque1, M. Jahangir Hossain2, Subodh Kumar Kundu3, Abu Mohd. Naser2, Mahmudur Rahman3, Stephen P. Luby1

1International Center for Diarrheal Disease Research, Bangladesh and Institute of Epidemiology, Disease Control and Research (IEDCR), Dhaka, Bangladesh, 2International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh, 3Institute of Epidemiology, Disease Control and Research (IEDCR), Dhaka, Bangladesh, 4Centers for Disease Control and Prevention, Atlanta, USA and International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh

In February 2011, an outbreak of severe diarrhea was reported at a tertiary medical college hospital campus in Bogra District in northwest Bangladesh. In April 2011, a similar outbreak was reported at 3 urban communities in the northeastern district of Kishorganj. We investigated these outbreaks to determine the etiology and pathways of transmission. We visited the tertiary hospital in Bogra and the secondary hospital serving the affected communities in Kishorganj. We listed the admitted cases of severe diarrhea (passage of ≥3 loose stools per day) from the affected areas. We interviewed the admitted cases, physically examined them and collected rectal swabs in bacterial transport media to test for enteric pathogens including Vibrio cholerae. We visited the affected communities to explore the water supply and sanitation. We collected water samples from selected cases’ household taps, tube wells and central pumping stations to test for microbes including Vibrio. We identified 21 cases from Bogra and 84 cases from Kishorganj. The median age was 23 years in Bogra and 21 years in Kishorganj. There were no reported deaths. We isolated Vibrio in 29% (5/17) of the rectal swab samples from Bogra and in 40% (8/20) of the rectal swab samples from Kishorganj. We found Vibrio in 1 out of 8 tap water samples from Bogra and both the tap water samples from Kishorganj. We did not find Vibrio in the water samples from central pumps or tube wells. Ground water extracted from deep tube wells was supplied intermittently through interconnected pipes without treatment in both outbreak areas. We found visible leakages in pipelines in Bogra. Though we found no visible leakages, but pipes passed through open sewers in Kishorganj. The rapid onset of severe watery diarrhea in adults and isolation of cholera organisms from their rectal swabs confirmed that the outbreaks were caused by Vibrio cholerae. The detection of Vibrio in the tap water samples but not from central pumps or tube wells, suggested water contamination in the pipelines. Safe water provision is difficult in municipalities where water supply is intermittent, and where pipes commonly leak; and requires actions outside of the health sector. Collaborative research exploring effectiveness of water purification strategies, including chlorination in areas with intermittent water supply, may identify appropriate approaches for ensuring safe water until improvement of the water and sanitation infrastructure.

Acceptability, Feasibility and Sustainability of Dual Pit Latrines for Rural Households in Bangladesh

Faruqe Hussain1, Stephen P. Luby1, Thomas Clasen2, Elii Leontsini2, Milan K. Barua3, Tania Naushin3, Mahfuzur Rahman3, Samir Ghosh4, Shahnnoor Akter4, Leanne Unicomb1

1International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh, 2Faculty of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London, United Kingdom, 3Department of International Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, 4BRAC Centre, Dhaka, Bangladesh

In Bangladesh, single pit pour-flush latrines that separate human feces away from a child’s environment are commonly installed. But latrine water seals are frequently broken by owners to reduce the volume of water used, thus extending the time for pits to fill. They also break the pit liner and allow latrine contents to overflow. Up to 70% of rural households have visible feces on or around latrines. Latrines with two pits, one for current use and a second for subsequent use when the first is filled and composting, may reduce fecal exposure. We investigated an on-going dual pit latrine construction project to identify the acceptability, feasibility, sustainability, perceived benefits and barriers among impoverished rural households. From October 2010 to April 2011 we enrolled households in a communities where BRAC, a non-governmental organization, implemented a project in 2007 providing dual pit latrines for households that met the Government of Bangladesh definition of ‘hardcore poor’ who shared the cost of transportation and labor. We conducted group discussions (5), interviewed household members (15) and observed their latrine use at four key stages: immediately after installation, upon switching to the second pit when the first was full, during decomposition of the first pit contents, and during emptying and disposing of the first pit contents. None of the households reported latrine overflow or breaking the pit liner or water seals and we observed no visible feces on or around latrines. Participants perceived the main benefit of using the dual pit latrine was when the first pit became full; households immediately started using the back-up pit without having to empty the fresh feces. After approximately one year, households could empty the decomposed pit contents themselves. This saved money and the decomposed excreta of the first pit could be used as manure. Disgust when switching pits was described as a barrier, but did not discourage switching. 8 households successfully completed pit switching at least once. Subsidized dual pit latrines were acceptable to impoverished households in rural Bangladesh and provided safe and effective separation of feces from the environment. The dual pit latrine should be evaluated among other groups on a larger scale.

Motivating Continued Use of Point of Use Water Treatment in Rural Bangladesh

Shaila Arman1, Leanne Unicomb1, Elii Leontsini2, Pavanii K. Ram3, Fazlul K. Chowdhury1, Md. A. Mamun2, Smriti Roy1, Subas C. Biswas4, Rouha Anamika Sarkar2, Sania Ashraf2, Thomas Clasen4, Peter J. Winch2, Stephen P. Luby1

1International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh, 2Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, 3University at Buffalo, State University of New York, Buffalo, NY, United States, 4Faculty of Infectious and Tropical Diseases, London School of Hygiene & Tropical Medicine, London, United Kingdom

Point of use (POU) water treatment can prevent diarrhea, though most interventions fail to achieve continued use in low income countries. In October 2010, we undertook a 13-month pilot POU water treatment intervention with sodium dichloroisocyanurate (NaDCC) tablets in 3 rural communities in Bangladesh. Trained local female community health promoters (CHPs) made 2 household visits and conducted 1 courtyard meeting per month. They encouraged water treatment by appealing to both health benefits and non-health values including convenience, nurture and modernity; addressed barriers; and provided a free supply of NaDCC tablets for daily use. At the last visit, CHPs gave study participants enough NaDCC tablets to last for two months. We assessed barriers to long term POU water treatment uptake and evaluated the effectiveness of the intervention in addressing these barriers during and at the end of pilot intervention activities. We assessed use by testing for residual free chlorine in stored drinking water in 4 households at the 2nd month (=129) and 14th month (=91). We also interviewed mothers of <5 children (n=30) and conducted group discussions (n=6) with both male and female study participants at 14 months. At months 2 and 14, 82% (106/129) and 62% (56/91) of households had detectable free residual chlorine in stored treated water, respectively. Respondents reported that they had become accustomed to the smell, taste and temperature of stored treated water and no longer perceived them as barriers. Respondents reported reduced episodes of illness, especially stomach aches, compared to the previous year, ease of dosing with provided storage vessel and clarity of treated water as factors that motivated continued use. Respondents welcomed regular household visits by CHPs whom they knew as neighbors. They
emphasized that the encouragement provided by the CHPs motivated them to use NaDCC tablets in spite of their initial reaction to stored treated water, their heavy workload, and the reluctance of males to drink treated water. Despite initial concerns with smell, taste and temperature, the majority of study participants continuously treated their water for 13 months and at least one month after active promotion ended. Combining access to effective, easy-to-use water treatment technology with trained, confident and knowledgeable local community health promoters can help improve the uptake of POU water treatment.

THE NEED FOR POINT OF USE WATER TREATMENTS IN AREAS OF PERI-URBAN POVERTY: CASE STUDY OUTSIDE IQUITOS, PERU

Kimberly F. Fal detta1, Derek A. Reighard1, Katie L. Dickinson1, Chloe Q. Wang1, Daniel R. George1, William H. Strosnider2

1Pennsylvania State College of Medicine, Hershey, PA, United States, 2Saint Francis University, Loretto, PA, United States

This study aimed to determine the water collection and home treatment methods in Belen, Iquitos, Peru to elucidate the cause of the high incidence of gastrointestinal diseases in this neighborhood. The results of this study emphasize the importance of point of use water treatment in the home. Belen is a sector of urban poverty on the outskirts of Iquitos, capital of the Loreto region of Peru, where people suffer from gastrointestinal diseases at higher rates than the rest of the city. While many previous studies have highlighted the prevalence of several specific pathogens in this neighborhood, there is little information regarding water-collection methods and treatment in this region. In July 2011, 50 households located in Belen were surveyed using stratified random sampling. Surveys were administered to the head of household in Spanish. In each house, a water sample was collected from the primary drinking water source in a sterile cup with an airtight screw cap then transported to the Universidad Nacional de Amazonica Peruana microbiology lab for fecal coliform (FC) testing. The American Public Health Association guidelines were used for FC testing. The overall rate of contamination was 11.1%. Most of the water samples (92.5%) that were negative for FC were untreated in the home, suggesting that treatment methods used by the local water provider are sufficient at the point of treatment. The positive FC sample results most likely represent contamination during the time of storage or use in the home. No sample that had been treated at home had a positive FC test. Therefore, it will be critical to emphasize to residents of Belen the importance of home water treatment before consuming water. It is possible that water is contaminated during storage, so residents should be urged to keep their water storage containers disinfected as well. Iquitos boasts a water plant with treated water, but the amount of chlorine may not be adequate to cover contamination en route or in the home. Although a region may have access to treated water, residents should continue to practice point of use treatment to ensure the safety of their drinking water. Point of use contamination could be a substantial source for fecal contamination and therefore point of use treatment should be encouraged in the homes of communities of peri-urban poverty similar to Belen.

MEASURING CONTAMINATION OF CHILDREN’S TOYS TO EVALUATE HOUSEHOLD SANITATION IMPROVEMENTS IN RURAL BANGLADESH

Jelena Vujicic1, Pavani K. Ram1, Leanne Unicomb2, Faruque Hussain1, Partha Sarathi Gope3, Zahid Hayat Mahmud2, Jaynal Abedin1, Md. Sirajul Islam2, Stephen P. Luby3

1University at Buffalo, Buffalo, NY, United States, 2International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh, 3Global Disease Detection Branch, Centers for Disease Control and Prevention, Atlanta, GA, United States

The impact of modest improvements in sanitation facilities and practices on community health are unknown. As one step to better understand the potential contribution of such modest improvements, we evaluated whether different levels of sanitation are associated with environmental contamination, as indicated by fecal contamination of children's toys, in rural Bangladesh. We assigned 100 households to the “clean” category if they had an improved latrine and no visible human feces in the living or adjacent space, or to the “less clean” category if they had an unimproved latrine and visible human feces in living or adjacent space. We distributed two non-porous toy balls to each household, washed each toy in 200 ml of Ringer’s solution 3-4 days later, and repeated the process with two new toys. We enumerated fecal coliforms and fecal streptococci in the wash fluid from each toy following standard procedures. Toys from 39 clean households had lower average fecal coliform contamination than toys from 61 less clean households (mean of log10-transformed values 2.4 versus 3.2, p= 0.03). Fecal streptococci contamination was not significantly different between clean and less clean households (mean of log10-transformed values 4.7 versus 4.8, p = 0.37). There was substantial variability in fecal coliform contamination of two toys in the household at the same time (Coefficient of Variation (CV)=36.5), and toys in the household at two different times (CV=37.6). In rural Bangladesh improved sanitation structures and practices were associated with less environmental contamination. Whether this level of difference in environmental contamination improves child health merits further study. The level of variation of this measure was typical for measures of environmental contamination, such as measures of water quality. Sentinel toy contamination may be a useful objective measure to assess the ability of sanitation interventions to reduce fecal contamination.

ETHNOGRAPHIC AND DIARRHEA PREVALENCE RESULTING FROM COMMUNITY BASED WATER TREATMENT SYSTEMS: A COMPARISON BETWEEN FINDING IN UGANDA AND HONDURAS

Jeffery L. Deal

Water Missions International, Charleston, SC, United States

Using a combination of ethnographic methods, healthcare facility chart reviews, and individual waterborne parasite tests, this paper presents the results of a three year investigation comparing the health impacts of providing water treatment systems for communities in Uganda versus Honduras. The Honduras project provided treated water and flush toilets for an approximated 340,000 people. Improvements in health were documented in Honduras by ethnographic findings, parasite surveys, and medical chart reviews, and were confirmed by local public health officials. In Uganda, no such impact was documented despite provision of access to treated water meeting US, EPA standards and the universal knowledge of waterborne illnesses and their causes within the six communities studied. Ethnographic data and subsequent KAP survey data confirmed accurate local understandings of water and health issues as well as significant gaps in the water safety behavior. A total of 19,420 patient interactions were searched for possible waterborne illnesses within both test and control communities and showed no significant differences in rates of diarrhea and/or dysentery. Random selection of subjects for parasite surveys by
rapid stool tests also showed no significant differences between test and control communities. Likely causes of these findings will be discussed including the probability that exposure to contaminated water in Lake Victoria, animal feces, and open-air food sources contribute to ongoing disease loads within the test communities. Methods developed and implemented for this study represent a significant advance over commonly used survey techniques.

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CLINICAL TOLERABILITY OF ARTESUNATE-AMODIAQUINE VS. COMPARATOR TREATMENTS FOR UNCOMPLICATED FALCIPIRUM MALARIA IN SUB-SAHARAN AFRICA, AN INDIVIDUAL PATIENT DATA ANALYSIS

Julien Zwang1, Grant Dorsey2, Djimédi Aboulaye3, Corine Karéma4, Andreas Mårtensson5, Jean-Louis Ndiaye6, Sodiomon Sirima6, Piero L. Olliaro7

1Drugs for Neglected Disease initiative, Geneva, Switzerland, 2Department of Medicine, University of California, San Francisco, CA, United States, 3Malaria Research and Training Center, Department of Epidemiology of Parasitic Diseases, Faculty of Medicine and Pharmacy, University of Science, Techniques and Technology of Bamako, Bamako, Mali, 4Malaria and Other Parasitic Diseases Division-RBC, Ministry of Health, Kigali, Rwanda, 5Infectious Diseases Unit, Department of Medicine, Karolinska University Hospital, Karolinska Institutet, Stockholm, Sweden, 6Department of Parasitology, Faculty of Medicine, Cheikh Anta Diop University, Dakar, Senegal, 7Centre National de Recherche et de Formation sur le Paludisme, Ministère de la Santé, Ouagadougou, Burkina Faso, 8UNICEF/UNDP/WB/WHO Special Programme for Research and Training in Tropical Diseases (TDR), Geneva, Switzerland

The wide-spread use of artesunate-amodiaquine (ASAQ) for treating uncomplicated malaria makes it important to gather and analyse information on its tolerability. An individual-patient tolerability analysis was conducted using data from eight randomized controlled clinical trials conducted at 17 sites in nine sub-Saharan countries comparing ASAQ to other antimalarial treatments. All patients who received at least one dose of the study drug were included in the analysis. Differences in adverse event (AE) and treatment emergent adverse event (TEAE - AE which were absent pre-treatment or worsened with treatment) were analysed by Day 28. A total of 6,179 patients were enrolled (74% <5 years of age), of whom 50% (n=3,113) received ASAQ, 20% (n=1,217) another ACT, and 30% (n=1,849) a non-ACT (combination or single-agent) treatment. Overall, 8,542 AEs and 3,943 TEAEs were recorded. The proportion of patients experiencing at least one gastro-intestinal AE on ASAQ was 43% (higher than with artemether-lumefantrine and dihydroartemisinin-piperaquine at two sites only), and was 23% for any other AEs (not different from other treatments). Specifically, the risk of diarrhoea, vomiting, cough and weakness was lower with artemether-lumefantrine (with the exception of dihydroartemisinin-piperaquine carried a higher risk of pruritus, chloroquine-SP of nausea. Parasitological recurrence increased the risk of occurrence of any AE. No other difference was detected. Comparing AE to TEAE in patients who had pre-treatment occurrence and grades of intensity recorded, AEs were significantly more related to the pre-treatment prevalence of the symptom (p=0.001, Fischer test); AEs overestimated TEAEs by a factor ranging from none to 5-fold. The overall incidence of serious AEs (SAEs) with ASAQ was nine per thousand (29/3,113) and a mortality of one per thousand (three deaths, none drug-related) and similar to other treatments. ASAQ was comparatively well-tolerated. Safety information is important, and must be collected and analysed in a standardised way.

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TOWARDS RATIONAL USE OF ANTIBIOTICS FOR SUSPECTED SECONDARY INFECTIONS IN BURULI ULcer PATIENTS

Ymke J Stienstra1, Yves Baroug3, Sandor Klisz2, Honore Sourou Bankole1, Ghislain Sopoh1, S. Mamo4, Lamine Baba-Moussa7, Willem L. Manson6, Christian Johnson3, Tijp S. van der Werf5

1University Medical Center Groningen, Department of Internal Medicine/ Infectious Diseases, Groningen, The Netherlands, 2Programme National de Lutte contre l’ulcère de Buruli, Cotonou, Benin, 3University Medical Center Groningen, Groningen, The Netherlands, 4Département de Génie de Biologie Humaine, Ecole polytechnique de l’université d’Abomey-Calavi, Cotonou, Benin, 5PNLB, Cotonou, Benin, 6Agogo Presbyterian Hospital, Agogo, Ghana, 7Laboratoire de biologie et de typage moléculaire en microbiologie, Faculté des sciences, Université d'Abomey-Calavi, Cotonou, Benin

The emerging neglected disease Buruli ulcer is treated with streptomycin and rifampicin and surgery if necessary. Frequently other antibiotics are used during treatment. Information on prescribing behavior of antibiotics for suspected secondary infections and prophylactic use was collected together with cultures from ulcers. Of 185 patients that started treatment for Buruli ulcer in different centers in Ghana and Bénin 51 were admitted. Forty of these 51 admitted patients (78%) received at least one course of antibiotics other than streptomycin and rifampicin during their admission. The median number (IQR) of antibiotic courses for admitted patients was 2 (1, 5). Only twelve patients received antibiotics for a suspected secondary infection, all other courses were prescribed for use as prophylaxis during 10 days on average after excision, debridement or skin grafting. Antibiotic regimens varied enormously per indication. Cultures from superficial swabs showed the expected bacteria from a chronic wound, but 13 of the 34 (38%) S. aureus showed to be MRSA. A guide for rational antibiotic treatment for suspected secondary infections or prophylaxis is needed. Adherence to the proposed guideline may reduce and tailor on antibiotic use other than streptomycin and rifampicin in Buruli ulcer patients. It may save costs, reduce toxicity and limit development of further antimicrobial resistance. This topic should be included in general protocols on the management of Buruli ulcer.

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THE ASSOCIATION BETWEEN MALARIA PARASITEMIA, ERYTHROCYTE POLYMORPHISMS, MALNUTRITION AND ANAEMIA IN CHILDREN LESS THAN 10 YEARS IN SENEGAL: A CASE CONTROL STUDY

Roger C. Tine1, Helle H. Hansson2, Magatte Ndiaye2, Michael Allifrango3, Babacar Faye1, Cheikh T. Ndour1, Jean L. Ndiaye1, Pascal Magnussen4, Ib C. Bygbjerg5, Oumar Gaye6

1Service de Parasitologie, Faculté de Médecine de Dakar, Dakar, Senegal, 2University of Copenhagen Faculty of Health Sciences Department of International Health, Immunology and Microbiology, Copenhagen, Denmark, 3Clinique des Maladies Infectieuses, Centre Hospitalier Universitaire de Fann, Sénégal, Dakar, Senegal, 4DBL - Centre for Health Research and Development, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark

Malaria and anaemia (Haemoglobin<11 g/dl) remain frequent in sub-Saharan Africa. The ethology of anaemia is known to be multi-factorial, most studies in malaria endemic areas, have been confined to analysis of possible associations between anaemia and individual factors such as malaria. A case control study involving children aged from 1 to 10 years was conducted to assess some assumed contributors to anaemia in the area of Bonconto Health post in Senegal. Study participants were randomly selected from a list of children who participated in a survey in December 2010. Children aged from 1 to 10 years with haemoglobin level below 11 g/dl represented cases (anaemic children). Control participants were eligible if of same age and their haemoglobin
level was = 11 g/dl. For each participant, a physical examination was done and anthropometric data collected prior to a biological assessment which included: malaria parasitemia infection, intestinal worm carriage, G6PD deficiency, sickle cell disorders, and alpha-talassemia. Three hundred and fifty two children < 10 years of age were enrolled (176 case and 236 controls). In a logistic regression analysis, anaemia was significantly associated with malaria parasitaemia (OR=5.23, 95%CI [1.1-28.48]), sickle cell disorders (OR=2.89, 95%CI [1.32-6.34]), alpha-thalasassaemia (OR=1.82, 95%CI [1.2-3.35]), stunting (OR=3.97, 95%CI [1.93-5.88]), age ranged from 2 to 4 years (OR=0.13, 95%CI [0.05-0.31]) and age = > 5 years (OR=0.03, 95%CI [0.01-0.08]). No association was found between G6PD deficiency, intestinal worm carriage and children's gender. Malaria parasitaemia, stunting and haemoglobin genetic disorders represented the major causes of anaemia among study participants. Anaemia control in this area could be achieved by developing integrated interventions targeting both malaria and malnutrition.

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ASSESSMENT OF THE ULTRASOUND EXAMINATION AS AN EPIDEMIOLOGICAL TOOL FOR THE SECONDARY AND TERTIARY PREVENTION IN A MALIAN RURAL AREA

Yaya I. Coulibaly1, Siaka Y. Coulilbaly2, Boubacar Fofana3, Modibo Keita4, Mahamadou M. Keita4, Ilo Dicko2, Moussa B. Sangare2, Seydou Doumbia2, Adama Dao1, Oumar Maiga1, Samba O. Sowi1, Thomas B. Nutman1, Amy D. Klion1, Adama D. Keita1

1Centre National d’Appui a la lutte contre la Maladie (CNAM), Bamako, Mali, 2Malaria Research and Training Center, Bamako, Mali, 3Hopital du Point G, Bamako, Mali, 4National Institutes of Health/National Institute of Allergy and Infectious Diseases, Bethesda, MD, United States

Rural populations have less access to preventive health care and routine medical tests than residents of large cities. Ultrasonography is a non-invasive method that can aid in the diagnosis of a variety of conditions that require medical intervention. To assess the utility of ultrasound as a diagnostic screening test in a rural setting, five ultrasound examination visits were held in the 11 villages of Sabougou health area in Kolokani district (population 10,999 inhabitants in 2010). The village chief provided the examination site in 10 of the study villages and the local health clinic (CSCOM) was used in Sabougou. The motorcycle of the CSCOM was used, as well as a power generator and a portable ultrasound machine equipped with two probes of different frequencies. All volunteers (n=782) underwent a brief medical examination and ultrasound examination of the abdomen and heart performed by two physicians, including a well-trained ultrasonographer. In addition, women older than 15 years of age (n=416) underwent uterine ultrasound, male subjects of all ages (n=272) underwent scrotal ultrasound and all individuals older than 15 years of age (n=588) underwent thyroid ultrasound. Of the 782 subjects examined, 194 (25%) were less than 15 years old, 64 (8%) were pregnant women and 53 (7%) were > 65 years old. The overall prevalence of cardiac valvular calcification was 5% (39/782), and 0.64% (5/782) subjects had evidence of ventricular dilatation. Among the 272 men examined, 22 cases (8.09%) of subclinical hydrocele, 11 cases (4.04%) of hydrocele, 5 cases of testicular cysts (1.84%), 3 cases of prostatic adenoma (1.1%) and 1 case of prostatic cancer were identified. Two of the 510 women (0.39%) examined had uterine fibromas and one case of uterine malignancy was detected. Among the 64 pregnant women, one case of fetal demise (1.6%) and one case of extra-uterine pregnancy (1.6%) were detected. One case of multiple abnormalities of the thyroid, heart and tests was also observed. A total of 117 and 28 subjects were referred for further management to the Sabougou community health center and the Kolokani district reference center, respectively. Given these results, ultrasound examination in remote rural areas is a practical and non-invasive method for the identification of individuals requiring referral for medical care in rural Mali and its use should be considered at a regional and national scale.

EVALUATION OF NEW TECHNOLOGY MULTIPLEX NUCLEIC ACID TESTS FOR EMERGING AND TROPICAL BLOODBORNE PATHOGENS

Elena Grigorenko1, Carolyn Fisher1, Sunali Patel1, Clark Tibbets1, Moussa Kourout1, Anjan Purkayastha3, Hira L. Nakhashi2, Robert C. Duncan2

1Life Technologies, Corp., Beverly, MA, United States, 2Food and Drug Administration/CBER, Rockville, MD, United States, 3TessArae, LLC, Potomac Falls, VA, United States

Testing of bloodborne pathogens has reduced the risk of transfusion-transmitted infections significantly and use of molecular diagnostic tools has further improved the accuracy of diagnosis. However, with increasing numbers of emerging pathogens that can impact blood safety and the potential for multiple infections in a tropical setting, it is becoming burdensome to conduct separate tests for each agent. Devices that allow simultaneous testing for multiple pathogens (multiplex testing) can potentially streamline blood donation and diagnostic testing. We evaluated two devices, the OpenArray® by Life Technologies Corp. (Carlsbad, CA), and the Resequencing Pathogen Microarray (RPM) by TessArae, LLC (Potomac Falls, VA), for their potential ability to enable multiplex testing in whole blood and plasma samples. The OpenArray® system can perform approximately 3,000 individual real-time polymerase chain reaction tests simultaneously on a microchip-based wafer. The RPM utilizes an Affymetrix® GeneChip® base and a particular arrangement of oligonucleotides. Hybridization to these oligonucleotides leads to sequence identification. We assembled and tested a blood pathogen OpenArray® with primer and probe sets for viruses (HAV-1, HCV, HBV, WNV), parasites (Trypanosoma cruzi, Leishmania, Plasmodium), and Gram negative bacteria. Simultaneous detection of these 4 viruses in plasma specimens and 5 bacterial or parasite species in whole blood specimens was achieved at limits of detection equivalent to individual assays. An RPM was designed and tested with tiles for 22 viruses, 53 prokaryotes and 25 eukaryotes. We correctly identified nucleic acid from 10 pathogens simultaneously. These two multiplex detection devices are highly specific for known bloodborne pathogens. Future testing will reveal whether the sensitivity of these platforms is adequate and whether they are feasible for use in clinical diagnostic and blood donation settings.

CENTRE-BASED CLINICAL MANAGEMENT OF CYSTIC ECHINOCOCOSIS

Thomas Junghanss
University Hospital, Heidelberg, Germany

Cystic echinococcosis (CE) is one of the world's most neglected diseases. The lesions, predominantly in the liver and lungs, develop clinically silently over long periods of time until complications suddenly precipitate. The challenge for health care services, in particular in low-resource settings, is twofold, early detection and treatment of cases and very demanding management of complications of late stage disease. In high-income countries mostly migrants from CE-endemic areas are affected. In this setting CE is not only neglected but also rare and health services are, as a rule, not experienced to diagnose, stage and manage this disease appropriately. A centre-based approach of CE is presented with our interdisciplinary clinical CE unit at Heidelberg University Hospital as an example. Infectious disease / tropical medicine physicians, radiologists, abdominal and thoracic surgeons, gastroenterologists and pathologists work very closely together to stage patients (ultrasound-based cyst classification) and to tailor currently available mostly expert-opinion based treatment options (medical treatment with albendazole, percutaneous cyst-sterilization techniques, surgery and ‘watch and wait’) to the needs of the individual patient. This approach can serve as a model for the clinical management of many other NTDs / NIDs in highly mobile global populations.
DENGUE AND DIARRHEAL DISEASE RISK FACTORS IN RURAL AND SUBURBAN VILLAGES IN THAILAND AND LAOS

Hans J. Overgaard¹, Nsa Dada¹, Nanthasane Vannavong¹, Razak Seidu¹, Ram Rangsin², Theeraphap Chareonviriyaphap³, Audrey Lenhart⁴, Thor Axel Stenström¹

¹Norwegian University of Life Sciences, Ås, Norway, ²Department of Military and Community Medicine, Pramongkutklao Medical College, Bangkok, Thailand, ³Department of Entomology, Faculty of Agriculture, Kasetsart University, Bangkok, Thailand, ⁴Liverpool School of Tropical Medicine, Liverpool, United Kingdom

Diarrheal diseases and dengue fever are major global health problems. Household drinking water (DW) storage can be a determinant for both diseases if water is fecally contaminated and the storage containers provide breeding sites for dengue mosquitoes. The aim of this project is to assess health risks associated with household water storage practices by identifying relationships between household water management, contaminated DW, and mosquito production. In 2011 we collected entomological, bacteriological, and socioeconomic data from one rural and one suburban village in northeastern Thailand and southern Laos, respectively. In rural Thailand, almost 100% of the study population use rainwater as DW. In rural Laos 83% use unprotected wells in the dry season and 92% use rainwater in the rainy season. In the suburban settings DW sources are rainwater and bottled water. There was an average of 2.5 DW containers per household. Only 6% of households in rural Thailand and 43% in rural Laos treat their DW. These figures were higher for the suburban areas (Thailand: 65%; Laos: 84%). Water holding containers were found in >93% of the households, of which ~19% were positive for Aedes aegypti immatures. The most productive containers were cement tanks in both countries, representing 15-17% of all encountered pupae. The Breteau index (BI) was higher in Thailand than in Laos (140 vs 845, p<0.01). In Thailand the BI was higher in the rural area than in the suburban area (147 vs 134, p<0.01), whereas in Laos the opposite was observed (112 vs 56; p<0.01). In Thailand almost 10% of the Aedes positive containers were used for drinking, whereas in Laos as many as 25% were used for drinking. Of the Aedes infested DW containers 26% in Thailand and 51% in Laos were also contaminated with Escherichia coli. The results suggest an intricate relationship between water contamination and mosquito production in household water storage containers. This relationship and the role of domestic water management practices as risk factors for both dengue and diarrheal disease will be discussed.
DISTRIBUTION OF RUBELLA INFECTIONS IN RWANDA SINCE 2003

Zena Uwimana, Jean-Frederic Flandin, Odette Mukabayire
National Reference Laboratory, Kigali, Rwanda

Rubella virus is the causative agent of the disease known more popularly as German measles and is predominantly a childhood disease, endemic throughout the world. Natural infections of Rubella occur only in humans and are generally mild but complications, most commonly polyarthralgia in adult women, do exist. RV infection of women during the first trimester of pregnancy can induce a spectrum of congenital defects in the newborn, known as congenital rubella syndrome (CRS). Since 2003, the National Reference Laboratory of Rwanda has been involved in the surveillance of Rubella infection throughout the country. Cumulative data show that of the 1,778 samples suspected of Rubella, 362 were positively identified by ELISA (21.5%). In Rwanda, geographical data indicates that the Rubella is equally distributed in all provinces of Rwanda with small pockets of infections in Kigali city and Ruhango district, close to the border of Burundi. According to sex and age, infections occur equally in males and females but the majority of infections were in patients older than 5 years old (69.9%).
DECREASING OCCURRENCE OF TROPIC NEUROINFECTIONS: CEREBRAL MALARIA, MENINGOCCOCCAL MENINGITIS AND SLEEPING SICKIES IN SOUTH SUDANESE RURAL HOSPITALS

Emilia Ceploova1, Alexandra Mamova2, George Benca3, Lubica Alumbasi Timon3, Pavlina Bukovinova2, Jana Krulova1, Eva Smrekova2, Jaroslava Sokolova2, Nada Kulkova3, Gertruda Mikolosova3, Inocent Nkonwa1, Vladimir Krcmery1

1Lady of Fatima AAA and SEU Hospital, Gordim, South Sudan, 2Marial Lou Hospital, Tropical programme of Trnava University and St. Elizabeth University, Mapuordit, South Sudan, 3Slovak Tropical Institute, St. Elisabeth University College of Health and Social Sciences, Bratislava, Slovakia, 4Tropical Program in Buikwe, St. Elizabeth University of Health and Social Sciences, Buikwe, Uganda, 5Department of Clinical Disciplines, School of Health Care and Social Work, Trnava University, Trnava, Slovakia, 6St. Elizabeth University of Health and Social Sciences, Bratislava, Slovakia

With advantages of AIDS treatment, 20 years ago some of tropical neuroinfections (e.g. cryptococcal meningitis, tuberculosis meningitis, CNS toxoplasmosis or CMV encephalitis) showed increasing incidence. However, with the era of HAART they again become to be rare. Also severe malaria, manifesting as cerebral malaria with hypoglycemia acidosis and severe anemia, is less frequent after the introduction of intermittent preventive treatment (IPT) and artemisinin in early treatment. In this study, two rural hospitals - Mapuordit in Yirrol country and Gordim in Aweil country (both with 5-20 beds) - were compared. Totally, 18 027 patients were treated in Mapuordit and 9358 in Gordim. Most patients (90%) were treated on outpatients’ basis, the rest as inpatients. Both hospitals offered two doctors with surgical and medical qualification and mobile team from Italy is coming for elective surgeons two times a year. Occurrence of severe malaria in Mapuordit (close to Nile River) cases was more common in Gordim. Trends in malaria occurrence correlated with dry and rainy season. Diarrheal diseases showed slight increase in 2011. Pneumonia represented about 20-25% of all respiratory tract infections. Skin and soft tissue infections (STTI) were fifth common infection after RTI, diarrheal diseases, malaria and sexually transmitted diseases with urinary tract infections showed stable trend and not significant difference between Mapuordit (40-50 a month in average) and Gordim (33-40 per month). Only 1 case of tetanus was detected in both hospitals during observational period. Diphtheria was also sporadic, as well as meningococcal meningitis, tuberculosis and leprosy appeared in 442 patients in Mapuordit and showed a relatively stable trend. HIV was only rarely detected in Mapuordit however only surgical patients were tested as a part of the hospital screening. Commonest ID of both south-Sudanese hospital was malaria and RTI, followed by diarrhea; however, these hospitals are located in different climatic and vegetation areas (close to Nile versus Savannah - Satel type vegetation). Sporadic cases of cholera, meningococcal meningitis and tetanus were observed in Gordim and outbreak of meningitis in 2009 in Aweil country (both with 5-20 beds) - were compared. Totally, 18 027 patients were treated in Mapuordit and 9358 in Gordim. Most patients (90%) were treated on outpatients’ basis, the rest as inpatients. Both hospitals offered two doctors with surgical and medical qualification and mobile team from Italy is coming for elective surgeons two times a year. Occurrence of severe malaria in Mapuordit (close to Nile River) cases was more common in Gordim. Trends in malaria occurrence correlated with dry and rainy season. Diarrheal diseases showed slight increase in 2011. Pneumonia represented about 20-25% of all respiratory tract infections. Skin and soft tissue infections (STTI) were fifth common infection after RTI, diarrheal diseases, malaria and sexually transmitted diseases with urinary tract infections showed stable trend and not significant difference between Mapuordit (40-50 a month in average) and Gordim (33-40 per month). Only 1 case of tetanus occurred during 2010 - 2011. There was observed only 1 case of cerebral malaria, 8 cases of meningococcal meningitis and 1 case of sleeping sickness in period of 2010 - 2011, which is very low in comparison with 32 cases of cerebral malaria, 119 cases of meningococcal meningitis and 9 cases of sleeping sickness in period of 2005 - 2006. We can conclude that in Gordim there was no significant difference in tropical ID incidence before and after South Sudan has been completely opened for foreign travellers. Also, occurrence of tropical neuroinfections, such as sleeping sickness, cerebral malaria and meningococcal meningitis, was sporadic.

SPECTRUM OF TROPICAL NEUROINFECTIONS AND OTHER INFECTIOUS DISEASES IN OUR LADY OF FATIMA HOSPITAL, GORDIM, SOUTH SUDAN, BEFORE AND AFTER DECLARATION OF INDEPENDENCE

Emilia Ceploova1, Alexandra Mamova1, Miriama Balazova1, Petra Stullerova1, Nada Kulkova3, Jaroslava Sokolova2, Vladimir Krcmery3

4Tropical Program in Buikwe, St. Elizabeth University of Health and Social Sciences, Buikwe, Uganda, 5Department of Clinical Disciplines, School of Health Care and Social Work, Trnava University, Trnava, Slovakia, 6St. Elizabeth University of Health and Social Sciences, Bratislava, Slovakia

For 10 years, the area of South Sudan was under control of Sudan’s People Liberation Army (SPLA) and medical services were served by Médecins Sans Fronières (MSF) and other humanitarian organizations after the declaration of independence in May 2011, when country was completely opened for foreign travel. Aim of this study is to compare the spectrum of tropical infectious diseases (ID) before and after independence declaration (2010 vs. 2011) in rural hospital in Gordim, South Sudan. In 2010, together 5097 outpatients were compared to 3612 outpatients treated in 2011. Diagnostic of malaria, geohelmints and tuberculosis (TB) has been performed by microscopy, as rapid diagnostic tests (RDT) were not available until spring 2012. HIV was not observed in none of outpatient department (OPD) patients. Trends in TB were stable with 30 - 60 new cases per month. However, trends in malaria correlated with rainy season, with maximum of 329 and 367 cases in August and September, respectively. Fortunately, complicated malaria (cerebral malaria, renal failure) were extremely rare, probably because of early treatment with artesunate or artesunate/fumefantrin. Second commonest diseases were respiratory tract infections (RTI) with mostly stable occurrence of 260 - 293 cases per month, followed by diarrhea and sexually transmitted diseases (STD). Only 1 case of tetanus occurred during 2010 - 2011. There was observed only 1 case of cerebral malaria, 8 cases of meningococcal meningitis and 1 case of sleeping sickness in period of 2010 - 2011, which is very low in comparison with 32 cases of cerebral malaria, 119 cases of meningococcal meningitis and 9 cases of sleeping sickness in period of 2005 - 2006. We can conclude that in Gordim there was no significant difference in tropical ID incidence before and after South Sudan has been completely opened for foreign travellers. Also, occurrence of tropical neuroinfections, such as sleeping sickness, cerebral malaria and meningococcal meningitis, was sporadic.

HEALING OF CUTANEOUS LARVA MIGRANS AFTER A SINGLE DOSE OF IVERMECTIN IS ACCOMPANIED BY CHANGES IN CYTOKINE PATTERNS IN PERIPHERAL BLOOD

Hermann Feldmeier1, Rieko Shimogawara-Furushima2, Nobuhide Hata2, Angela Schuster1, Nobuo Ohta2, Nobuaki Akao2, Silas Guedes3, Ralf Ignatius4

1Department of Microbiology and Hygiene, Charité University Medicine, Berlin, Germany, 2Tokyo Medical and Dental University, Tokyo, Japan, 3Fundação de Medicina Tropical do Amazonas, Manaus, Brazil, 4Institute of Tropical Medicine and International Health, Berlin, Germany

Cutaneous larva migrans (CLM) is a neglected tropical skin disease caused by the migration of animal hookworm larvae in the epidermis. The disease is common in resource-poor communities in developing countries. Patients with CLM were identified through active case finding in two resource-poor communities in Manaus, Brazil. Patients were diagnosed clinically, and severity of the disease was assessed using a semi-quantitative severity score. Clinical pathology was assessed and hematological and immunological investigations were performed before, and two and four weeks after treatment with ivermectin (200µg/kg). Leucocytes and eosinophils were counted and total serum IgE was determined. The concentration of IL-4, IL-5, IL-10, IFN-γ, TNF-α and TGF-β1 was determined in serum using commercially available ELISA kits. 92 patients were included in the study: 69.6% were male and 30.4% were female. Median age was 9.5 years (IQR 5-44). At baseline, 93.4% of all patients complained about severe pruritus and 73.6% about insomnia. The median severity score was 4 points (IQR 3-6). 87.8% of the patients had eosinophilia. Patients with CLM had significant higher concentrations of IgE, eosinophils, IL-4, IL-5 and IL-10 in serum than age- and sex- matched controls living in the same community. Four weeks after treatment, clinical pathology and eosinophilia decreased significantly. While the serum concentration of IL-4, IL-5 and IL-10 decreased, the concentration of IFN-γ increased significantly. It is concluded that in an impoverished community CLM is associated with considerable morbidity. After treatment with ivermectin, clinical pathology, eosinophilia and cytokine patterns normalize rapidly.
EPIDEMIOLOGY OF FEBRILE ILLNESSES AMONG INFANTS: A CASE CONTROL STUDY IN KINTampo NORTH AND SOUTH DISTRICTS

Princess R. Mahama, Harry Tagbor
Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

Information on the burden and risk factors of febrile illnesses in infancy is scarce. Young infants are relatively protected against infant illnesses during the first six months of life due to the presence of maternal antibodies and foetal haemoglobin, and have received relatively little attention with respect to research and treatment guidelines. To add to the limited data available, this study therefore sought to determine the predisposing factors to febrile illnesses among infants (0-11 months). A case control study was conducted in Kintampo North Municipality and South District. We randomly selected 230 cases and 454 controls from infants with and without infant febrile illnesses and were participants of an ongoing study. Standard questionnaires were administered by blinded interviewers to randomly selected cases and controls. Variables compared in both groups included birth weight, breastfeeding practices, immunization status, household background characteristics and socio-economic status of mothers. Data collected was entered on Microsoft Access and analysed using STATA Version 11. Results of the study showed that malaria was the most prevalent febrile illness. Analysis showed that 70% of febrile cases were above 6 months of age, whilst 91.85% were exclusively breastfed.

A significant difference was shown between cases and controls in terms of age and breastfeeding status. Apparent similarity was shown between cases and controls with respect to birth weight, household background characteristics, immunization status, ITN use and socio-economic levels of mothers. The study showed that infants above 6 months and those not exclusively breastfed are more likely to develop febrile illnesses. Information on the epidemiology of febrile illnesses among infants will be essential for designing and interpreting results of clinical trials of drugs, vaccines and other interventions for this vulnerable group.

PROVIDING HIV EDUCATION TO HEALTH CARE PROVIDERS IN COCHABAMBA, BOLIVIA IN EXCHANGE FOR AMERICAN STUDENT ROTATION AT THE MAIN LOCAL PUBLIC HOSPITAL

Wayra Y. Salazar Moreno1, Rosario Castro2, Edgar Valdez3, Andres Vargas4, Douglas Golenbock1
1University of Massachusetts, Worcester, MA, United States, 2Hospital Clínico Viedma, Cochabamba, Plurinational State of Bolivia, 3Instituto de Desarrollo Humano, Cochabamba, Plurinational State of Bolivia

American physicians in training seldom experience the florid variety of infectious diseases found in developing countries and tropical areas. The management of patients is also different in limited resource settings. The number of reported cases of HIV in Bolivia is growing exponentially, but the number of HIV providers is not. Hospital Clínico Viedma is a public hospital in the heart of South America that serves the most underprivileged population in the city. It also is a referral center for the surrounding tropical areas. In February, 2012, the Division of Infectious Diseases at the University of Massachusetts coordinated an elective for UMass Medical Students in Cochabamba Bolivia. The elective consisted of 3 medical students, 4 faculty members and a Bolivian Infectious Diseases fellow who served as the course coordinator/director. A major goal of the interchange between the two institutions is to provide basic HIV education for Bolivian health care practitioners in the Cochabamba area in Spanish, the main native language. We accomplished this by teaching an intensive course, involving 10 hours of didactics and 5 hours of case presentation. This course was judged to be outstanding by the participants, although they felt that the course should be expanded to teach the care of pediatric patients with HIV. This year we are expanding our course to pediatrics and live state of the art recording. A secondary benefit of expanding our HIV course will be that UMass faculty members will be on site to precept UMass students on elective in Cochabamba in the diagnosis and management of infectious illnesses not commonly seen in the US setting. This DVD will then be available for distribution to additional health care providers in Bolivia and other Spanish speaking countries.

THE HEALTH AND DEMOGRAPHIC SURVEILLANCE SYSTEM IN RURAL WESTERN KENYA: RELEVANCE TO PUBLIC HEALTH AND RESEARCH ENDEAVORS

Peter M. Sifuna, Louis Macareo
USAMRU-K, Kisumu, Kenya

The Health Demographic Surveillance System (HDSS) set up in Kisumu West district, rural western Kenya, is designed to track on a bi-annually basis, evolving health status, demographics and health threats within the catchment area. The program has GPS-located every dwelling unit that exists within the catchment area and has conducted baseline population and housing survey that is monitored through regular surveys. The primary goal for the program is to provide an exhaustive health and demographic data set throughout the catchment area that would be of great interest to potential research collaborators and the local ministry of Health. This paper examines the contributions made by the program towards advancement of public health and research agenda in the catchment area. The Kisumu West HDSS has provided the first steps in developing the linkage of extensive demographic and health data that is tracked over time to patient health care records, beginning with PEFAR subjects and later expanding to all patients in the study area. The linkage will greatly aid in subject/patient tracking and linking of disease and patient to specific locations with a view of designing targeted interventions. During a recent polio outbreak in western Kenya, the Kisumu West HDSS provided information towards the successful implementation of the immunization campaign. The information included baseline population figures for the target population and village maps to aide movement of the MOH staff in the field. The KWHDSS provides an ideal research platform for clinical and epidemiological studies. Specific examples of how the KWHDSS supports these studies-including the Phase III Malaria vaccine trial will be provided to showcase its relevance to research endeavors. In conclusion, the KWHDSS continues to provide a central analytical framework for work on clinical trials, disease surveillance and public health intervention in the Kisumu West District. The longitudinal nature of the KWHDSS allows better matching of volunteers for clinical trials such as those involving post-marketing surveillance and studies assessing the impact of other health care interventions.

BEYOND SIMPLE PREVALENCE: ENHANCED DISCRIMINATION OF INFECTIOUS DISEASE-RELATED DATA PATTERNS BASED ON THREE-DIMENSIONAL ANALYSIS

Ariel Rivas1, Gabriel Leitner2, Daniel Schwarz3, Kevin Anderson4, Renata Piccinini5, Jeanne Fair6, Almira Hoogesteyn7, Prakash Kempaiah1, John Ong’echa1, James Hittner8, Douglas Perkins1
1Center for Global Health - University of New Mexico, Albuquerque, NM, United States, 2National Mastitis Reference Center, Kimron Veterinary Institute, Bet Dagan, Israel, 3Division of Microbiology and Animal Hygiene, Department of Animal Sciences, Faculty of Agricultural Sciences, Goettingen, Germany, 4Population Health and Pathobiology, North Carolina State University, Raleigh, NC, United States, 5Animal Pathology, Università degli Studi di Milano, Milan, Italy, 6Biosecurity and Public Health, Los Alamos National Laboratory, Los Alamos, NM, United States, 7Human Ecology, CINVESTAV, Merida, Mexico, 8Department of Psychology, College of Charleston, Charleston, SC, United States

One important challenge in infectious disease research is to reduce the rate of information loss and/or errors associated with data analysis, including those generated by prevalence-based analyses. To develop and evaluate an alternative approach that addresses these issues, leukocyte
data collected from humans, birds, and bovines affected by different pathogens were assessed with two approaches: (i) non-structured indicators, such as the neutrophil percent, which were determined with bi-dimensional plots and considered the overall (population) disease prevalence, and (ii) structured indicators (indices designed to generate a single line of observations), which were explored with three-dimensional (3D) plots and considered subset-specific prevalence. These approaches revealed that population-based prevalence analyses did not distinguish the leukocyte profiles of disease-negative (D-) and disease-positive (D+) subsets. In contrast, structured indicators assessed with 3D plots revealed patterns which, when used to partition the data, enhanced discrimination of infection: (1) non-overlapping D- and D+ subsets were generated, (2) observations suspected to be false were detected, and (3) in humans infected with malaria, four disease classes were distinguished. Results presented here demonstrate that patterns previously unrecognized in D+ and D- individuals can be identified with structured, 3D analysis, leading to more informative, subset-specific prevalence estimates.

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METRICS OF SUCCESS FOR SOCIAL DETERMINANTS OF HEALTH AND TROPICAL DISEASES

Eileen Stillwaggon1, Larry Sawers2
1Gettysburg College, Gettysburg, PA, United States, 2American University, Washington, DC, United States

Numerous measurement problems emerge when considering neglected tropical diseases (NTDs) on their own, and especially in the social determinants of health (SDH) framework. Burden of NTDs is miscalculated because of underestimation of mortality, long-term sequelae, effects on fertility and on pregnancy, cross-generational effects, and synergies of multiple morbidities. In addition, burden-of-disease methodology specifically abstracts from socio-economic context. Disability-Adjusted Life Years (DALYs) attempt to aggregate the effects of every disease on mortality and morbidity, based on prevalence and specific effects of each disease. DALYs were derived so that a life has the same value, and a disease has the same burden, regardless of place of residence, occupation, or income. The SDH framework embodies a different principle of fairness that requires society to prioritize problems of poor and marginalized people. In the SDH approach, it is necessary to allocate investment disproportionately to diseases of poverty and also to the structural determinants that promote poor health in poor populations. Invisibility of socially excluded populations and their health concerns is another methodological challenge. National and subnational averages can show important progress in achieving disease-reduction goals, while obscuring the persistence of NTDs and the concentration of multiple NTDs in family and community clusters. Global campaigns, including the Millennium Development Goals, state targets as national and global averages. Reliance on them as sole indicators of progress in disease reduction reinforces invisibility of persistent clusters afflicted with multiple morbidities of diseases of poverty, even while national statistics improve. ‘Elimination as a public health problem’ is a term that definitionally could be at odds with the spirit and practice of reducing health inequities. Continued existence of a public health problem’ is a term that definitionally could be at odds with the spirit and practice of reducing health inequities. Continued existence of a public health problem’ is a term that definitionally could be at odds with the spirit and practice of reducing health inequities. Continued existence of a public health problem’ is a term that definitionally could be at odds with the spirit and practice of reducing health inequities. Continued existence of a public health problem’ is a term that definitionally could be at odds with the spirit and practice of reducing health inequities.

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RECLAIMING THE ‘BETTER HEALTH FOR ALL’ MANDATE: A CASE FOR INTEGRATING GLOBAL HIV PROGRAMMING WITH COMPREHENSIVE PRIMARY HEALTH CARE SYSTEMS IN SUB-SAHARAN AFRICA

Kenechukwu O. Chudy-Onwugaje
Simon Fraser University, Burnaby, BC, Canada

Twelve years into the twenty-first century, our world is still grappling with an HIV/AIDS epidemic that has placed great strain on global human and material resources and compounded human suffering; especially in sub-Saharan Africa. While the discovery and improved access to antiretroviral therapy and allied treatment has significantly reduced mortality and morbidity from this disease, it is still positioned to continue to garner prime attention in health discourse and in the allocation of global resources. However, competing health demands in developing countries such as the rising threat of non-communicable diseases and often-neglected communicable diseases amidst a slowly recovering global financial economy are timely prompts for a careful reconsideration of the prevailing approach to HIV funding and programming which has so far privileged this disease to the detriment of overall health. At this critical juncture, a re-evaluation is invaluable if we are to make the smart health investment decisions that would protect better health in the future. I argue that a departure from the current vertical nature of HIV programming is needed to curb its detraction from the development of effective health systems that are sensitive to the totality of local health realities and needs in sub-Saharan Africa. Using support from relevant literature, I trace the evolution of this vertical handling of HIV, its origins in the selective health care model that eclipsed the Alma Ata affirmation and its deleterious effects on health systems; present policy options and recommendations in making the case for an integration of HIV programming with comprehensive primary health care and discuss some of the few available cases that have pursued integration in various forms. Despite the litany of practical difficulties that may dissuaded a global adaptation of such integration, this step is vital if developing countries are to achieve sustainable, efficient and locally owned comprehensive health systems capable of safeguarding better overall health.

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COMBINING HIV/AIDS AND MALARIA INDICATOR SURVEYS IN TANZANIA TO LEVERAGE EXPERTISE AND MAXIMIZE EFFICIENCY IN LARGE HOUSEHOLD SURVEYS

Erasmus Malekela1, Albina Chuwa2, Mohamed Rajab3, Anne Cross4, Joanna Lowell4, Aldegunda Komba5, Geoffrey Somi6, Renata Mandike6, Abdul-wahid Al-mafazy7, Mary Kibona8, Jessica Kafuko9, Ritha Njau10, Gilly Arthur8, Raz Stevenson1, Peter McElroy11
1United States Agency for International Development/Tanzania, Dar es Salaam, United Republic of Tanzania, 2National Bureau of Statistics, Dar es Salaam, United Republic of Tanzania, 3Office of Chief Government Statistician, Zanzibar, United Republic of Tanzania, 4ICF Macro, Calverton, MD, United States, 5National AIDS Control Programme, Dar es Salaam, United Republic of Tanzania, 6National Malaria Control Programme, Dar es Salaam, United Republic of Tanzania, 7Zanzibar Malaria Control Programme, Zanzibar, United Republic of Tanzania, 8Centers for Disease Control and Prevention-Tanzania, Dar es Salaam, United Republic of Tanzania, 9United States Agency for International Development/Tanzania and President’s Malaria Initiative, Dar es Salaam, United Republic of Tanzania, 10WHO/Tanzania, Dar es Salaam, United Republic of Tanzania, 11Centers for Disease Control and Prevention-Tanzania and President’s Malaria Initiative, Dar es Salaam, United Republic of Tanzania

Development assistance to Tanzania for HIV/AIDS and malaria ($2.3 billion in 2005-10) has permitted intensive scale-up of multiple interventions. Separate, labor-intensive, nationally-representative household surveys are a cornerstone of monitoring and evaluation (M&E) for National AIDS and Malaria Control Programs. In 2007/8 and 2011/12, the National Bureau of Statistics (NBS) and Zanzibar’s Office of Chief Government Statistician (OCGS) succeeded in meeting the needs of multiple stakeholders by creating a combined Tanzania HIV/AIDS and Malaria Indicator Survey (THMIS). The THMIS used a two-stage probability sample design implemented by NBS/OCGS, overseen by the Tanzania Commission for AIDS, with technical guidance from Mainland and Zanzibar malaria and AIDS control programs, and MEASURE DHS. Demographic data and HIV/AIDS and malaria knowledge and attitudes, risk behaviors, and intervention use were collected from adults aged 15-49 years. Dried blood spots for HIV testing of adults and malaria rapid tests, thick blood films, and hemoglobin measurement for children were prepared from...
fingerstick capillary blood specimens. In December 2011, data collection for the 2011/12 TMHS was initiated in over 9,700 households, with approximately 10,800 women and 8,000 men expected to be interviewed and tested for HIV and 7,500 children tested for malaria and anaemia by April 2012. The 2007/8 HIV and malaria prevalence (5.7% and 17.7%, respectively) will be compared to 2011/12 estimates. The TMHS required four months to complete data collection compared to three to four months per each stand-alone survey. In-country costs for 2011/12 TMHS ($2.1 million vs. $900,000 for 2003/4 AIDS survey) were shared by two U.S. Government initiatives (74%), Government of Tanzania, and others.

April 2012. The 2007/8 HIV and malaria prevalence (5.7% and 17.7%, approximately 10,800 women and 8,000 men expected to be interviewed) were fingerstick capillary blood specimens. In December 2011, data collection for the 2011/12 TMHS was initiated in over 9,700 households, with approximately 10,800 women and 8,000 men expected to be interviewed and tested for HIV and 7,500 children tested for malaria and anaemia by April 2012. The 2007/8 HIV and malaria prevalence (5.7% and 17.7%, respectively) will be compared to 2011/12 estimates. The TMHS required four months to complete data collection compared to three to four months per each stand-alone survey. In-country costs for 2011/12 TMHS ($2.1 million vs. $900,000 for 2003/4 AIDS survey) were shared by two U.S. Government initiatives (74%), Government of Tanzania, and others. Careful coordination and planning by multiple stakeholders from HIV/AIDS and malaria control produced a single, mutually appealing, nationally representative household survey. This efficiency helps conserve resources needed to document progress toward Millennium Development Goals.

EVALUATIONS OF HEALTH RESEARCH CAPACITY DEVELOPMENT: A REVIEW OF THE EVIDENCE

Donald Cole1, Ritsuko Kakumaa, Sharon Fonn3, Chimaraoke Izugbara4, Margaret Thorogood5, Imelda Bates5
1University of Toronto, Toronto, ON, Canada, 2University of Melbourne, Melbourne, Australia, 3University of Witwatersrand, Johannesburg, South Africa, 4African Population and Health Research Center, Nairobi, Kenya, 5University of Warwick, Warwick, United Kingdom, 6Liverpool School of Tropical Medicine, Liverpool, United Kingdom

Local research and innovation capacity is essential to improving health outcomes prompting significant investment in strengthening health research capacity (HRCS) in low and middle income countries LMICs. Although funding agencies need to show value for money and implementers want to demonstrate HRCS impact, empirical evaluation evidence on HRCS seems scarce. We conducted a scoping review of published evaluations of HRCS to learn lessons about how to assess its effectiveness and impact especially in the longer term. We searched electronic bibliographic databases, reference lists of relevant articles, reports of funding agencies, and websites, and consulted ‘experts’ to identify relevant publications using search terms covering training, mentorship, collaborations, partnerships and networks. We assessed the quality of these evaluations using an instrument developed for reviews of community interventions, and synthesized information about the types and design of the evaluation, and the measurement tools and indicators that were used. We identified 593 publications from health, education and management literature that focused on evaluating the development of health research capacity. 31 were primary studies; only 4 (0.7%) were from LMICs; Ghana (2), Vietnam and Pakistan and the quality of these four studies was variable. None used a comparator group; two were retrospective and two used validated tools. All four studies specified objectives and outcome measures, and stressed the importance of engaging senior managers in developing research capacity. Most provided descriptive analyses including both qualitative and quantitative results. HRCS literature is dominated by recounting of programs and experiences with little published evaluation. A much more substantial evidence base on HRCS interventions reported in peer-reviewed publications is needed before we can develop robust evaluations of impact and value for money of investments in HRCS.

STRENGTHENING RESEARCH CAPACITY WITHIN A GHANAIAN TEACHING HOSPITAL: TEN YEAR PROSPECTIVE STUDY

Daniel Ansong1, Sue Purnell2, George Bedu-Addo1, Alex Yaw Osei-Akoto1, Patrick Karikari1, Tsiri Agbenyega3, Imelda Bates2
1Komfo Anoyke Teaching Hospital, Kumasi, Ghana, 2Liverpool School of Tropical Medicine, Liverpool, United Kingdom, 3Kwame Nkrumah University of Science, Kumasi, Ghana

A strong research culture within teaching hospitals, supported by robust research infrastructure and the ability to demand and utilise research, is essential to promote evidence-based practice and improve health outcomes. For 10 years senior managers and academics at Komfo Anoyke Teaching Hospital (KATH), Ghana have been strengthening the hospital’s research systems and creating a critical mass of research expertise among mid-career health professionals (eg. doctors, nurses, managers, ancillary cadres). The research capacity strengthening programme was designed prospectively using a rigorous implementation research approach for designing and monitoring complex interventions. We adapted a published framework for institutional change and used this to design and monitor the programme in collaboration with key stakeholders. The framework enabled us to use mixed methods flexibly and systematically to plan, regularly review and adapt the programme, to identify and prioritise gaps in KATH’s research systems and infrastructure, and to derive and use indicators to monitor progress in closing the gaps. One component of the programme was an innovative 1 year, part-time Professional Diploma course (UK award) which taught ~20 students/year to undertake a research project important to their department. Our published course evaluation demonstrated graduates were competent and confident to design and conduct research. Through the programme KATH now has a dedicated research support office and administrators, a biostatistics unit and better successes with exams, grants and publications. By 2007 the Diploma course was managed and taught entirely by Ghanaian faculty (KNUST) and it is sustainable through locally generated funds. KATH/university faculty have extended the course to other sites in Ghana and Zimbabwe (2009-12). There has been some impact on clinical practice (eg. birth injury prevention programme; reduced needlestick injuries). Next priorities are to strengthen systems for utilisation of research results and to consolidate departmental research incentives.

STRENGTHENING PATIENT-CENTERED COMMUNICATION THROUGH WORKSHOPS AND SELF-REFLECTION: A CLUSTER RANDOMIZED TRIAL AT PUBLIC HEALTH CENTERS IN UGANDA

Susan Nayiga1, Deborah DiLiberto2, Lilian Taaka1, Christine Nabirye1, Anie Haaland3, Sarah Staedke4, Clare Chandler4
1Infectious Disease Research Collaboration, Kampala, Uganda, 2London School of Hygiene and Tropical Medicine, Kampala, Uganda, 3Institute of Health and Society, University of Oslo, Oslo, Norway, 4London School of Hygiene and Tropical Medicine, London, United Kingdom

The quality of health care at African health facilities is often reported to be poor, linked to low patient attendance and poor health outcomes. In western settings, patient centred approaches, focused on communication between health workers and care seekers, have been advocated to improve quality of services. Such approaches have received little attention in Africa. Rigorous evaluations are required to inform best practice for improving quality of care in resource limited settings. A cluster randomised trial of the PRIME intervention to enhance quality of services is underway at health facilities in rural Uganda. One component of the intervention is a Patient Centred Services package, intended to improve communication with health care seekers, increase attendance at health facilities and improve overall population health indicators. This paper presents the first step in this hypothesised mechanism of change: the
impact of the intervention on health worker communication. We assessed communication between health workers and care seekers at baseline and immediately after the implementation of the intervention at 20 health facilities randomly assigned to intervention or standard care. A total of 26 health care workers and 213 health care seekers participated. Consultations were recorded and rated using the Measurement of Patient Centred Communication method and care seekers were interviewed on exit to provide their assessment of the quality of communication. Patient-centred communication was rated 10% higher (p<0.008) by care seekers consulting with health workers who had recently participated in the PRIME intervention compared with those in the standard care arm. A per protocol analysis suggests this increase may be plausibly attributed to the Patient Centred Services component of the intervention. Improvements to quality of care in resource limited settings may be achieved by approaches that reorient services towards patients.

**INSCALE CLUSTER RANDOMIZED TRIAL EVALUATING THE EFFECT OF INNOVATIVE MOTIVATION AND SUPERVISION APPROACHES ON COMMUNITY HEALTH WORKER PERFORMANCE AND RETENTION IN UGANDA AND MOZAMBIQUE: INTERVENTION DESIGN**

Karin Källander1, James Tibenderana1, Betty Kirkwood2, Zelee Hill3, Daniel Strachan3, Seyi Soremekun2, Raghu Lingam2, Anna Vassal2, Frida Kasteng2, Sylvia Meek4

1Malaria Consortium, Kampala, Uganda, 2London School of Hygiene and Tropical Medicine, London, United Kingdom, 3University College London Centre for International Health & Development, London, United Kingdom, 4Malaria Consortium, London, United Kingdom

If properly trained, equipped and utilized, community health workers (CHWs) delivering integrated community case management (ICCM) for children with diarrhea, pneumonia and malaria can potentially reduce deaths from these infections by 60%. To achieve this outcome it is essential to maintain CHW performance and retention. The inSCALE project aims to increase sustainable coverage of ICCM in Uganda and Mozambique by designing and evaluating innovations for increased CHW supervision and motivation. A combination of participatory research methods were used to identify program gaps, best practices and potential interventions. Quantitative baseline surveys with household members, CHWs and health facility staff were conducted to establish key outcomes and to inform the randomization process. Following extensive formative research and national stakeholder consultations, two interventions were developed in Uganda and one in Mozambique. In Uganda approximately 3500 CHWs in 39 clusters were randomized into a mobile health (mHealth) arm, a community engagement arm, and a control arm. In Mozambique 300 CHWs in 12 clusters will be randomized into a mHealth arm and a control arm. The mHealth interventions in Uganda and Mozambique encompass three main activities: 1) closed user groups to enable free two-way communication between CHWs and their supervisors; 2) weekly ICCM data submission using phones with automated motivational feedback, SMS to supervisors flagging problems for target supervision, and summary ICCM statistics made accessible online to district statisticians; and 3) monthly motivational and constructive SMS to CHWs. The community engagement arm in Uganda will establish health clubs which seek to improve child health and identify health challenges through a community led model with the CHW as its focal point, potentially resulting in 1) improved status and standing of CHWs as key health assets; 2) increased demand for CHW services, and 3) communication to CHWs and other village members that CHW work is important, of value and appreciated. In both countries process evaluation will be conducted and endline surveys will establish impact after 12 months. Main outcomes will be the proportion of sick children appropriately treated, CHW performance and motivation, and cost effectiveness of interventions.

**EVALUATION OF LOW-COST OPEN-SOURCE MHEALTH TOOLS TO SUPPORT A LONITUDINAL PEDIATRIC DENGUE AND INFLUENZA COHORT STUDY IN NICARAGUA: IMPROVING QUANTITY, QUALITY, TRACEABILITY AND TIMELINESS OF DATA COLLECTION AND MANAGEMENT**

William Avilés1, Heather Zornetzer1, Brenda Lopez1, Guillermína Kuan2, Lionel Gresh1, Angel Balmaseda1, Aubree Gordon1, Eva Harris5

1Sustainable Sciences Institute, Managua, Nicaragua, 2Centro de Salud Socrates Flores Vivas, Ministerio de Salud, Managua, Nicaragua, 3Centro Nacional de Diagnóstico y Referencia, Ministerio de Salud, Managua, Nicaragua, 4Division of Epidemiology, School of Public Health, University of California, Berkeley, Berkeley, CA, United States, 5Division of Infectious Diseases and Vaccinology, School of Public Health, University of California, Berkeley, Berkeley, CA, United States

Dengue and influenza are major problems worldwide. The Pediatric Dengue Cohort Study began in 2004 as a collaboration between the University of California, Berkeley, the NGO Sustainable Sciences Institute, and the Ministry of Health in Managua, Nicaragua, to study the natural history and transmission of dengue in children in a community setting in a developing country. In 2007, the Nicaraguan Influenza Cohort Study was added to study the burden and seasonality of pediatric influenza. These studies provide critical epidemiology and transmission data to support detection and prevention approaches around the world, and clinical data and biological samples are used to study viral and immunological determinants of protection and pathogenesis and for development of novel diagnostic assays and algorithms. Currently, ~3700 children aged 0-14 receive medical care through the studies, and data from all clinical visits are systematically recorded. Participants with suspected dengue, influenza or undifferentiated fever are tested by serological, virological, and molecular biological assays, and yearly blood samples are analyzed to detect apparent infections. To facilitate the logistics and operations, a set of information technologies have been implemented by the study team since 2004. These eHealth tools - electronic medical records, patient and sample tracking systems using barcode and fingerprint IDs, and field logistics support tools for household visits - help to maintain quality control and facilitate compliance with established Good Clinical Practice (GCP) and Good Laboratory Practice (GLP) standards. In the 2012 annual blood collection conducted in the study Health Center and through household visits, a mixed methods approach was used to assess the impact of a new mobile data collection and management technology package using low-cost Android tablets and cell phones with the free open-source software ODK Collect and OpenMRS. Specific advantages in data entry/processing time, accuracy and accessibility, user experience, and cost savings were observed compared to paper and PDA-based tools. Results were shared with the Ministry of Health, along with lessons learned about implementation, for potential scale-up for routine data collection needs in the national public health system.

**PREVALENCE OF PURCHASE OF ANTIBIOTICS WITHOUT PRESCRIPTION IN CHILDREN UNDER FIVE IN PRIVATE PHARMACIES CLOSE TO PRIMARY CARE CENTERS IN PERI-URBAN AREAS OF LIMA, PERU**

Lucie Ecker1, Theresa J. Ochoa2, Martha Vargas1, Luis J. Del Valle3, Joaquin Ruiz2

1Instituto de Investigación Nutricional, Lima, Peru, 2Universidad Peruana Cayetano Heredia, Lima, Peru, 3CRESIB, Hospital Clinical/DIBAPS, Barcelona, Spain, 4Universitat Politècnica de Catalunya, Castelldefels, Spain, 5Fundación Clinic para la investigación Biomédica/CIBER Epidemiología y Salud Pública (CIBERESP), Barcelona, Spain

The overuse of antibiotics is associated with the increase of resistant pathogens. In developing countries, antibiotics are commonly purchased
at private pharmacies, which are important suppliers of health tips and low-cost medicines. The objective of this study was to determine the prevalence of purchase of antibiotics without a prescription for use in children under 5 years in private pharmacies of peri-urban areas of Lima, Peru. A survey was applied in adults who bought an antibiotic for a child up to five years in a private pharmacy close to a health center in a peri-urban area of Lima, Peru. 287 of the surveyed bought an antibiotic. The prevalence of purchase without prescription was 13.2%. From these, 1.7% were due to self-medication and 11.5% were due to indication of the pharmacist. The diseases that were most often associated with the use of antibiotics were 45.8% common cold (45.8%) and acute diarrhea (18.5%) and bronchospasm (18.5%). Diseases that were mostly associated with use of antibiotics without prescription was common cold (50%) and watery diarrhea (28.9%). An overuse of antibiotics in children less than 5 years exists in this setting, especially in diagnoses as watery diarrhea, common cold and bronchospasm, mainly due to medical prescription. Self-medication was found in a very low percentage as well as pharmacy personnel recommendation. Training of medical personnel should be prioritized and legislative measures in relation to the purchase of prescription antibiotics should also be strengthened.

**BUILDING CAPACITY FOR CONDUCTING CLINICAL TRIALS IN VIETNAM**

**Peggy L. Coyle**1, Giang D. Dao2, Quang N. Nguyen3, Thang C. Tran4, Dzung V. Truong4, Tien K. Nguyen4, Stephen Mills2

1FHI 360, Durham, NC, United States, 2FHI 360, Hanoi, Vietnam, 3Ministry of Health, Hanoi, Vietnam

Vietnam has seen the emergence of new diseases such as SARS and highly pathogenic avian influenza. Other infectious diseases are endemic. Many parties, including the Vietnamese Ministry of Health (MOH) have a strong interest to conduct clinical trials in Vietnam. The MOH is faced with an urgent challenge to develop a system of oversight that follows the Principles of Good Clinical Practice (GCP), which protects the rights and well-being of human subjects. The MOH also recognizes a need to develop the capacity of local research institutions and their personnel to conduct quality research. With the cooperation of FHI 360 and other partners, MOH has made several interventions to improve capacity to conduct clinical trials in Vietnam. As part of the Southeast Asia Influenza Clinical Research Network (SEA CRN), a new role titled Clinical Trial Support Specialist was developed within FHI 360. Local health professionals were trained in clinical trial regulatory, ethics and operations processes and then provided formal as well as side by side training to study staff of the local hospitals. Existing health system structure and operation were challenges. In a separate but complimentary effort, MOH partnered with FHI 360 to host a series of workshops which included government regulators, and other stakeholders. Open discussions among the groups revealed and prioritized gaps in ethics knowledge, systems, and infrastructure, from which an MOH strategy to build capacity developed. In 2008, MOH issued a GCP document for Vietnam and a regulation to define and operate an Independent Ethics Committee (IEC). The MOH committed to developing an independent ethics system consistent with international standards. Further workshops hosted by the MOH built capacity within the ministry for ethics review. In conclusion, the oversight of clinical trials in Vietnam and the related capacity of Vietnamese institutions have shown significant improvement since 2005. This has been enabled through a coordinated and strategic approach by the MOH and included partnerships with several international institutions. For next steps in the emerging model the MOH will need continuing partnerships that provide technical assistance, monitoring, and support in order to continue this growth.

**REDDUCING HEALTH AND HEALTH SERVICE DISPARITIES IN AN ETHNICALLY DIVERSE, HIGH MIGRATION AREA ON THE THAI-MYANMAR BORDER**

**Peter Kunstadter**

Program for HIV Prevention and Treatment, A. Muang, Chiang Mai, Thailand

Trans-border migration is increasing rapidly worldwide and already involves almost one-quarter billion people. Ethnic diversity among migrants and between migrants and national majority populations into which they move, plus legal eligibility for residence and access to services pose numerous problems for providing health services and control of transmissible diseases. Transborder migration to Thailand, mostly from Myanmar, now accounts for at least 3 million people from many different ethno-linguistic backgrounds. Transborder migration is expected to increase markedly in 2015 following the opening of borders between ASEAN countries. Migrants to Thailand have significantly higher prevalence of malaria, TB and probably HIV than non-migrant residents. PHTP’s Access to Care Project to date has surveyed 998 women and men from Chinese, Hmong and Lahu minorities and from the ethnic Northern Thai majority. Survey data show statistically highly significant differences between different ethnic groups, between minorities and the ethnic majority in socioeconomic characteristics (e.g., income, household possessions, education, Thai language ability, health insurance), and between migrants and non-migrant members of the same ethnic group living in the same communities, with respect to: health information, (e.g., knowledge of HIV transmission, prevention, diagnosis and treatment); use of health services (antenatal care, HIV counseling and testing); and reported constraints to use of health services (e.g., service delays, transportation, direct and indirect costs, lack of knowledge of health and health services, language). Analysis of hospital records allow analysis of differences in delays and interruptions in services and severity of illness associated with ethnicity, location and migration status. Effects of interventions (e.g., to date, health education) tailored by results from surveys (e.g., ethnicity, education, Thai language ability, knowledge of health and health services, migration status) are evaluated by before and after assessment.

**KNOWLEDGE, ATTITUDE AND PRACTICES OF HEALTH CARE WORKERS TOWARDS MALARIA CASE MANAGEMENT IN CHANGING MALARIA TRANSMISSION IN NAMIBIA**

**Davis R. Mumbengegwi**1, Michael L. Conteh2

1University of Namibia, Windhoek, Namibia, 2Namibia Institute of Public Administration and Management, Windhoek, Namibia

Malaria cases in the last 7 years are on the decline in Namibia due to interventions in malaria control implemented by the Ministry of Health and Social Services (MoHSS). There is a shift from control of malaria to its elimination; hence it is necessary for interventions to reflect this shift. Early and proper malaria diagnosis and case management are of paramount importance in reducing the parasite reservoir for elimination of the disease. The knowledge and perceptions of Health Care Workers (HCWs) regarding the prevalence of malaria, its diagnosis and treatment were investigated to provide a basis for aligning the training of health care workers to the objective of elimination of malaria. Three malaria endemic regions of Namibia namely Omusati, Caprivi and Kavango representing different malaria risk strata as well as cultural differences and practices within the country were selected. Six Focus Group Discussions (FGDs) and 7 Key informants Interviews (KI) were conducted. FGDs consisting of 6-10 participants were conducted using semi-structured questions to collect data. Three FGDs were conducted in Caprivi region (Katima Mulilo), 2 in Kavango region (Andara) and 1 in Omusati (Onesi). Each group was primarily composed of registered and enrolled nurses from rural clinics, health centres and regional hospitals. Staff members who were managers
were excluded from the FGDS but were still included in the study and interviewed as key informants. There was a general perception that less malaria cases were presented with 90% of Health Care workers having knowledge of the four species of Plasmodium although 80% of had not participated in a formal, organized malaria case management training session. Only 60% adhered to negative RDT results regardless of persistent symptoms of malaria. There is a need for initial and continuous training of HCWs on malaria diagnosis using RDTs, differential diagnosis and unambiguous case management guidelines to increase their confidence in handling negative results and adherence to RDT results.

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ANALYSIS OF THE AGGREGATE AND DISTRIBUTIONAL WELFARE EFFECTS FROM VACCINE DIFFERENTIAL PRICING, POOLED PROCUREMENT AND POOL MEMBERSHIP

Prashant Yadav1, Natalie Privett2
1WDI, University of Michigan, Ann Arbor, MI, United States, 2New York University, New York, NY, United States

A well designed vaccine pricing architecture can ensure more equitable vaccine prices and result in greater access to new vaccines globally. Pooled procurement is a mechanism that is commonly used to achieve lower vaccine (and medicine) prices for lower income countries and consequently allow country programs to immunize more people with a given budget. Under pooled procurement, several countries bargain collectively as one unit to achieve one (supposedly) lower price. Currently, there is very little if any analytical or empirical research to guide policy around the optimal buy-side market structure for vaccines. Existing vaccine procurement pools are organized in several different ways, each with different implications in terms lowering prices for countries in the pool, and for ensuring more equitable vaccine access globally. Some procurement pools, such as PAHO and GCC, are organized regionally (geographical proximity-based), with high income heterogeneity among the countries in the pool. Other pools such as UNICEF are organized by country income level (income-proximity based), but have to rely on a third party i.e. a UN or multilateral agency led procurement structure. Using game theoretical models this research attempts to answer the following questions: (1) What form of buy-side market structure (single purchasing pool vs. multiple purchasing pools; pools organized by income vs. pools organized by geo-spatial proximity; differential pricing within pools vs. single price within pools) maximizes overall social welfare and vaccine availability? (2) For each type of purchasing structure what is the distribution of welfare benefits across countries in different income groups? and (3) What opportunities exist for improvement in the current organization of global vaccine pricing and procurement that will increase total social welfare, create more equitable distributions of welfare/benefits across the manufacturer(s), low income countries and middle income countries?

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A PROTOCOL TO OPTIMISE MICROSATELLITE DNA AMPLIFICATION OF Trypanosoma brucei gambiense FROM BODY FLUIDS

Jacques Kabore1, Vincent Jamonneau2, Thierry De Meeüs2, Hamidou Ilboudo1, Paul Capevell2, Mamady Camara4, Adrien Marie Belem5, Bruno Bucheton2, Annette Macleod1
1CIRDOS URBIO, Bobo-Dioulasso, Burkina Faso, 2IRD/CIRDOS, Bobo-Dioulasso, Burkina Faso, 3University of Glasgow, Glasgow, United Kingdom, 4PNLTHA, Conakry, Guinea, 5Université Polytechnique de Bobo-Dioulasso, Bobo-Dioulasso, Burkina Faso

Microsatellite genotyping of Trypanosoma brucei gambiense, the causative agent of African trypanosomiasis or sleeping sickness, and population genetics tools, are useful for inferring population parameters such as population size and dispersal. Amplifying parasite DNA directly from body fluids (i.e., blood, lymph or cerebrospinal fluid) allows avoiding costly and tedious isolation phases. It is however associated to increased frequencies of amplification failures (allelic dropouts and/or null alleles). In this paper, we present a study focused on improving microsatellite loci amplification of T. brucei gambiense from Guinea sleeping sickness foci. We checked for the real nature of blank and of apparent homozygous genotypes of parasite DNA directly amplified from body fluids. We tested the effect of three different DNA quantities for different microsatellite loci of trypanosomes from different body fluids. Our results show that some initially blanks and homozygous genotypes happen to be actual heterozygous genotypes. In Guinea, lymph from the cervical lymph nodes, known to contain the highest concentrations of parasites, appeared to provide the best amplification results. Simply repeating the PCR may be enough to retrieve the correct genotype, but we also show that increasing initial DNA content provides better results while undertaking first amplification. We finally propose an optimal protocol for amplifying T. brucei DNA directly from body fluids that should be adapted to local characteristics and/or constraints.

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ECOLOGICAL NICHE MODELS FOR CUTANEOUS AND VISCERAL LEISHMANIASIS IN BRAZIL BASED ON MAXIMUM ENTROPY (MAXENT)

Maria E. Bavia1, Moara S. Martins2, John B. Malone2, Marta M. Silva1, Luciana L. Cardim1, Deborah D. Carneiro1, Jennifer McCarrill2, Michael T. Kearney2
1Universidade Federal da Bahia, Salvador, Brazil, 2Louisiana State University, Baton Rouge, LA, United States

Leishmaniasis are diseases of great medical, social, and economic importance in endemic areas and are considered serious public health problems due to its clinical impact and epidemiological diversity. They belong to the group of neglected diseases intrinsically associated with poverty as well as health iniquities. The goal of this work was to identify environmental and socioeconomic factors that may be associated with the occurrence of cutaneous (CL) and visceral (VL) leishmaniasis in Brazil from 2005 to 2009, using ecological niche models to predict the risk of disease at the municipality level. A GIS database was constructed using records of CL cases by municipality available in the national notifiable diseases information system (SINAN) database. Records from the Brazilian Institute of Geography and Statistics (IBGE) and the Pan-American Health Organization (PAHO) unsatisfied basic needs data for people (UBNp) and housing (UBNh) were used as socioeconomic data variables. Environmental data included long-term normal monthly climate from WorldClim and MODIS remote sensing annual composite image data. Probability distribution models for CL and VL based on environmental and socioeconomic features were executed using Maxent and ArcGIS 10. From 2005 to 2009, a total of 96,351 cases of CL and 13,563 cases of VL were registered by SINAN. No cases of either disease were reported in 83% of municipalities; CL was reported in approximately 13% of the municipalities, mainly in the North and Northeast regions, and VL was reported in less than 1% of the municipalities and mostly in the Northeast. Maxent results showed that variables that contributed most to the environmental model for CL were precipitation of September (26.2%) and annual precipitation (17.3%)(AUC 0.80); for VL, precipitation in October (11.6%) and mean temperature of warmest quarter (14.5%) were the most influencing variables (AUC 0.948). The Maxent socioeconomic model was most influenced by the variables UNBp education (39.6%), UNBH plumbing (11.3%) and number of health units (8.8%) for CL (AUC 0.864) and the variables that most contributed in the VL scenario were human development index (25.7%), literacy rate (24%) and sewage services (18.9%) (AUC 0.928). Results suggest Maxent can be used to generate the probability distribution maps based on limited distribution point data and that models can be used to improve the allocation of resources in control programs.
ASSOCIATION BETWEEN DISSEMINATED LEISHMANIASIS AND POLYMORPHISMS IN LEISHMANIA BRAZILIENSIS STRAINS

Albert Schriefer1, Adriano Queiroz1, Rosana Sousa1, Claudia Heinke1, Luiz Henrique Guimarães1, Paulo Roberto L. Machado1, Edgar M. Carvalho1, Lee W. Riley1, Mary E. Wilson1

1Hospital Universitário Professor Edgard Santos, Universidade Federal da Bahia, Salvador, Brazil, 1Division of Infectious Diseases, School of Public Health, University of California - Berkeley, Berkeley, CA, United States, 2Departments of Internal Medicine and Microbiology, University of Iowa and the Veterans Affairs Medical Center, Iowa City, IA, United States

We have previously described a multiclonal population structure among genotypically polymorphic Leishmania braziliensis from an area with high endemicity for American tegumentary leishmaniasis (ATL) in Bahia, Brazil, named Corte de Pedra. Based on RAPD (Randomly amplified polymorphic DNA) profiles, we also found an association between clinical outcome of ATL and parasite genotypes in this region, indicating a role for the intra-species variability among these microorganisms on form of disease. In order to further explore the hypothesis of association between form of ATL and strain of L. braziliensis, we cloned, sequenced and compared homologous RAPD bands previously explored for genotyping the L. braziliensis of Corte de Pedra. With this strategy we found six genomic loci that were polymorphic between representatives of the different clades (i.e. subpopulations) of parasites described in that region. PCR primer sets were designed for the specific targeting of each locus identified. Using these primers each locus was re-amplified, electrophoresed and had the band corresponding to the ampiclon gel extracted and cloned into pCRII vectors. Then six clones of each locus were sequenced per leishmania isolate. The cloned amplicons permitted identify that the SNPs and indels defining the polymorphisms at each locus segregate within the population of L. braziliensis in Corte de Pedra according to haplotypes. Several SNPs, indels and haplotypes displayed significant associations with disseminated leishmaniasis (DL). In particular, patients infected with L. braziliensis containing certain SNP genotypes and haplotypes found in the locus starting at position 425451 in chromosome 28 presented significantly increased risk ratios for developing DL. Thus this rapidly emerging form of ATL may have its outcome driven in part by the infecting L. braziliensis strain.

ECOLOGY AND TRANSMISSION DYNAMICS OF VISCERAL LEISHMANIASIS IN ETHIOPIA: RESULTS OF A PROSPECTIVE STUDY TO DETERMINE HUMAN INFECTION RATES IN AN ENDEMIC AREA OF NORTH ETHIOPIA

Asrat Hailul1, Workagegnehu Hailul1, Welelta Shiferaw1, Shewaye Belay1, Ayshehem Kassahun1, Asrat Bezuneh1, Hirut Wonglim1, Ibrahim Abassi2, Samar Aramin3, A. Saldaña2, C. Rigg2, R. Rojas2, A. Valderrama2, Jose E. Calzada2
1University of Buenos Aires, Buenos Aires, Argentina, 2Emory University, Atlanta, GA, United States

Visceral leishmaniasis is considered an emerging disease in north Ethiopia where it is associated with seasonal migration of agricultural laborers to endemic areas and HIV/AIDS. A prospective cohort study of VL in North Ethiopia was designed to elucidate the VL infection dynamics in an endemic setting. A cross-sectional survey was conducted around the town of Sheraro during March 2011 involving 4,883 individuals living in 18 villages. Participating households (1,386) were numbered and their coordinates were recorded. Demographic and socio-economic data were collected. Screening for VL by physical and laboratory examination was performed and previously treated VL cases were noted. Exposure to Leishmania was assessed by Leishmanin Skin Test (LST). Sera and dried blood spots were tested by Direct Agglutination Test (DAT) and RT-PCR. The LST rate among 4,554 individuals was 10.1% and remained surprisingly low (35%) among 126 PTCs. Serological screening of 4,788 individuals by DAT identified 3.9% positives. Of 4,757 dried-blood samples tested by RT-PCR, 680 samples (14.3%) were found positive for Leishmania DNA. Of those, 119 (2.5%) harbored over 100 parasites per ml of blood. To validate these findings ITS1/PCR products were sequenced and 90% (19 of 21) were confirmed to be L. donovani. From March 2011 to February 2012, a total of 34 new VL cases (22 males, 12 females) were found amongst the study population. The mean age of these patients was 16.8 ±12.5. Of these 34 cases, 38.2% were DAT positive in March 2011. Similarly, 15.6%, were positive by LST, and 27% were positive by kDNA RT-PCR. Based on these data, the annual incidence of VL in the study localities is at least 7.0/1000. The study is ongoing, more data will accrue and the results of an in-depth analysis will be reported.

SAND FLY DENSITY REDUCTION IS LESS MARKED IN PRECARIOUS HOUSING AFTER INSECTICIDE THERMAL FOGGING

Luis F. Chaves1, A. Saldaña2, C. Rigg2, R. Rojas2, A. Valderrama2, Jose E. Calzada2
1Hokkaido University, Sapporo, Japan, 2Instituto Conmemorativo Gorgas de Estudios de la Salud, Panama, Panama

Insecticide thermal fogging (ITF) is a tool to control vector borne diseases. It is generally assumed that ITF reduces vector density independently of housing conditions. Few studies have been focused on Sand Flies while also looking at housing characteristics. We conducted a 15 month longitudinal study that included two deltamethrin based ITF interventions in 12 of the 24 houses at Comunidad de Trinidad Las Minas, Capira, Panama, an hyperendemic cutaneous leishmaniasis transmission village. During the study we followed sand fly (SF) abundance. We found a 50 to 80% reduction in SF density at fogged houses when compared with control houses, while controlling for seasonal changes in SF abundance associated with rainfall. We found some heterogeneities in the reductions, as abundance changed according to SF species, with Lutzomyia gomezi, L. panamensis, L. dysponeta and L. triamula reducing their density between 40% and 90% after ITF; in contrast to L. trapidoi whose density increased 5% after the ITF. Differences in the impact of ITF were associated with housing quality, the most precarious houses, i.e., those with features that ease insect entrance, had a disproportionally larger SF abundance, in some cases with an increased domiciliary SF density following the ITF. Our results suggest that ITF success potential to control SF density and Leishmaniasis transmission could be dependent on housing quality.

THE ECO-EPIDEMIOLOGY OF TRYPANOSOMA CRUZI INFECTION IN RURAL COMMUNITIES OF THE HUMID CHACO OF ARGENTINA

Victoria Cardinal1, Marcela Orozco1, Gustavo Enriquez1, Leonardo Ceballos1, María Sol Gaspe1, Julián Alvarado-Otegui1, Juan Gurevitz1, Uriel Kitron2, Ricardo E. Gürtler1
1University of Buenos Aires, Buenos Aires, Argentina, 2Emory University, Atlanta, GA, United States

The eco-epidemiology of the domestic transmission of Trypanosoma cruzi in the humid Chaco region has seldom been investigated. We assessed the household distribution of bug, dog and cat infection in two local ethnic groups (Tobas and Creoles) and investigated differences in transmission risks between them, tested the role of domestic dogs and cats as reservoir hosts, and identified transmission risk factors. We conducted a cross-sectional survey of house infestation with Triatoma infestans bugs and T. cruzi infection in bugs, dogs and cats in a well-defined rural area in northeastern Argentina including 323 households.
Bug infection prevalence among 1,869 bugs examined was highest in domiciles (43%) and in storerooms and kitchens (24%), and was marginal elsewhere (<3%). The composite prevalence of infection was similar for 481 dogs (26%) and for 87 cats (29%) that were examined using serology and/or xenodiagnosis. Vector and host infections were highly aggregated at the household level. Using a catalytic irreversible model, the annual force of infection in dogs was three times higher in Toba than in Creole households, in agreement with other transmission indices. The demography of dogs and cats differed between ethnic groups in several respects. Random-coefficient multiple logistic regression analysis showed that infection in dogs increased significantly with age of the dog, number of infected dogs or cats in a household, and the relative abundance of infected bugs. The fraction of infected bugs increased significantly and in a dose-response fashion with number of infected dogs in a household. Infected cats also increased transmission to bugs when no infected dog was present. Our results reveal the persistence of domestic transmission of T. cruzi in northern Argentina, especially among Tobas; the occurrence of a peridomestic transmission cycle, and the key role of dogs and cats as domestic reservoir hosts, risk factors and control targets in the humid Chaco.

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CHAGAS DISEASE: KNOWLEDGE, ATTITUDES, AND PRACTICES AMONG LATIN AMERICAN IMMIGRANTS LIVING IN LOS ANGELES
Mahmoud I. Traina, Daniel R. Sanchez, Salvador Hernandez, Haneen Khamag, Aiman M. Smer, Sheba K. Meymandi
Olive View-University of California Los Angeles Medical Center, Sylmar, CA, United States

This study was undertaken to examine the knowledge, attitudes, and practices associated with Chagas disease (CD) among Latin American immigrants living in Los Angeles. Background: It is estimated that more than 300,000 individuals are infected with CD in the United States (US), though most were infected via vector-borne transmission in Latin America where it is endemic. We assessed the knowledge, attitudes, and practices associated with CD among 2712 individuals in Los Angeles County, ages 18 to 60 years, who had previously resided in Mexico, Central America, or South America for at least 6 months. Sixty-two percent of participants recall seeing triatomine insects in Latin America, and 27% of participants reported being bitten by triatomine insects at least once per year while living in Latin America. Eighty-six percent of participants had never heard of CD. These results were significantly affected by the participant’s country of birth. Of the 13% of participants who had heard of CD, 62% thought CD was a problem in their native country, 55% thought CD was a problem in the US, and 81% thought CD was not serious. Over 90% of participants who had heard of CD would want to be tested and treated for it. In conclusion, the majority of Latin American immigrants residing in Los Angeles recall seeing the insects that transmit CD in their native country, yet they have never heard of CD. Of the participants who had heard of CD, the majority believe it is a problem in their native country and the US but do not believe it is a serious problem overall. Nevertheless, they would want to be tested and treated for CD.

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MODELING THE DISTRIBUTION OF CUTANEOUS LEISHMANIASIS IN BRAZIL BASED ON ENVIRONMENTAL AND SOCIOECONOMIC RISK FACTORS
Maria E. Bavia1, Moara S. Martins2, John B. Malone3, Marta M. Silva1, Luciana L. Cardim1, Deborah D. Carneiro1, Jennifer McCarroll1, Michael T. Kearney2, Joara S. Santos1

1Federal University of Bahia, Salvador, Brazil, 2Louisiana State University, Baton Rouge, LA, United States

Cutaneous leishmaniasis (CL) presents a variety of casual agents, reservoirs and vectors with different transmission patterns. Out of the 12 new world species known to cause disease in humans, seven can be found in Brazil which makes control of this endemic difficult. Thus, the necessity of a new methodology which would consider a better definition of transmission and risk areas. This study aimed to model the distribution of CL at the municipality level in Brazil based on environmental and socioeconomic factors. The GIS database was constructed using records of CL cases available in the national notifiable diseases information system SINAN from 2005-2009; records from the Brazilian Institute of Geography and Statistics (IBGE) and the Pan-American Health Organization (unsatisfied basic needs for people (UBNh) and housing (UBNh)) were used to compile the socioeconomic data. The environmental database was constructed using long-term normal monthly climate data from WorldClim and MODIS annual composite data. Distribution models for CL were executed using Maxent and maps of spatial distribution and prediction models were created in ArcGIS 10. A total of 96,351 cases of CL were registered at SINAN (13% of the municipalities). Cases of CL increased as the number of health facilities and UNBp education increased (p<0.0001) but notification of disease decreased as UNBn improved (drinking water; plumbing; sanitation and electricity (p<0.0001)). CL was inversely correlated with Temperature Seasonality (p<0.0001) and directly correlated with annual precipitation (p<0.0001). The environmental variables that most contributed in the Maxent model were precipitation of September (26.2%) and annual precipitation (17.3%) (AUC 0.80). From the socioeconomic data the most influencing variables were gross domestic product per capita (23%) and literacy rate (22%) (AUC 0.795, IBGE model); sanitation (83.9%; AUC 0.76, UBNB model); subsistence (33.7%) and unemployment (26%; AUC=0.77, UBNB model). A final model was executed combining environmental and socioeconomic data and it was found that the variables contributing in the model were UNBn sanitation (39.6%), UNBp subsistence (11.3%) and annual precipitation (8.8%) (AUC 0.86). Socioeconomic factors may be playing an important role in the occurrence of CL in Brazil and together with environmental features may provide a better understanding of the dynamics of this endemic in Brazil.

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CONGENITAL TRANSMISSION OF TRYPSANOSOMA CRUZI IN ARGENTINA, HONDURAS, AND MEXICO: AN ONGOING STUDY
Pierre Buekens1, Maria-Luisa Cafferata2, Jackeline Alger3, Fernando Althabe4, Jose Belizan4, Yves Carlier4, Alvaro Ciganda2, Eric Dumonteil5, Rubi Gamboa-Leon5, Elizabeth Howard5, Maria Luisa Matute5, Sergio Sosa-Estani5, Carine Truyens5, Dawn Wesson6, Concepcion Zuniga6

1Tulane University, New Orleans, LA, United States, 2UNICEM, Montevideo, Uruguay, 3Instituto Antonio Vidal, Tegucigalpa, Honduras, 4IECS, Buenos Aires, Argentina, 5Université Libre de Bruxelles (ULB), Brussels, Belgium, 6Universidad Autónoma de Yucatán, Mérida, Mexico, 7National Laboratory, Ministry of Health, Tegucigalpa, Honduras, 8Instituto Nacional de Parasitología “Dr. Mario Fatala Chaben”-ANLIS, Buenos Aires, Argentina

This study is to determine the rate of congenital transmission of TcI and non-TcI (including lineages II-VII). TcI is predominant in Mexico and Central America, while non-TcI is predominant in most of South America, including Argentina. Little is known about congenital transmission of TcI. The specific aim of this study is to determine the rate of congenital transmission of TcI compared to non-TcI. We are conducting a prospective study to enroll at delivery, 10,000 women in Argentina, 7500 women in Honduras, and 10,000 women in Mexico. We are measuring transmitted maternal T. cruzi antibodies by performing two rapid tests in cord blood (Stat-Pak, Chembio, Medford, New York, and Trypanosoma Detect, InBios, Seattle, Washington), and, if at least one of the results is positive, we are identifying infants who are congenitally infected by performing parasitological examinations on cord blood and at 4-8 weeks, and serological follow-up at 10 months. We will also perform standard PCR, real-time quantitative PCR, and T. cruzi genotyping on maternal venous blood and on cord blood, and serological examinations on siblings. Study
enrollment has been staggered and began in Tucuman, Argentina in April 2011, and in Intibuca and Santa Barbara, Honduras in May 2011. Study enrollment began in July 2011 in Merida and Valladolid, Mexico. As of April 2012, recruitment numbers per country are as follows: Argentina - 4,280 births; Honduras - 3,634 births (1,739 in Intibuca and 1,895 in Santa Barbara), and Mexico - 4,002 births (2,161 in Merida and 1,841 in Valladolid). Argentina has reported 80 (1.9%) births of seropositive mothers (with at least one positive serological rapid test result in cord blood), Honduras has reported 144 (4.0%) births of seropositive mothers (96 (5.5%) in Intibuca and 48 (2.5%) in Santa Barbara), and Mexico has reported 28 (0.7%) births of seropositive mothers (21 (1.0%) in Merida and 7 (0.4%) in Valladolid).

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GENETIC DIVERSITY AND POPULATION STRUCTURE OF TRYPANOSOMA BRUCEI IN UGANDA: IMPLICATIONS FOR THE EPIDEMIOLOGY OF SLEEPING SICKNESS AND NAGANA

Richard Echodu1, Mark Sistrom2, Jon S. Beadell2, Loyce M Okedi2, Serap Aksoy4, Chineme Enyioha2, John C. K Enyaru6, Elizabeth Opio1, Wendy Gibson6, Adalgisa Caccone2

1Faculty of Science, Gulu University, Gulu, Uganda, 2Department of Ecology and Evolutionary Biology, Yale University, New Haven, CT, United States, 3National Livestock Resources Research Institute, Tororo, Uganda, 4Yale School of Public Health, Yale University, New Haven, CT, United States, 5Department of Biochemistry and Sports Science, College of Natural Sciences, Makerere University, Kampala, Uganda, 6School of Biological Sciences, University of Bristol, Bristol, United Kingdom

Human African Trypanosomiasis has remained a major and long term public health problem in Uganda characterized by recurrent sporadic outbreaks in the traditional endemic areas and continued spread to new unaffected areas. Uganda harbors the two forms of Trypanosoma brucei subspecies; Trypanosoma bbrucei rhodesiense and T. b. gambiense causing two forms of sleeping sickness, the acute and the chronic forms respectively. The third T. brucei subspecies; T. b. brucei is the third leading cause of African Animal Trypanosomiasis or nagana and has a wider geographical distribution. T. b. gambiense remains localized in North West Uganda while T. b. rhodesiense is currently restricted to Central and Eastern regions, although it continues to spread towards the T. b. gambiense foci. All three forms of parasites are closely related subspecies and remain a major challenge to human health and animal production in Uganda. This is the only country where all three taxa occur. Human African Trypanosomiasis has remained a major and long term public health problem in Uganda characterized by recurrent sporadic outbreaks in the traditional endemic areas and continued spread to new unaffected areas. Uganda harbors the two forms of Trypanosoma brucei subspecies; Trypanosoma bbrucei rhodesiense and T. b. gambiense causing two forms of sleeping sickness, the acute and the chronic forms respectively. The third T. brucei subspecies; T. b. brucei is the third leading cause of African Animal Trypanosomiasis or nagana and has a wider geographical distribution. T. b. gambiense remains localized in North West Uganda while T. b. rhodesiense is currently restricted to Central and Eastern regions, although it continues to spread towards the T. b. gambiense foci. All three forms of parasites are closely related subspecies and remain a major challenge to human health and animal production in Uganda. This is the only country where all three taxa occur. Thus, understanding the population structure of T. brucei in Uganda is critical for disease control. We use a newly developed set of microsatellite loci to investigate two important hypotheses regarding the population processes affecting T. brucei in Uganda: 1) Do temporally distinct disease foci originate from similar or distinct populations of T. brucei? 2) Does host species influence the genetic population structure of T. brucei? By investigating these hypotheses we aim to inform on evolutionary processes at the population level, which will assist in developing effective control measures and treatment of T. brucei. Results are based on isolate collections from 18 Ugandan sites including 300 Trypanosoma isolates from infected tsetse, vertebrates and humans.

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INFECTIONOUSNESS OF SMALL RODENTS TO THE SANDFLY LUTZOMYIA LONGIPALPIS REINFORCES THEIR ROLE AS SOURCES OF INFECTION LEISHMANIA (VIANNIA) BRAZILIENSIS

Maria S. Andrade1, Maria E. Brito2, Francisco G. Carvalho3, Ana I. Araujo2, Fabia C. Soares2, Ericka L. Almeida2, Helio F. Valença2, Fernando J. Silva2, Pietra L. Costa2, José C. Miranda1, Jeffrey J. Shaw2, Sinval P. Brandão Filho1

1Universidade de Pernambuco, Recife, PE, Brazil, 2Centro de Pesquisas Aggeu Magalhães, Recife, PE, Brazil, 3Centro de Pesquisas Gonçalo Muniz, Salvador, BA, Brazil, 4São Paulo University, Brasilia, DF, Brazil

There are many records of leishmanial infections detected in wild animals by molecular methods but a major question is: Are they infectious to vectors? The present study was aimed at characterizing the infectiousness of experimental infections of Leishmania (Viannia) braziliensis in the small rodents Necromys lasiurius, Rattus rattus and Nectomys squamipes. These animals are incriminated as the major reservoir hosts of cutaneous leishmaniasis in an endemic area of Pernambuco, northeast Brazil. For these experiments we established colonies of the three rodent species and a colony of Lutzomyia longipalpis in an endemic area of Pernambuco, northeast Brazil. For these experiments we established colonies of the three rodent species and a colony of Lutzomyia longipalpis in an endemic area of Pernambuco, northeast Brazil. For these experiments we established colonies of the three rodent species and a colony of Lutzomyia longipalpis. A total of 30 animals (10 Rattus rattus, 10 Necromys lasiurius and 10 Nectomys squamipes) and a control group of golden hamster Mesocricetus auratus were infected with cultured promastigotes of L. (V). braziliensis (MBOL/BR/2000/CpAM95); a stock previously isolated from the rodent Bolomys lasiurius (Syn. Necromys lasiurius) captured in the endemic study area, as reported previously. An average of 25 female sand flies was used to perform the xenodiagnosis. Ten days after feeding the sand flies were dissected and their intestinal tract was examined for the presence of promastigotes. Samples of the intestine were also preserved and were subsequently tested by Polymerase chain reaction (PCR) tests that were specific for the Lutzomyia cacophony gene and for the subgenus L. (Viannia) spp. Samples of skin, spleen and liver of each experimentally infected animals were tested by PCR for the presence L. (Viannia) DNA. Three Necromys lasiurius, 3 Nectomys squamipes and 5 Rattus rattus were infective for phlebotomine sand flies. The visualization of promastigotes in phlebotomine sand flies was confirmed by the PCR specific for the subgenus L. (Viannia). The results show that these 3 rodent species are infectious and strengthen their incriminated importance as natural reservoirs of L. (V.) braziliensis. They also indicate the potential use of molecular techniques to determine reservoir host infectiousness by comparing parasite load with xenodiagnoses results.

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THE SYLVIC transmission CYCLE OF TRYPANOSOMA CRUZI IN THE HUMID CHACO OF ARGENTINA

Marcela Orozco1 Orozco1, Gustavo Enriquez2, Julián Alvarado-Otegui1, Victoria Cardinal1, Alejandro Schijman1, Uriel Kitron2, Ricardo Gürtl1

1University of Buenos Aires, Buenos Aires, Argentina, 2Emory University, Atlanta, GA, United States

A wide variety of wild mammals (e.g., marsupials, edentates, rodents and primates) are reservoir hosts of Trypanosoma cruzi. Understanding the complex epidemiology of T. cruzi and the variety of transmission cycles requires a representative picture of parasite genetic diversity – currently classified into six Discrete Typing Units (DTUs). We estimated the prevalence and diversity of T. cruzi infection in wild mammals of a well-defined rural area (Pampa del Indio) in Chaco, northern Argentina. A total of 195 mammals from 20 identified species were captured in four surveys conducted between 2008 and 2011 and examined for infection by xenodiagnosis and kDNA-PCR. A total of 27 (14%) were xenodiagnoses-positive: 12 of 31 (39%) Didelphis albiventris opossums and among armadillos, 12 of 29 (46%) Dasypus novemcinctus, 2 of 15 (13%) Tolypeutes matacus, and one of 16 (6%) Chaetophractus
vellerosus. A total of 15 xenodiagnosis-negative animals were kDNA-PCR-positive, including 4 D. albiventeri opossums, 1 Euphractus sexcinctus and 3 D. novemcinctus armadillos, 5 Thylamys pusilla (Chaco fat-tailed opossum), and 2 small rodents (unidentified species). Using SAT-DNA-PCR we confirmed T. cruzi infection in one D. novemcinctus and two T. pusilla positive by kDNA-PCR only. These are the first findings of T. cruzi in T. pusilla and T. c. cattus in Argentina. A PCR-based strategy showed that all opossums were infected with DTU Tcl and all armadillos with TcIII, implying separate parasite transmission cycles. Wild mammals had no evidence of parasite DTUs infecting local domestic dogs, cats or Triatoma infestans bugs (TcV and TcVI). Sylvatic transmission cycles of T. cruzi in the dry and humid Chaco differ in the composition of the main reservoir hosts.

**LEISHMANIA BRAZILIENSIS IS THE ETIOLOGICAL AGENT OF CUTANEOUS LEISHMANIASIS IN LOS MONTES DE MARÍA, COLOMBIA**

Eduar E. Bejarano, Lily Martínez, Margaret Paternina-Gómez, Luis E. Paternina, Alveiro Pérez-Doria

Universidad de Sucre, Sincelejo, Colombia

Cutaneous leishmaniasis (CL) and visceral leishmaniasis (VL) constitute important public health problems in the Caribbean coastal region of Colombia. These clinical presentations of leishmaniasis are endemic in rural and urban areas of the departments of Sucre and Bolívar, especially in Los Montes de María, an area that constitutes the most important CL macrofocus of the Caribbean coastal region. The objective of the present study was to use sequencing of the subtelomeric region to determine the Leishmania species producing CL among the inhabitants of Montes de María. Thirty-six CL patients from the municipalities of Carmen de Bolívar, Macayepo, Morroa, Sincelejo and Ovejas were analyzed, each receiving a direct parasitological examination before samples were taken for parasite culture in NNN medium to allow molecular identification of the species involved. A sequence from the subtelomeric region of approximately 388 bp was obtained, presenting a 99-100% similarity with sequences of the subtelomeric region of three reference strains of L. (V.) braziliensis. It was thus determined that is the species responsible for CL in the Montes de María area. Its presence in the area has important implications in selecting the correct medical treatment to be administered.

**HEALTHY LIVING TO CONTROL CHAGAS DISEASE IN ECUADOR**

Claudia P. Nieto-Sanchez1, Darwin F. Guerrero-Jimenez2, Esteban G. Baus2, Mario J. Grijalva3

1Center for International Studies, Ohio University, Athens, OH, United States, 2Center for Infectious Disease Research, Catholic University of Ecuador, Quito, Ecuador, 3Tropical Disease Institute, Ohio University, Athens, OH, United States

Chagas disease is caused by the parasite Trypanosoma cruzi and transmitted mainly by the feces of triatomine insects. This disease affects ~10 million people mostly in Latin America. Studies conducted since 2002 by our group have described the biological and epidemiological factors that facilitate transmission of T. cruzi in Southern Ecuador, and have determined that insecticide-based control strategies are effective only in the short term due to frequent bug re-infection of treated dwellings. To address this issue, we designed in 2010 the Healthy Living Initiative (HLI), a holistic process aimed at facilitating the socioeconomic development of rural communities affected by Chagas disease. The final goal of the HLI is to design, implement and evaluate a sustainable model to eliminate vectorial Chagas disease transmission in Loja province via improvement of the houses and the peri-domestic areas. This model is based on community organization and socioeconomic participative development as basic conditions to promote human health. So far it has been possible to facilitate process in five areas: health (community promoters and entomological surveillance network); infrastructure (land entitlement and improvements to local water systems and access road); income generation (ecotourism, artisans’ groups, and local products commercialization); capacity building, and safety/security. Based on these advances, the current phase of the HLI identifies characteristics of a Healthy Housing Model adapted to the cultural and social realities of this area. Positive Deviance methodological framework was used with particular attention to existing knowledge, attitudes, and practices (KAP) of houses that have remained bug free during the last four years. In this process the HLI seeks to unite the efforts of various local, national and international organizations active in Loja by integrating their activities to government plans, as well as facilitating families’ participation through critical analysis of their own reality.

**EPIDEMIOLOGY OF ACTIVE TRYPANOSOMA CRUZI TRANSMISSION AND IMPACT OF INSECTICIDE SPRAYING IN AREA IN THE BOLIVIAN CHACO**

Aaron M. Samuels1, Eva Clark2, Gerson Galdos-Cardenas3, Ryan Wiegand1, Lisbeth Ferrufino4, Silvio Menacho5, Jose Gil6, Jennifer Spencer7, Julia Budde1, S. Pamela Cajal8, Ruben Cimino6, Michael Z. Levy9, Ricardo W. Bozo Gutierrez10, Robert H. Gilman3, Caryn Bern1, Working Group on Chagas Disease in Bolivia and Peru1

1Centers for Disease Control and Prevention, Atlanta, GA, United States, 2University of Alabama School of Medicine, Birmingham, AL, United States, 3Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, 4Hospital Universitario Japones, Santa Cruz, Plurinational State of Bolivia, 5Centro de Salud, Eity, Gutierrez, Plurinational State of Bolivia, 6Universidad Nacional de Salta, Salta, Argentina, 7Emory University School of Medicine, Atlanta, GA, United States, 8Universidad Nacional de Salta, Oran, Argentina, 9University of Pennsylvania, Philadelphia, PA, United States, 10Hospital Municipal de Camiri, Camiri, Plurinational State of Bolivia

An estimated 8-10 million people are infected with Chagas disease. Prevention strategies rely primarily on insecticide spraying against vectors and risk factor reduction including housing improvements. We performed a cross-sectional survey to evaluate the prevalence and risk factors for disease acquisition in seven contiguous villages in an area of the Bolivian Chaco where active transmission persists despite an insecticide spraying program that operated from 2000-2007. Furthermore, we attempted to evaluate the effectiveness of the insecticide spraying program by modeling disease incidence on age-specific prevalence using a catalytic model. Survey teams performed a census of the 7 evaluated villages, and collected demographic, socio-economic, and risk factor data. We collected venous blood from 1578 persons aged ≥ 2 years, and performed Indirect Hemagglutination and Weiner ELISA on each sample for Trypanosoma cruzi diagnosis. Discordant results were confirmed by Weiner recombinant antigen ELISA. The population prevalence of T. cruzi infection was 51.8%. We limited our analyses of risk factors to the ≤15 year age group, in which prevalence was 19.5%, and assumed that infection was acquired relatively recently. Preliminary univariate analyses demonstrated statistically significant associations between T. cruzi seropositivity and village of residence (P < 0.0001), roofing material of metal as compared to straw (Odds ratio [OR] 0.57; 95% Confidence Interval [CI]: 0.33-0.99), and household ownership of ducks (OR 1.77; CI: 1.22-2.57). No association between serostatus and history of household insecticide spraying (OR 1.03; CI: 0.63-1.67) was found in univariate analysis, and no significant decrease in risk of infection associated with the spraying campaign was detected in the catalytic model (g(11) = 0.42; P = 0.52). Failure of spraying to yield a decrease in transmission may be due to inadequate spraying, insufficient duration or frequency of the program, insecticide resistance, or reinfection by sylvatic vectors. Multivariate analyses are forthcoming.
EXAMINING LEVEL OF USE OF CHEMOTHERAPY, CHEMOPROPHYLAXIS AND INTERMITTENT-PREVENTIVE TREATMENT OF MALARIA IN PREGNANCY BY PREGNANT WOMEN IN NIGERIA

Obinna Onwuujeke1, Oguchukwu Onwuujeke2, Rebecca Soremekun3

1University of Nigeria, Enugu, Nigeria, 2University of Nigeria Teaching Hospital, Enugu, Nigeria, 3University of Lagos, Lagos, Nigeria

The study assessed the nature of health seeking for chemotherapy and chemoprevention for malaria-in-pregnancy (MIP), especially the acceptability and use of intermittent preventive treatment of MIP by pregnant women attending public and private health facilities. The study was undertaken in Enugu, southeast Nigeria. A total of 647 consenting pregnant women (321 in the public hospitals and 326 in the private hospitals) were administered with structured questionnaires. Data was analyzed for the levels of perceptions, acceptability and use of IPTp amongst the pregnant women. Bivariate analysis was used to examine whether the differences in the variables between pregnant women attending public and private facilities were statistically significant. The results showed that the knowledge about MIP was high among the pregnant women. Pregnant women attending private hospitals were less aware of IPTp as a preventive strategy for MIP (p<0.05), but there was no significant difference in the acceptability of IPTp by the pregnant women in public and private facilities (p>0.05). IPTp was consumed more by pregnant women in the private facilities (76.9%) compared to those in the public facilities (27.6%) (p<0.05). Blood tests were used more by consumers in the private facilities (71.3%) compared to those in the public facilities for diagnosis of MIP (50.2%) (p<0.05). It is concluded that health seeking behaviour for MIP by pregnant women attending private facilities was better than for those attending public facilities. Hence, interventions are needed to improve the management of MIP in public facilities, and also enhancing the services of private providers.
take any malaria prophylactic. None of the patients had used malaria prophylaxis with AP (neither regular nor short AP course). Between 2005-2007, 2095 travelers to Sub-Saharan Africa consulted the Sheba Medical Center pre-travel clinic (total travel days to Sub-Saharan Africa = 134,488). There were no reports of malaria among these travelers. In conclusion, we retrospectively studied a large group of travelers exposed to highly endemic malaria areas. Despite cessation of AP prophylaxis one day after leaving the endemic area none of the travelers developed malaria. In addition, analyzing the passive surveillance data of malaria cases in Israel did not show any *falciparum* malaria case which occurred after AP prophylaxis (regular or short course). Based on pharmacokinetic properties and *falciparum* malaria pathophysiology it is reasonable to recommend use of AP prophylaxis ending one day after leaving the endemic area. Further prospective validation of our findings in larger number of travelers should follow.

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**IMPACT OF INTERMITTENT PREVENTIVE TREATMENT IN PREGNANCY WITH SULFADOXINE-PYRIMETHAMINE ON PLACENTAL INFECTION AND INFANT BIRTH OUTCOMES IN MALAWI**

Julie Gutman1, Dyson Mwandama2, Ryan Wiegand1, Doreen Ali3, Don P. Mathanga2, Jacek Skarbinski1

1Centers for Disease Control and Prevention, Atlanta, GA, United States
2Malaria Alert Center, University of Malawi College of Medicine, Blantyre, Malawi
3National Malaria Control Program, Ministry of Health, Lilongwe, Malawi

Malaria in pregnancy is associated with severe maternal anemia, placental parasitemia, low birth weight, and increased perinatal mortality, especially among primi- and secundi-gravidae. Sulfadoxine-pyrimethamine (SP) is currently recommended for intermittent preventive treatment in pregnancy (IPTp), despite increasing prevalence of SP resistance that might compromise its effectiveness. HIV-uninfected women with a singleton pregnancy were enrolled at delivery and data on number of SP doses during the pregnancy collected via interview and review of the woman’s antenatal care card. The primary outcome was evidence of past or current placental infection by placental histology. Secondary outcomes included malaria parasitemia at the time of delivery in the cord blood, placenta or maternal peripheral blood, and composite birth outcome (any of small for gestational age (SGA) as assessed by Ballard exam, low birth weight, or preterm). Of 713 women enrolled, 22% received <2 SP doses; 33% were primigravid. About one-third reported sleeping under a bednet the previous night. Receipt of <2 SP doses versus ≥2 doses had no impact on placental infection as measured by placental histology (31.5% vs 31.8%, P=0.94) or blood films (3.8% vs 5.9%, P=0.30) at the time of delivery. Receipt of IPTp-SP was associated with a dose dependent protective effect in primigravid women only on the composite birth outcome due to a reduction in SGA; using 0 doses as the comparison, adjusted prevalence ratio (aPR) =0.69 (95% confidence interval (CI) 0.5-1.01), aPRr=0.43 (95% CI 0.3-0.6), and aPR=0.32 (95% CI 0.1-0.9) for 1, 2, and 3 doses, respectively. Receipt of SP was not associated with stillbirths or adverse delivery outcomes. IPTp-SP did not reduce placental infection, but was associated with improved birth outcomes in primigravidae in Malawi, suggesting that IPTp-SP may work primarily by treating infection, rather than prophylaxis. Very few women received 0 doses of SP, so these results may underestimate the true effect of IPTp-SP. IPTp-SP should continue to be provided to pregnant Malawian women, but given the high prevalence of SP resistance in Malawi, alternative antimalarials should be investigated for IPTp.

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**EFFICACY, SAFETY AND TOLERABILITY OF DIHYDROARTESININ-PIPERAQUINE FOR TREATMENT OF UNCOMPLICATED FALCIPARUM MALARIA IN PREGNANCY IN GHANA: A RANDOMIZED, NON-INFERIORITY TRIAL**

Joseph Osarfo1, Harry Tagbor1, Pascal Magnussen2

1Kwame Nkrumah University of Science and Technology, Kumasi, Ghana
2University of Copenhagen, Copenhagen, Denmark

Pregnancy-associated malaria remains a challenge in endemic areas, resulting in low birth weights, maternal anaemia, foetal loss and increased infant morbidity and mortality. A major intervention recommended is effective case management with artemisinin-based combination therapy. One such option is dihydroartesinin-piperaquine for which there is limited efficacy and safety data in pregnancy. With its introduction on the Ghanaian market and anticipated access by pregnant women, we assessed its use for treatment in pregnant women. Pregnant women of gestation 15-32 weeks attending antenatal clinics in a moderate-to-high transmission zone of Ghana were screened for peripheral *falciparum* parasitaemia using rapid diagnostic test (RDT) and microscopy. Those positive in both and eligible were randomized to receive dihydroartesinin-piperaquine or artesunate-amodiaquine. Baseline clinical, haematological and ultrasonographic assessments are conducted. They are actively followed up on days 1, 2, 3, 7, 14, 28, 42, at delivery and at 6 weeks postpartum to ensure adherence to study drugs, assess adverse events, collect blood samples for haematological and parasitological assessments and gather data on neonatal morbidity and mortality. Of a sample size of 904, 254 (28.1%) have been recruited giving a baseline RDT positivity of 14% (254/ 1805) and mean haemoglobin concentration of 10.0mg/dl. Of the day 3 blood films, 23.4% (43/ 184) had parasitaemia while 7.9% (14/177), 1.0% (2/193), 2.3% (4/172) and 0.6% (1/154) of the day 7, 14, 28 and 42 blood films respectively showed parasitaemia. Geometric mean parasite density decreased from 147/ul(CI 134, 162) at baseline to 88/ul(CI 71, 109) on day 3. Polymerase chain reaction work on filter paper blots to differentiate reinfection and recrudescence is yet to be done. Upon completion of the study, we will compare parasitological efficacy at days 28 and 42, low birthweight, maternal haemoglobin, adverse events and foetal loss in the two treatment arms for significance of differences.

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**MALARIA CASE MANAGEMENT PRACTICES IN PHARMACIES AND LICENCED CHEMICAL SHOPS IN GHANA**

David Y. Mensah, Kezia L. Malm, Vivian A. Aubyn, Constance Bart-Plange

Ghana Malaria Control Programme, Accra, Ghana

Malaria is the single most important cause of morbidity and mortality in Ghana, especially among children under five years. Among pregnant women, malaria accounts for 28.1% of OPD attendance in Ghana. About half of the people first seek help from the pharmacies and licensed chemical sellers (LCSs) when they are sick. This study sought to assess the practices of pharmacies and LCSs with respect to malaria in Ghana. A random sample of 216 pharmacies and 306 LCSs were drawn from the register of pharmacies and LCSs in Ghana. A mystery client approach was employed in gathering information. The interviewers who acted as mystery clients were trained using a structured mystery client guide. Scenarios of children under five years, adults and pregnant women with malaria were presented at the pharmacies and licenced chemical shops, based on which purchases of ACTs were made. The data was captured in epidata and analyzed in SPSS. About 63% of LCSs and 56.9% of pharmacies asked the clients about the age of the patient (child under-five, pregnant women and other adults). About 54% of LCSs and 57% of pharmacies visited asked about the symptoms of the patient. Also, 26% of LCSs and 35% of pharmacies asked about the medication history of the patient. About 88% of LCSs and 90% of pharmacies visited recommended some drugs to the
clients after they had presented their symptoms. The rest did not. The rate of purchase of anti-malarials was not associated with the type of facility. The same proportion of LCSs and pharmacies (i.e. 2.3%) referred the clients to a clinic for diagnostic tests. Management practices of pharmacies and LCSs were encouraging however most of them did not ask about the medication history of the clients. Pharmacy council of Ghana should educate the pharmacies and LCSs on the need to ask their clients about the medication history of their illness before prescribing anti-malarials to them.

HOW PATIENTS TAKE MALARIA TREATMENT: A SYSTEMATIC REVIEW OF THE LITERATURE ON ADHERENCE TO ANTIMALARIALS
Katia Bruxvoort1, Catherine Goodman1, S. Patrick Kachur2, David Schellenberg1
1London School of Hygiene and Tropical Medicine, London, United Kingdom, 2Centers for Disease Control and Prevention, Atlanta, GA, United States
Artemisinin-based combination therapies (ACTs) are the first-line drugs for treatment of malaria throughout sub-Saharan Africa, and are becoming increasingly available in the private sector. However, there are concerns about sub-optimal patient adherence which may have consequences for treatment efficacy and the development of antimalarial drug resistance. In order to identify patterns in how patients use antimalarial drugs and highlight gaps in current knowledge, a systematic literature review was performed. A search was conducted in PubMed using MeSH and free text terms. Of 1242 studies initially identified, 40 met the inclusion criteria of providing quantitative data on patient adherence to antimalarials obtained for treatment. Manual search of reference lists and contacting researchers in the field yielded 11 additional studies. Patient adherence to ACTs was assessed in 23 studies, non-artemisinin-based combinations in 12, and chloroquine and other monotherapies in 20. Only two studies involved the private sector. Adherence measurement methods included self-report with and without dose timing, pill counts and biological assays. Although some studies found very high adherence to ACTs, others endeavouring to capture “real life” situations reported adherence of 64-88%. Overall, adherence was higher in studies where consent was obtained at enrolment versus at follow-up, and in studies where patient consultations were observed by the study team. Comparison of results based on different measurement methods showed higher adherence when biological assays were used, but no other clear patterns. Multivariate models in 10 studies found 28 factors associated with adherence, but no factor was significant in more than one study. The suboptimal patient adherence to ACTs obtained in the public sector and the current dearth of data from the private sector represent significant challenges to ensuring ACTs are used appropriately and remain effective. To strengthen future studies, there is a clear need for awareness of the impact of study procedures on adherence outcomes, and the identification of improved measurement methods that are less dependent on self-report.

SONTOCINH AS A GUIDE TO DEVELOPMENT OF DRUGS AGAINST CHLOROQUINE RESISTANT MALARIA
Michael Riscoe1, Sovitj Pou1, Rolf Winter2, Aaron Nilsen1, Jane Xu Kelly1, Yuxin Li1, Joseph Stone Doggett1, Erin Riscoe1, Keith Wegmann1, David Hinrichs1
1Veterans Affairs Medical Center, Portland, OR, United States, 2Oregon Health Sciences University, Portland, OR, United States
Sontochin was the original chloroquine replacement drug, arising from research by Andersag two years after chloroquine (known as “Resochin” at the time) had been shelved due to the mistaken perception that it was too toxic for human use. We were surprised to find that sontochin, i.e., 3-methyl-chloroquine, retains significant activity against chloroquine-resistant strains of Plasmodium falciparum in vitro. We prepared derivatives of sontochin, “pharmacins”, with alkyl or aryl substituents at the 3-position and with alterations to the 4-position side chain to enhance activity against drug resistant strains. Modified with an aryl substituent in the 3-position of the 7-chloro-quinoline ring PH-203 exhibits low nanomolar IC50 values against drug sensitive and multidrug resistant strains and in vivo efficacy against patent infections of P. yoelii in mice that is superior to chloroquine. Our findings suggest that novel 3-position aryl pharmacin derivatives have the potential for use in treating drug resistant malaria. A detailed structure-activity profile will be presented.

WEIGHT BASED DOSING CAUSES SIGNIFICANTLY LOWER CHLOROQUINE CONCENTRATIONS IN YOUNGER CHILDREN
Johan Ursing1, Poul-Erik Kofoed2, Amabelia Rodrigues3, Staffan Eksborg4, Lars Romro5
1Karolinska Institutet, Stockholm, Sweden, 2University of Southern Denmark, Kolding, Denmark, 3Bandim Health Project, Bissau, Guinea-Bissau, 4Centre for Clinical Research, Sörmland County Council, Eskilstuna, Sweden
Chloroquine (CQ) was previously the main drug for treatment of Plasmodium falciparum malaria but it is no longer recommended due to resistance. In Guinea-Bissau, West Africa double dose CQ (50mg/kg) split into 2 daily doses for 3 days was well tolerated, as efficacious as artemether-lumefantrine and eradicated 87% of P. falciparum with resistant genotypes in 2008. As part of 3 previous clinical trials, 100 µl of blood was collected on day 7 from children aged 6 months-15 years that had taken 25 or 50 mg/kg of CQ. Whole blood CQ concentrations were analysed using high performance liquid chromatography. CQ concentrations were obtained from 188 and 293 children after intake of 25 and 50 mg/kg of CQ, respectively. CQ concentrations after intake of 25 mg/kg and stratification by age were: 545 (10y) nmol/l. Using the same age groups, concentrations after 50mg/kg were 834, 1220, 1164, 1573, 1565 and 1546 nmol/l. The increases with age were significant, nonparametric test for trend P<0.008 and P<0.0001, respectively. Using the same age groups, the dose of CQ taken according to body surface area ranged from 489-702 and 978-1405 mg per square meter after intake of 25 and 50 mg/kg, respectively. The CQ concentration in children 10 years of age after intake of 25 and 50 mg/kg, respectively. CQ concentrations were only 21% higher in children <2 years taking 50mg/kg compared to children >10 years taking 25mg/kg. Dosing according to body weight rather than body surface area most probably accounts for the lower concentrations seen in younger children. Vomiting and spitting among the youngest children are unlikely explanations as treatment was repeated after vomiting and because it does not explain the trend throughout the age groups. Chloroquine should be dosed according to body surface area and the effect of dosing according to body weight should be assessed for other antimalarials.

RANDOMIZED, CONTROLLED TRIAL OF TREATMENT OF FEBRILE CHILDREN WITH A NEGATIVE MALARIA RAPID DIAGNOSTIC TEST WITH ARTEMETHER-LUMEFANTRINE VS. NO ANTIMALARIAL IN BAGAMOYO DISTRICT, TANZANIA
Meredith McMorrow1, Saumu Ahmed2, Peter Lyaruu3, Musa Maganga2, Thomas Lymo2, Salim M. Abdulla1, S. Patrick Kachur1
1Centers for Disease Control and Prevention, Atlanta, GA, United States, 2Ifakara Health Institute, Bagamoyo, United Republic of Tanzania, 3Ifakara Health Institute, Dar es Salaam, United Republic of Tanzania
Until recently, most national malaria control programs recommended treating all febrile children less than five years of age with first-line antimalarial drugs to prevent severe malaria, disability and death. In 2010, WHO recommended uniform confirmation of malaria parasitemia by blood smear or rapid diagnostic test (RDT) prior to treatment. Unfortunately
little is known about the epidemiology of other common causes of non-malarial fevers in malaria endemic areas. Moreover, the effect of withholding malaria treatment from febrile children, even when they test negative, may have unintended public health consequences. From January 2010 to December 2011, we enrolled 1000 children aged 6 to 59 months with uncomplicated febrile illness and negative malaria RDTs from two health facilities in Tanzania. Subjects were randomized to receive either artemether-lumefantrine (AL) or no treatment and followed for 91 days to document symptom resolution, time to next malaria infection, and frequency of hospitalization or death. Subjects who missed more than two follow-up visits were not included in per protocol analyses. Preliminary results are available for 708 (70.8%) subjects. Among these 708 subjects, 353 (49.9%) were randomized to AL, 457 (64.6%) completed 91 days of follow-up per protocol without developing malaria, 15 (2.1%) were healthy to day 91 but missed more than two visits, 14 (2.0%) withdrew consent, 8 (1.1%) were given a non-study antimalarial, 118 (16.7%) were lost to follow-up, 93 (13.1%) developed malaria during follow-up, and 3 (0.4%) died of non-study related illness. Children randomized to receive AL had a lower risk of developing malaria during follow-up (RR=0.72, 95% confidence interval 0.49-1.04, p=0.09). The time to malaria infection by 10% of subjects in each arm was 56 days for the AL arm and 28 days

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AN INHIBITOR OF MULTIPLE CYTOCHROME P450S, 1-AMINOBENZOTRIAZOLE, ALTERS THE PHARMACOKINETICS OF PRIMAQUINE AND CHLOROQUINE IN A RHESUS MODEL OF MALARIA RADICAL CURE

Charlotte A. Lanteri1, Susan Charman2, Montip Gettayacamin1, Rawiwan Imerbsin1, Brandon Pybus3, Jason Sousa3, Pattaraporn Vanachayangkul1, Larry Walker4, Colin Ohta5

1 Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand, 2Monash Institute of Pharmaceutical Sciences, Parkville, Victoria, Australia, 3Walter Reed Army Institute of Research, Silver Spring, MD, United States, 4University of Mississippi, University, MS, United States

An unidentified metabolite of primaquine (PQ) is suspected to exert anti-hypnozoite activity to prevent Plasmodium vivax relapse. We previously showed that a nonselective inhibitor of multiple cytochrome P450s (CYP450s), 1-aminobenzotriazole (1-ABT), blocks PQ’s malaria causal prophylaxis activity in mice. Subsequently, we attempted to use this inhibitor to explore if CYP450 metabolism is involved in PQ’s anti-relapse activity in P. cynomolgi-infected Rhesus monkeys. Infected monkeys were administered 1-ABT prior to treatment with a 7 day radical curative regimen of PQ plus chloroquine (CQ). Efficacy +/- 1-ABT administration was determined via daily parasitemia readings and safety was assessed using clinical laboratory results, including % methemoglobin (metHgb). The 7 doses of 1-ABT and primaquine planned were halted after the second dose because some monkeys had elevated alanine aminotransferase levels, which returned to baseline after stopping dosing. Increases in metHgb occurred only in monkeys treated with PQ plus CQ. In contrast, metHgb decreased daily in animals pre-dosed with 1-ABT suggesting 1-ABT blocks PQ-induced metHgb formation. Blood draws were included to assess plasma pharmacokinetics (PK) of PQ and CQ +/- 1-ABT. Pre-treatment with 1-ABT decreased PQ and CQ levels and prolonged half-lives. Animals pre-dosed with 1-ABT had a 4 to 7 day delay in onset of malaria relapse, relative to controls given CQ only, presumably because 1-ABT inhibits metabolism of the antimalarial active CQ parent drug. Besides PK interactions of 1-ABT with PQ and CQ, we noted a link between PQ and CQ metabolism. Animals given PQ plus CQ had a two-fold greater plasma exposure to CQ’s major CYP450 metabolite (desethyl-CQ) after 1 dose and 8-fold higher levels after 7 daily doses relative to animals given CQ alone. In contrast, pre-dosing with 1-ABT precluded formation of desethyl-CQ. We report that the CYP450 inhibitor, 1-ABT, alters PK properties of PQ and CQ, and that PQ potentially induces CQ metabolism. Our results also suggest PQ metabolism is linked to metHgb generation.

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EXPLORING THE PERFORMANCE OF RETAIL SECTOR PROVIDERS EXPOSED TO A PILOT INTERVENTION TO PROVIDE SUBSIDIZED ARTEMETHER-LUMEFANTRINE THROUGH THE PRIVATE SECTOR IN WESTERN KENYA

Beth B. Kangwana1, Sarah Kedenge1, Abdisalan Noor1, Victor Alegana2, Andrew Nyandigisi2, Jayesh Pandit3, Gregory Fegan1, Jim Todd4, Simon Brooker1, Robert Snow4, Catherine Goodman4

1 Kenya Medical Research Institute - Wellcome Trust Research Programme, Nairobi, Kenya, 2Division of Malaria Control, Nairobi, Kenya, 3Pharmacy and Poisons Board, Nairobi, Kenya, 4London School of Hygiene and Tropical Medicine, London, United Kingdom

To improve the quality of care received for presumptive malaria from the highly accessed private retail sector in Western Kenya, subsidised pre-packaged artemether-lumefantrine (AL) was provided on a pilot basis to private retailers, together with a one day training for retail staff on malaria diagnosis and treatment. The pilot was assessed using a cluster randomised, controlled design with nine control and nine intervention sublocations, equally distributed across three districts. Provider and mystery shopper cross-sectional surveys were conducted at baseline and eight months post intervention to assess the impact of the intervention on retailer performance. Data were analysed based on cluster-level summaries, comparing control and intervention arms, while adjusting for covariates, including baseline values where a difference of 5% or more was observed between the arms at baseline. On average 564 retail outlets were interviewed per year. At follow up, 43% and 1% of respondents reported that at least one staff member had attended the intervention training, in the control and intervention arm, respectively. The intervention significantly increased the percentage of providers knowing the first line treatment for uncomplicated malaria by 24.2% points (confidence interval (CI): 14.8%, 33.6%; adjusted p=0.0001); the percentage of outlets stocking AL by 31.7% points (CI: 22.0%, 41.3%; adjusted p=0.0001); and the percentage of providers prescribing AL for presumptive malaria by 23.6% (CI: 18.7%, 28.6%; adjusted p=0.0001). No significant difference was observed between the arms at endpoint in the percentage asking for specified danger signs that determine need for referral to a health facility, and there remained substantial room for improvement in provision of appropriate dispensing advice to those who bought AL.

Overall, subsidizing ACTs and retailer training can significantly increase the percentage of outlets stocking and selling AL for the presumptive treatment of malaria, but further research is needed on strategies to improve the provision of counselling advice to retail customers.

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Comparative Efficacy and Acceptability of Artemether-Lumefantrine versus Dihydroartemisinin-Piperaquine in Kenyan Children with Uncomplicated Falciparum Malaria

Bernhards Ogutu1, Kevin Omondi Onyango2, John Michael Ongecha3, Elizabeth Juma3, Godfrey Allan Otieno1, Charles Obonyo3, Lucas Otieno1, Fredrick Eyase4, Jacob D. Johnson3, Douglas Jay Perkins5, Willis Akhwale6

1Walter Reed Project-Centre for Clinical Research, Kenya Medical Research Institute, Nairobi, Kenya, 2Centre for Clinical Research, Kenya Medical Research Institute, Kisumu, Kenya, 3Centre for Global Health Research, Kenya Medical Research Institute, Kisumu, Kenya, 4Walter Reed Project, Kenya Medical Research Institute, Kisumu, Kenya, 5University of New Mexico, Albuquerque, NM, United States, 6Department of Disease Prevention and Control, Ministry of Public Health and Sanitation, Nairobi, Kenya

The primary objective was to compare the corrected Acceptable Clinical and Parasitological Responses (ACPR) on Day 28 of artemether-lumefantrine (AL) and dihydroartemisinin-piperaquine (DP) in children with uncomplicated falciparum malaria. This open-label, comparative trial study in Western Kenya randomized 454 children with uncomplicated falciparum malaria of age 6-59 months to receive either AL (n=227) or DP (n=227). Children were hospitalized for 3 days for observed treatment and 72-hour parasite kinetic monitoring, and actively followed up at scheduled visits after discharge from hospital on Days 7, 14, 28 and 42. Genotyping for determining treatment outcome was performed on Day 0 and any other day the study participant had a recurrence of parasitemia. No significant differences were observed for the corrected ACPR rates on Day 3, 14, 28 and 42 for AL (99.1%, 100%, 97.8%, 96.8%) and DP (100%, 100%, 99.1%, 98.7%). Similarly, for the uncorrected ACPR rates no significant differences were seen on Day 3, 14, 28 and 42 for AL (99.1%; 98.7%; 81.1%; 67.8%) and DP (100%; 100%; 87.7%; 70.5%), (p>0.05 for all comparisons). Both AL and DP are efficacious treatments for uncomplicated falciparum malaria in Kenyan children. No signs of P. falciparum resistance to artemisinins were noted.

Drug-Drug Interactions between Primaquine and Chloroquine: Pharmacokinetic and Transporter Inhibition Studies

Xiannu Jin, Brandon S. Pybus, Jason C. Sousa, Heather Gaona, ThuanLuong, Theresa Bettger, Nicholas McCulley, Sean Marcislin, Qigui Li, Colin Ohrt, Victor Melendez

Walter Reed Army Institute of Research, Silver Spring, MD, United States

The long established potentiation of primaquine’s liver stage activity when co-administered with chloroquine is still poorly understood after more than six decades (Alving et al., 1955). In the present study we have compared the pharmacokinetics of primaquine (8.8 mg/kg PO in C3H mice) and its primary plasma metabolite carboxyprimaquine after co-administration of 90 mg/kg of chloroquine (CQ). The overall effect observed was a decrease in Cmax with a corresponding decrease in C0f and increase in AUC. To better understand these effects, transporter inhibition studies were carried out using both MDR1-MDCK and Caco2 cell lines. Permeability experiments with increasing levels of CQ showed a marked dose dependence in B-A permeability, indicative of MDR1 inhibition. Results for a larger screen of the effects of CQ on various efflux and uptake transporters will be presented.

Inhibitors of Primaquine Metabolism as Modulators of Efficacy and Hemolytic Toxicity

Brandon Pybus, Jason C. Sousa, Gregory Deye, Xiannu Jin, Qigui Li, Colin Ohrt, Victor Melendez

Walter Reed Army Institute of Research, Silver Spring, MD, United States

The 8-aminoquinoline drug primaquine (PQ) is the only drug approved for the treatment of relapsing relapsing malaria. However, PQ is known to cause hemolytic toxicity in G6PD deficient individuals. Proposed mechanisms of both efficacy and toxicity suggest a role for transient reactive oxygen species formed as a byproduct of metabolism. We previously showed that CYP 2D6 plays a major role in the production of the redox active metabolites most likely to produce oxidative stress, however the relevance of this role in vivo was not clear. To this end, the effects of Paroxetine (PX), a potent selective inhibitor of CYP 2D6 metabolism, co-administration was tested in models of both in vivo liver stage efficacy and G6PD deficient hemolytic toxicity. In C57BL/6 mice, co-administration of PX at 25 mg/kg with PQ at 2.5 mg/kg showed a reduction in liver stage potency at both 24 and 48 hr post infection with P. berghei sporozoites. Co-administration of PQ with the MAO-A inhibitor Clorgiline (CG) however, resulted in enhanced liver stage efficacy. Further, significant mitigation of the hemolytic toxicity associated with PQ dosing in a G6PD deficient strain of C3H mice was also observed after co-administration of PX. These data suggest that CYP 2D6 plays an integral role in the metabolic pathways necessary for PQ’s efficacy and hemolytic toxicity. While the effects of MAO-A inhibition on toxicity remain unknown, metabolic compensation may account for increased efficacy as a result of decreased primaquine clearance.

Reported Adverse Events Associated with Artemisinin Combination Therapies (ACTs) in Northern Ghana

Nana Akosua Ansah1, Frank Atuguba1, Timothy Awine1, Patrick Ansah1, Victor Asaol1, Thomas Anyorigiya1, Abraham Hodgson2, Alex Dodoo3, Fred N. Binka4

1Navrongo Health Research Centre, Navrongo, Ghana, 2Ghana Health Service, Research and Development, Ghana, 3Ghana Health Service, Accra, Ghana, 4University of Ghana, School of Public Health, Legon, Ghana

Many African countries have adopted artemisinin derivative based combination therapy (ACT) as treatment for uncomplicated malaria, offering an opportunity to assess the safety of these drugs when in real life setting. Knowledge of side effects of these drugs is important for improved management of malaria. This study was conducted to document adverse events associated with Artesunate Amodiaquine (ASAQ), Artether Mefufenantline(AL) and Dihydroartemisin Piperaquine (DHP) through comprehensive pharmacovigilance in the Kassena Nankana districts of Northern Ghana. As part of INDEPTH Effectiveness and Safety studies, a cohort event monitoring study was conducted at selected public and private health facilities after administration of artesiminin combination therapy to patients to treat uncomplicated malaria during visits to the hospital. Participants were recruited if they were prescribed an ACT. Each participant was followed up between the 3rd and 7th day after enrolment to document adverse events. A total of 4951 participants with uncomplicated malaria prescribed ASAQ, AL and DHP were recruited across all age groups from August 2010 to June 2011. Out of the 4951 participants recruited, 26.0 % reported at least one adverse event; none had a serious adverse event. Of the 1288 participants reporting an adverse event 78.0%, 19.4% and 2.6% took ASAQ, AL and DHP respectively. 27.1% of females reported an adverse event compared to 19.8% of males recruited. Participants in the 15-49 years age group reported 43.3% of adverse events. The most reported adverse events were dizziness (24.2%) and weakness (23.5%) and these were more associated with ASAQ. The unadjusted odds ratio for participants who took AL are 2.3 (95% CI: 1.9-
EVALUATION OF TWO QUALITY ASSURANCE APPROACHES FOR MALARIA RAPID DIAGNOSTIC TESTS IN PERIPHERAL HEALTH FACILITIES IN RURAL TANZANIA

Irene M. Masanja1, Mussa Maganga1, Debora Sumari1, Naomi Lucchi2, Venkatachalam Udhayakumar2, Meredith McMorrow2, Peter McElroy3, Patrick Kachur2

1Ifakara Health Institute, Dar es Salaam, United Republic of Tanzania, 2Centers for Disease Control and Prevention, Atlanta, GA, United States, 3U.S. President's Malaria Initiative, Dar es Salaam, United Republic of Tanzania

Who recommends parasitological confirmation of malaria before treatment. Limited availability of microscopy in malaria endemic countries has resulted in increased use of antigen-detecting malaria rapid diagnostic tests (mRDTs), but appropriate quality assurance (QA) systems for mRDTs remain a concern. Tanzania has begun a national scale-up of mRDTs at all government health facilities. We evaluated mRDT performance in two districts of Tanzania with low malaria transmission using two QA methods: a) reference microscopy and b) detection of parasite DNA by real-time quantitative polymerase chain reaction (qPCR). Blood samples were collected from patients undergoing mRDT during two to three consecutive days each month in 12 health facilities between January and August 2010. Thick blood films were examined at the district headquarters and the Ifakara Health Institute (IHI) Bagamoyo Laboratory. A third blood film reader was consulted for discordant results. Molecular analysis involved extraction of parasite DNA from dried blood spots tested for presence of Plasmodium falciparum DNA with a pilot real-time assay targeting the tubulin gene. The assay was performed at IHI and about 40% of the DNA aliquots were sent to CDC for validation of IHI results. Samples from 1,837 patients were analyzed. Malaria positivity rates were 6.5%, 3.4%, and 2.7% for mRDT, qPCR, and microscopy, respectively. When qPCR was a gold-standard, mRDTs had higher sensitivity (68.6%; 95% CI: 55.0-79.7) than microscopy (53.7%; 95% CI: 38.7-68.0), but the difference was not significant. When microscopy was the gold standard, mRDT sensitivity was the highest (85.3%; 95% CI: 70.0-93.6). With qPCR as a gold standard, positive predictive values were significantly different between the two tests: microscopy vs qPCR-IHI (75.9%; 95% CI: 58.0-78.8), and mRDTs vs. qPCR-IHI (36.5%; 95% CI: 27.5-46.4). Higher inter-observer agreement (kappa=0.75) was seen amongst the microscopists. We identified many technical problems with qPCR analysis. QPCR may not be an appropriate QA tool to assess mRDT performance for routine care in this setting. A microscopy-based QA system may be a more suitable option.

THE ROLE OF BASELINE ASSESSMENTS ON MALARIA MICROSCOPY TOWARDS IMPROVING THE QUALITY OF MALARIA DIAGNOSTICS IN TANZANIA

Awalludin Sutamihardja1, Sarah Chiduo1, Derryck Klarkowski1, Edward Sekonde2, Tiffany Hamm2, Colin Ohrt2, Robert Gramzincki2

1Walter Reed Programs, Dar es Salaam, United Republic of Tanzania, 2Walter Reed Army Institute of Research, Silver Spring, MD, United States

Well performed malaria microscopy has high sensitivity, can identify malaria species and quantitate parasitaemia which in turn will lead to appropriate clinical malaria management. Poor microscopic diagnosis of malaria results in both false positive and false negative results that directly result in poor clinical management and loss of physician confidence in laboratory results. We performed baseline assessments at 22 district hospital laboratories in three regions in Tanzania (Coastal, Kagera, and Zanzibar) to determine the status of staff experience, malaria diagnostic knowledge, the availability of quality equipment and supplies, quality of malaria smear staining, laboratory documentation procedures, supply chain management, and other parameters. Our analysis of the baseline assessments indicate a need for focused improvement in malaria smear preparation and staining procedures, distribution of high quality microscopes, use of high quality malaria smear staining reagents, use of standardized laboratory operating procedures, the development of a
robust supply chain management system, and implementation of QA/QC procedures. Areas that were performed well include laboratory registry documentation, generally consistent power supply, and patient safety procedures. The effectiveness of malaria microscopy training will be significantly augmented if the laboratory infrastructure enables microscopists to apply their training in routine practice. To this end there is an immediate need to improve the quality of laboratory equipment, supplies and improved standardized methodology to consistently prepare good quality stained malaria slides. The dual implementation of strengthened laboratory infrastructure and training will increase both malaria diagnostic capacity and competency that will directly lead to increasing accurate diagnosis. The duel focus on training and infrastructure strengthening will be the focus of ongoing WRAIR contributions to the PMI efforts in Tanzania and will directly improve the effectiveness of treatment and prevent over- or misuse of antimalarials.

**BASELINE ASSESSMENTS ON THE USE OF MALARIA RAPID DIAGNOSTIC TESTS (mRDT) IN HOSPITALS AND DISPENSARIES IN TANZANIA**

Derryck Klarkowski, Awalludin Sutamihardja, Sarah Chiduo, Edward Sekonde, Tiffany Hamm, Colin Ohrt, Robert A. Gramzinski, Fidelis Mgoahmwendwe, Sigsbert Mkude

**Walter Reed Program, Dar es Salaam, United Republic of Tanzania; Henry M. Jackson Foundation Medical Research International, Rockville, MD, United States; Walter Reed Army Institute of Research, Silver Spring, MD, United States; United States; Walter Reed Army Institute of Research, Silver Spring, MD, United States; National Malaria Control Program, Dar es Salaam, United Republic of Tanzania**

A key Tanzanian National Malaria Control Program (NMCP) objective is to increase the percentage of malaria microscopy and malaria rapid diagnostic test (mRDT) confirmed cases of malaria in public health facilities from 20% to 80% by 2012. Malaria diagnosis by microscopy requires well trained technicians and quality equipment, supplies and procedures. Increasingly Tanzania is relying on mRDTs for point-of-care malaria diagnosis in hospitals, health centres and peripheral dispensaries. Similar to microscopy, mRDTs can suffer from logistical supply chain problems, lack of quality assurance/quality control (QA/QC) procedures, and infrastructure deficiencies. In 20xx we conducted baseline assessments of healthcare workers’ performance of mRDT in 6 district hospitals in the Coastal Region, plus 7 regional/district hospitals and 13 health centers/dispensaries in the Kagera Region. Parameters assessed included testing procedures and performance, supply chain management, QA/QC, staff training, documentation, and storage and waste management. Significantly 44% (7/16) of health facilities scored ≤50% for testing performance and only one of 16 health facilities achieved 90%. Our overall analysis of the baseline assessments indicate need for focused improvement in the support provided to testing staff, including job aids, timers and adequate ambient lighting; increased supervision of testing performance; increased availability of training; strengthened training in test interpretation; and the implementation of QA/QC procedures. Improving mRDT testing and supply management will directly lead to increasing accurate diagnosis to improve the effectiveness of treatment in Tanzania. Implementing these changes will be the focus of ongoing efforts to strengthen malaria diagnostic services in Tanzania.

**MISCLASSIFICATION OF PLASMODIUM SPECIES BY CONVENTIONAL MICROSCOPY**

Peter Obare1, Bernhards Ogutu2, Christine Adhiambo1, Seth Owusu2, Fred Binka3, Jacob Johnson1

1United States Medical Research Unit, Kitumu, Kenya; 2Kenya Medical Research Institute, Nairobi, Kenya; 3Kintampo Health Research Center, Kintampo, Ghana; 4Malaria Clinical Trials Alliance, INDEPTH Network, Accra, Ghana

Microscopic differentiation of *Plasmodium* species relies on morphological characteristics manifested in stained blood films. Proficiency levels of microscopists as well as morphological variations within and between *Plasmodium* species may lead to misclassifications. Ten- day microscopy workshops were conducted from 2009 to 2010. Proficiency of the participants was assessed at the start and the end of each workshop using mono infection slide sets of *P. falciparum*, *P. malariae*, *P. ovale* and *P. vivax*. Each slide with densities between 1,000 to 30,000 parasites/µL was examined for 5 minutes. Errors observed on each of the *Plasmodium* species were false negatives, positive results with no species indicated, inability to differentiate between *Plasmodium* species and reports of mixed infections. Pre-workshop misclassification of *P. falciparum* as positive was significantly higher (p < 0.05) than all other reported misclassifications except *P. malariae*. Misclassification of *P. malariae* as negative was significantly higher than all other reported misclassifications. Misclassification of *P. ovale* and *P. vivax* as mixed infections was significantly lower than all the reported misclassifications. Post workshop misclassification of *P. falciparum* as mixed infections was significantly higher than all other reported misclassifications and there was no clear misclassification of *P. malariae*. *P. ovale* was highly misclassified as *P. vivax* and *P. vivax* equally misclassified as *P. ovale*. Microscopy workshops can minimize observed errors and improve reliability of both clinical and epidemiological data. Confirmation of results by expert microscopy in addition to molecular characterization of species is highly recommended.

**MALARIA MICROSCOPE QUALITY ASSURANCE USING A SMALL NUMBER OF SLIDES**

Luis Benavente1, Nicole Whitehurst1, Chris Petruccelli1, Sean Fennell1, Joseph Valadez2

1Medical Care Development Inc., Silver Spring, MD, United States; 2Liverpool School of Tropical Medicine, Liverpool, United Kingdom

WHO’s “Universal Access to Malaria Diagnostic Testing, an Operational Manual” released in 2011 recommends “cross-checking of 10 to 20 slides if time (during a supervisory visit) allows.” ‘Weak infrastructure in most Sub-Saharan African countries’ will hinder adherence to the previous WHO’s Malaria Microscopy Quality Assurance (MMQA) protocol released in 2009 of selecting five negatives and five weak positives per lab and per month and sending them to a certified microscopist at a referral lab for cross-checking. For health facilities in many African countries, there are not enough slides available and no fuel or transportation to reference labs. Even if slides can be sent, there are not enough skilled microscopists at the reference labs to read all the slides received. The backlog of unread slides contributes to long delays feedback for their work. The Improving Malaria Diagnostic (IMaD) project tested if slide cross-checking during Outreach Training and Support Supervision (OTSS) visits could be used to identify underperforming labs and engage in on-the-spot problem-solving to address any deficiencies in slide preparation, staining or examination. As lab supervisors stayed in the lab for only one or two days, the number of slides cross-checked during the supervisory visit was on average 9.15 (standard deviation 1.96 slides) 76% of facility visits saving slides of QA. In Benin, 78% of laboratories visited for OTSS cumulated 12-20 slides in two consecutive visits. A sample size of 12 slides per health facility identified facilities under the decision rule for 90% parasite detection, and/or below average parasite detection. If OTSS is done quarterly, the minimum annual
aggressive number of slides would be 24, out of a target number of 40 slides (tens), as opposed to 120 done by following the standard WHO MMQA 2009 protocol. Forty slides cumulated in a year selected randomly as per the LQAS stratified random sampling -with 50% slide positivity ratio-give a sufficiently precise estimate of parasite detection at laboratory level, allowing calculation of the % of laboratories attaining 90% agreement, and aggregate measurements at health zone level to focus MMQA efforts where they are needed the most. Resource-poor countries would be better served by considering a smaller sample size for MMQA selected with LQAS as opposed to not doing MMQA at all or doing MMQA in a way that fails to deliver feedback to participating labs.

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COMPARATIVE LABORATORY-BASED EVALUATION OF DIAGNOSTIC TESTS FOR G6PD

Cori A. Barfield, Jay Zimmers, Maria Kahn, Kathy Tietje, Gonzalo J. Domingo

PATH, Seattle, WA, United States

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common human enzyme deficiency in the world, affecting over 400 million people worldwide. It is characterized by abnormally low levels of G6PD, an enzyme involved in red blood cell metabolism. Individuals with diminished G6PD activity are susceptible to cellular oxidative damage, and can exhibit symptoms including hemolytic anemia and jaundice in response to a number of causes, most commonly infection or exposure to certain medications. In particular, treatment with anti-malarial drugs such as those in the 8-aminooquinoline group (e.g. Primaquine, Pamaquine and Tafenoquine) can cause acute hemolysis in people with G6PD deficiency. Because of this risk it is imperative to identify individuals with G6PD deficiency prior to administering these anti-malarial agents. As such, there is a need for a test that is appropriate for G6PD deficiency screening in areas of the developing world where malarial treatments are frequently administered. To explore the suitability of G6PD tests for use in conjunction with malarial management we conducted a laboratory-based evaluation to assess the performance and operational characteristics of several existing G6PD diagnostic tests. Tests evaluated included both qualitative and quantitative tests, utilizing a variety of test formats (fluorescent spot test, rapid point-of-care tests, dye reduction tests, and spectrophotometry-based tests). Our findings indicate that most of the currently available diagnostic tests for G6PD appear to have technical or operational shortcomings that may limit their applicability to low-resource malaria management settings. Further adaptation and/or modification of existing tests or development of new tests to better meet the needs of clinicians and laboratory staff involved in malaria-case management in the developing world may be needed. We present data from this evaluation and critical design inputs to guide development of new diagnostic tests for G6PD testing.

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DEVELOPMENT OF A READY-TO-USE GELIFIED REAL-TIME PCR ASSAY FOR SIMULTANEOUS SPECIFIC IDENTIFICATION OF PLASMODIUM FALCIPARUM, P. MALARIAE, P. VIVAX, AND P. OVALE IN CLINICAL SAMPLES

Cheysa Biondo1, Marco Krieger1, Viviane M. Goes1, Manuphet Xayavong2, de Almeida E. Marcos2, Naomi Lucchi2, Alexandre J. da Silva2

1FIOCRUZ, Curitiba, Brazil, 2Centers for Disease Control and Prevention, Atlanta, GA, United States

The use of PCR for identification Plasmodium spp. represents an attractive alternative for diagnosis of malaria. Some robust PCR techniques exist for this purpose, but they still based on complex procedures. This is not only time consuming, but increases the cost of PCR applications limiting its usefulness for laboratories in developing countries. Nevertheless, this can be drastically changed with PCR techniques designed to be executed under minimal quality control standards. We selected a previously published TaqMan assay and converted into a gelified format for robust, specific and simplified multiplex identification of P. falciparum, P. malariae, P. vivax, and P. ovale. Gelification consists in a process where the components of enzymatic reactions are stabilized by addition of different agents. In order to execute the procedure the laboratory simply needs to add water and the DNA sample to the reaction tubes coated with all chemicals required for PCR amplification. Next, the vessels containing the re-solubilized mixture and the template are inserted into the real-time PCR thermal cyclers for DNA amplification. The preliminary evaluation assay's liquid format indicated that it was very specific compared to the nested PCR, since it did not produce any cross-amplification with samples containing other Plasmodium species such as P. cynomolgi, P. hylobatii, P. inui and P. knowlesi (N=14); nested PCR primers for P. vivax cross-amplified P. cynomolgi (N=4). No false negative or false positive results were verified when this assay was compared to the nested PCR using approximately 100 blood specimens sent to CDC for confirmatory diagnosis of malaria. This evaluation showed that the gelified assay had more efficient amplification profiles in addition to being simple to execute and providing results within 2 hours, including preparation time. Also, the gelifed format of the assay was stable for 7 days at room temperature and for 2 months at 4 C. We believe that the gelifed assay format can streamline the use of real-time PCR for confirmatory diagnosis of malaria.
ANALYSIS OF DISCORDANT RESULTS BETWEEN MALARIA RAPID DIAGNOSIS TESTS (TDRS) AND MICROSCOPY

Laetitia C. Offouga, Denise P. Mawili, Marielle K. Bouyou Akotet
Faculty of Medicine University of Science of the Sante, Libreville, Gabon

The thick smear, a recommended blood test when diagnosing malaria, is a technique with some limits and that still is out of reach for people living in remote zones from endemic regions. Perfecting TDRs, means an easy and fast technique for malaria diagnosis, could, however help to make up these shortcomings. Nevertheless, their efficacy and effectiveness should be assessed in order to determine their performance. During a study conducted in Gabon, an HRP2 TDR (Acon® and the pLDH TDR Optimal-IT, 15% of discrepancies were found between TDRs and the thick smear. Our study aimed at analyzing these discordant results using the nested PCR, for amplification of the gene representing the small under-unit of the ARN 185. Out of the 415 analyzed samples (307 differences and 108 correspondences), 28.4% (38/171) were positive with the PCR. The Plasmodium falciparum infection was detected in 22.2% (38/171) of the positive samples with the Acon test, corresponding to 77.8% of false positive results and to more than 80% of bands with low intensity. The proportion of false negatives was 25.6%. The proportion of false positive with the test Optimal-IT (78.2%), was due to false detection of non falciparum species; that of the false negatives was lower (33%).

Sensitivity, specificity and negative predictive value of both TDRs with the thick smear corrected by the PCR considered as the reference exceeded 90%, except for the detection of the non falciparum species with Optimal-IT. Acon® and Optimal-IT remain of good interest for the biological diagnosis of malaria in areas where thick smears and well trained microscopists are not available.

MOLECULAR DIAGNOSIS OF MALARIA BY PHOTO-INDUCED ELECTRON TRANSFER FLUOROGENIC PRIMERS (PET-PCR)

Naomi W. Lucchi1, Jothikumar Narayanan1, Maniphet Xayavong1, Simon Kariuki2, Alexandre J. Dasilva1, Vincent Hill1, Venkatachalum Udhayakumar1
1-Centers for Disease Control and Prevention, Atlanta, GA, United States, 2-Kenya Medical Research Institute/Centers for Disease Control and Prevention, Kisumu, Kenya

Malaria control and elimination programs require the use of diagnostic tools that are sensitive, cost effective and able to detect multiple species simultaneously in a simple and accurate manner. The real-time PCR methods are particularly robust for large scale screening and there is scope for improving these methods for field applications. Here, we have designed novel self-quenching real-time PCR primers for the detection of Plasmodium spp. and P. falciparum. This PCR assay uses the photo-induced electron transfer (PET) chemistry and therefore does not require internal probes, which are usually very expensive or intercalating dyes, which are often non-specific. A total of 115 clinical samples consisting of different malaria species and some mixed infections (9 malaria negative samples, 81 P. falciparum, 9 P. vivax, 1 P. malariae, 9 P. ovale, 2 P. falciparum/P. malariae, 1 P. vivax/P. ovale, 2 P. falciparum/P. ovale mixed infections and 1 P. knowlesi) were used to test the utility of the novel PET-PCR primers in diagnosis of clinical samples. The sensitivity and specificity was calculated using a nested PCR as a gold standard. Both primer sets showed 100% sensitivity and specificity. This malaria PET-PCR method can detect parasite densities as low as 10 parasites/µL of both Plasmodium spp. and P. falciparum. In addition, the reaction can be duplexed to detect both Plasmodium spp. and P. falciparum in a single reaction. Further validation of this technique in field settings will help to assess its utility for large scale screening for malaria parasite, potentially important for control and elimination programs.

DEVELOPMENT OF A FLUORESCENCE IMMUNOASSAY FOR SEMI-QUANTITATIVE OF THE DIAGNOSIS MALARIA: PLASMODIUM FALCIPARUM AND PLASMODIUM VIVAX

Taek kyu Oh
Boditech Med Inc., Chuncheon, Republic of Korea

The rapid and accurate diagnosis of malaria is key to the central to clinical management and the prevention of drug-overuse, which may lead to resistance development, toxicity and economic loss. So far, microscopy of Giensa-stained thin or thick blood smears is the gold standard. Rapid diagnosis tests provide an alternative, although they cost more and give qualitative instead of quantitative results. A fluorescence (FL) dye-incorporated immunochromatographic assay (ICA) might offer a higher sensitivity than rapid device which can be used at the point of care testing(POCT). The fluorescence immunoaassay was employed to detect and semi-quantitative Plasmodium falciparum (P.f) and Plasmodium vivax (P.v) malaria-infected whole blood. It consisted of a FL antibody detector buffer, a test strip housed in a disposable cartridge, and a laser fluorescence scanner. The whole blood mixed with detector, loaded onto a cartridge, incubated 10 minutes, and the semi-quantitative of Pf and Pv malaria parasites were measured in a fluorescence scanner. The comparability of the new method was examined with microscopy check and rapid device malaria diagnosis. By microscopy, Plasmodium was detected successfully in all 81 clinically suspected malaria patients, including 59 individuals with low parasitemia (1-100 parasites/µL) and 22 individuals with middle parasitemia (101-500 parasites/µL). At low parasitemia (1-100 parasites/µL), sensitivities for FL-ICA and microscopy check were 80% and 100%, respectively. The accuracy of semi-quantitative was 90%. At middle parasitemia (1-100 parasites/µL), sensitivities for FL-ICA and microscopy check were 95% and 100%, respectively. In conclusion, while the approximate accuracy of semi-
MODELING HEALTH SYSTEMS BARRIERS TO SUCCESSFUL MALARIA MANAGEMENT
V. Bhargavi Rao1, David Schellenberg2, Azra Ghani1
1Imperial College London, London, United Kingdom, 2London School of Hygiene and Tropical Medicine, London, United Kingdom

A functioning and efficient health system is required to maintain reductions in malaria disease and transmission. Few models demonstrate how to deliver a proven intervention most effectively through an existing system. The “systems effectiveness framework” has previously been used to describe how a cascade of interacting health systems barriers may sequentially reduce the effectiveness of treatment interventions. We contrasted this approach with a decision analysis model of malaria treatment in the public sector. A common set of parameters for malaria management in Africa including access to care, diagnosis and treatment were obtained from the literature. The decision analysis model more accurately reflected reported levels of appropriate management of fever (malarial and non-malarial) in the public sector (>50% attendees) compared with a systems effectiveness approach (<15%). Modeling increases in availability and usage of rapid diagnostic tests (RDTs) improved overall management of fever (up to 80% attendees) and reduced overtreatment of non-malarial fevers with anti-malarials (<12%), but had less impact on the proportion of malaria cases treated (<57%). In contrast, reducing stockouts of first-line anti-malarials had a substantial impact on the proportion of malaria cases treated (68%) even without increased RDT use. Improving adherence to test results was not predicted to substantially improve appropriate treatment rates for malaria since the risk of under-treatment is low, and baseline utilisation of RDTs was assumed to be only 40% as per the literature. Under conditions of perfect availability and use of RDTs, test adherence and drug availability, appropriate treatment rates were predicted to rise to 95%. Simple decision analysis models can provide insight into which aspects of delivering care are most likely to impact on care quality and treatment effectiveness, and at different transmission intensities. Further work into the amenability of health systems to change is required to explore the most cost-effective targets in expanding the delivery of anti-malarials.

RAPID DIAGNOSTIC TEST PERFORMANCE IN THE SETTING OF DIFFERING TRANSMISSION INTENSITIES: THE MALAWI ICEM EXPERIENCE
Atupele Kapito-Tembo1, Don Mathanga1, Jacqueline Fiore2, Karl Seydel2, Mike Liomba1, Andrew Bauleni1, Paul Pensulo3, Rabia Mukadam4, Osward Nyrenda5, Terrie Taylor2, Miriam Lauffer2
1Malaria Alert Centre, University of Malawi College of Medicine, Blantyre, Malawi, 2College of Osteopathic Medicine, Michigan State University, East Lansing, MI, United States, 3Blantyre Malaria Project University of Malawi College of Medicine, Blantyre, Malawi, 4University of Malawi College of Medicine, Blantyre, Malawi, 5Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, MD, United States

In Malawi, like many malaria-endemic countries, rapid diagnostic tests (RDTs) are conducting surveillance for malaria in three transmission settings using RDTs (Paracheck®), microscopy and molecular detection of malaria infection. Among all surveillance sites during the rainy season, 25-30% of people with symptoms compatible with malaria had had a positive RDT. We conducted a preliminary evaluation comparing RDT results to expert microscopy. Overall, the positive predictive value (PPV) of a RDT compared to microscopy was 76.1%. The RDT PPV was inversely related to transmission intensity. In the moderate transmission intensity regions of the rural highlands and urban highlands, PPV was 91.7% and 72.3%, while in the lowland area with intense malaria transmission the PPV was 66.7%. In the areas of moderate transmission, the PPV was higher in adults compared to children under five years of age (97.9% vs. 84.2% and 80.5% vs. 55.6% in the highlands and urban setting respectively). In contrast, in the most intense transmission region, PPV was slightly lower in adults compared to children (64.2% vs. 73.5%). Microscopy is being conducted on additional slides collected from patients with positive RDTs in both the rainy and dry seasons. Sensitivity and specificity compared to molecular diagnosis will also be reported. The rate of false positive RDTs is high and is related to transmission intensity. This raises the concern that alternative causes of illness will not be pursued in patients with a positive RDT.
PROTEIN KINASE 5

KAMPALA, UGANDA, 5

DEPARTMENT OF CHEMISTRY, UNIVERSITY OF NAIROBI, 6

OF BIOLOGICAL SCIENCES, COLLEGE OF NATURAL SCIENCES, MAKERE UNIVERSITY, KAMPALA, 7

OF CHEMISTRY, UNIVERSITY OF NAIROBI, NAIROBI, UGANDA, 8

Walter and Molecular Biosciences, University of Queensland, St. Lucia, Queensland, Australia, 1

Diversity of Experimental Therapeutics, Walter Reed Army Institute of Research, Silver Spring, MD, United States, 4

School of Chemistry, University of Nairobi, Nairobi, Kenya, 3

Department of Chemistry, Makerere University, Kampala, Uganda, 2

Department of Immunology and Medicine, U.S. Army Medical Component, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand, 8

National Malaria Control Programme, Dar es Salaam, United Republic of Tanzania, 5

World Health Organization, Country Office, Dar es Salaam, United Republic of Tanzania, 7

World Health Organization, Dar es Salaam, United Republic of Tanzania, 6

National Institute for Medical Research, Tanga, United Republic of Tanzania, 3

Ifakara Health Institute, Ifakara, United Republic of Tanzania, 2

Ifakara Health Institute, Dar es Salaam, United Republic of Tanzania, 1

National Institute for Medical Research, Tanga, United Republic of Tanzania, 1

Department of Parasitology-School of Public Health, MUHAS, Dar es Salaam, United Republic of Tanzania, 4

National Malaria Control Programme, Dar es Salaam, United Republic of Tanzania, 3

World Health Organization, Country Office, Dar es Salaam, United Republic of Tanzania, 2

Tanzania mainland through the ministry of health and social welfare (MoHSW) introduced artemisinin combination therapy (ACT) with artemether-lumefantrine (AL) as first line treatment policy for uncomplicated falciparum malaria since 2006. Despite good profile of ALu on malaria control, there is a threat of ACT drug resistance. Due to recent report on the emerging drug resistance to ACT along the Thai-Cambodia border it is critical to our region to monitor the spread of drug resistance to ACT. Five years after countrywide policy implementation in Tanzania there has been no systematically designed and implemented efficacy monitoring study to assess ACT. In 2011 Ifakara Health Institute in collaboration with other country based researchers conducted a round of assessment of anti-malaria through 4 out of 8 invivo MOHSW monitoring sentinel sites that recorded good performance of both ALu and amodiaquine-artesunate (ASAQ). Because coverage of ACT profile across the remaining 4 sentinel sites is envisaged to portray a countrywide ACT performance, beginning in May 2012 another round of efficacy monitoring is planned to be implemented. We therefore set up to conduct an invivo monitoring study at four sentinel representative National Malaria Control Programme (NMCP)’s sites in May-September 2012 to assess efficacy of ALu. This study will be conducted at sites in Kyela, Masasi, Chamwino and Butimba in mainland Tanzania. Participants are febrile patients aged 6 months-10 years presenting at the health facility to be followed up during 28 days. It is intended to elicit treatment performance using 2010 WHO protocol. Results of this study will be out by the time of American Society of Tropical Medicine and Hygiene conference in November 2012. We will elucidate the occurrence of recrudescence by PCR using msp1 and glurp. Results from this study will be used to assist the MoHSW assess the current national treatment guidelines for uncomplicated falciparum malaria and update the global initiatives to containment of ACT drug resistance.
CHLOROQUINE-RESISTANT PLASMODIUM FALCIPARUM MALARIA IN TRAVELERS FROM HAITI AFTER THE 2010 EARTHQUAKE

Myriam Gharbi1, Jennifer Flegg2, Magatte Ndiaye3, Bruno Pradines4, Cally Roper5, Véronique Hubert6, Eric Kendjo7, Philippe Brasseur4, Gunmar Gaye8, Abdoulaye Djimdé9, Ako Berenger10, André T. Offianan9, Louis Penali10, Jacques Le Bras1, Philippe J. Guérin2

1WWARN - IRD - Université Paris-Descartes, Paris, France, 2WWARN - University of Oxford, Oxford, United Kingdom, 3Centre National de Référence du Paludisme, Paris, France, 4Laboratoire National de Santé Publique, Port-au-Prince, Haiti, 5WWARN, Paris, France, 6WWARN - University of Oxford, Oxford, United Kingdom

Chloroquine (CQ) associated with primaquine is recommended as first-line treatment for uncomplicated malaria in Haiti. CQ in vitro and molecular surveillance data collected over the past two decades suggest continued Plasmodium falciparum sensitivity. However, a 2006-2007 study showed around 6% (5/79) of P. falciparum isolates had the CQ resistance-associated pfcrtK76T genotype. The January 2010 earthquake and flooding following Hurricane "Tomas" later in the year may have created conditions for increased malaria infections. We have investigated the CQ sensitivity of P. falciparum parasites isolated from travelers recently returned from Haiti to France and Canada, using genotypic and phenotypic methods. Retrospective data was collected from the French National Malaria Reference Centre (CNR) and the Public Health Ontario, 1988-2010 and 2007-2010, respectively. The definition of an infection probably acquired in Haiti was recent travel to the country prior to diagnosis with P. falciparum positive thin and thick blood smear. Basic demographic and epidemiologic data, clinic and parasitologic information, treatment, history of travel and malaria infection were collected systematically. Prior to the earthquake, all isolates (n=29) had the wild-type pfcrtK76 allele, analysed by PCR-RFLP. The mean of the 50% growth inhibition (IC50) of CQ of the isolates (n=24) was 27nM (95% confidence interval[C], 23 to 31). After the earthquake, two of ten isolates showed CQ resistance in vitro after culture adaptation. Both isolates had high CQ IC50 (506nM and 708nM) and high CQ IC50 isolate:PF3D7 (CQ susceptible clone) ratio (20 and 27). Resistance was confirmed by the molecular analysis demonstrating the presence of the CQ-resistant associated pfcrtK76T allele (mixed K+T) only in these two isolates. Our data confirm the presence of CQ-resistant strains in Haiti. They highlight the importance to implement a therapeutic efficacy study for assessing in vivo CQ-sensitivity, essential for informing rational control strategies and guiding prophylaxis recommendations in Haiti.

TRAVELER’S SURVEILLANCE: A TOOL FOR DETECTING EMERGENCE OF ANTIMALARIAL DRUG RESISTANCE IN ENDEMIC COUNTRIES

Myriam Gharbi1, Jennifer Flegg2, Magatte Ndiaye3, Bruno Pradines4, Cally Roper5, Véronique Hubert6, Eric Kendjo7, Philippe Brasseur4, Gunmar Gaye8, Abdoulaye Djimdé9, Ako Berenger10, André T. Offianan9, Louis Penali10, Jacques Le Bras1, Philippe J. Guérin2

1WWARN - IRD - Université Paris-Descartes, Paris, France, 2WWARN - University of Oxford, Oxford, United Kingdom, 3University Cheikh Anta Diop, Dakar, Senegal, 4Institut de Recherche Biomédicale des Armées, Marseille, France, 5London School of Hygiene and Tropical Medicine, London, United Kingdom, 6Centre National de Référence du Paludisme, Paris, France, 7Institut de Recherche et Développement, Dakar, Senegal, 8Malaria Research and Training Center - University of Sciences, Techniques and Technologies, Bamako, Mali, 9Institut Pasteur de Côte d’Ivoire, Abidjan, Côte D’Ivoire, 10WWARN, Dakar, Senegal

There is growing concern about the emergence of resistance in Southeast-Asia to artemisinin-based combination therapy (ACT), the first-line treatment for malaria. In the time since the widespread adoption of ACTs, a decrease in the systematic surveillance of antimalarial drug resistance has been observed in many endemic countries. Furthermore, high levels of host immunity complicate the identification of treatment failures associated with resistance. The aim of this project was to validate the use of travelers returning from Africa with malaria as an additional surveillance system for the emergence of drug resistance. We compared data collected between 1998-2011, from the French Malaria Reference Centre for traveler's data versus field data from the literature and within the WWARN database. We compared temporal trends of the proportion of wildtype-genotype isolates for CTR76 and DHFR108 molecular markers, as well as the in vitro response to chloroquine (CQ) of isolates using generalized linear models. Three countries were selected for the analysis: Senegal (SN), Mali (ML) and Cameroon (CM) based on a required sample size of 600 isolates per group. For CTR76, no significant (NS) difference is shown between travelers and field studies in CM (slope=0.03, respectively, p<NS) and ML (β1=0.19, β2=-0.17, respectively, p=NS) and SN (β1=0.17, β2=0.21, respectively, p=NS). An increase of CQ-sensitive isolates is observed, except for ML where only data up to 2004 was included. For DHFR108, no significant difference is shown between travelers and field studies in CM (β1=-0.24, β2=0.10, respectively, p=NS), ML (β1=-0.17, β2=-0.11, respectively, p=NS) and SN (β1=-0.09, β2=0.06, respectively, p=NS). A decrease of wildtype-genotype isolates is observed. Our results show similar trends in resistance extracted from the Demographic Health Surveys (DHS) and Multiple Indicator Cluster Surveys (MICS). Logistic regression models were used to detect trends in the susceptible isolates proportions. From 2000 to 2011, around 700 isolates were genotyped for each country. The frequency of the pfcrtK76 wild-type significantly increased for Cameroon (CM) (from 10% to 41%, Slope=0.09, p<0.01), Cote d’Ivoire (CI) (from 37% to 69%, Slope=0.14, p<0.01) and Senegal (SN) (from 22% to 53%, Slope=-0.17, p<0.01). The mean of the 50% growth inhibition (IC50) of CQ decreased from 314nM (95% confidence interval, 102-526) to 101nM (71-131) in CM, from 109nM (70-148) to 47nM (28-66) in CI and from 144nM (91-196) to 75nM (36-115) in SN. Meanwhile, CQ use among children with fever significantly decreased during this period. An increase of CQ susceptibility following official withdrawal is observed in travelers returning from Cameroon, Cote d’Ivoire and Senegal. The length of time between policy changes and their subsequent implementation, as well as the cross resistance between antimalarial drugs, may affect the time for a significant recovery of CQ sensitivity. This information should be compared to country level CQ efficacy data.
between travelers and field studies. This work highlights the value of an international traveler’s database to assess and monitor the emergence of drug resistance in endemic areas where information is limited.

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**MULTIPLE INSECTICIDE RESISTANCE IN WESTERN KENYA: IMPEDIMENT TO INSECTICIDE-BASED MALARIA VECTOR CONTROL PROGRAMS IN KENYA**

Christine L. Wanjala1, Yaw Afrane1, Andrew K. Githeko1, Guiyun Yan2  
1Kenya Medical Research Institute, Kisumu, Nairobi, Kenya, 2University of California, Irvine, CA, United States

Indoor Residual Spraying (IRS) and long-lasting insecticidal nets (LLINs) have been extensively used for malaria prevention and control in Kenya. However, the development of resistance by mosquitoes to recommended insecticides for IRS and/or ITNs/LLINs would affect insecticide-based malaria vector control. We assessed the effect of extensive use of IRS and LLINs on development of resistance in Anopheles gambiae from western Kenya. Wall bioassays were performed on artificial walls and filter papers sprayed with ICON and deltamethrin using mosquitoes collected from different sites from western Kenya and Kisumu strain as a control. Net cone bioassays were also performed on nets collected from the fields using mosquitoes from two sites and Kisumu susceptible strain as a control. Chemical analysis of the netting material was also done using HPLC to determine the concentration of insecticides on the net. Kisumu strain was susceptible to all the insecticides with 100% mortality. Mosquitoes from Chulambho, Ahero, chulambho, Emakakha and Kisian shows susceptibility to both deltamethrin and ICON with the mortality rates ranging between 80% - 85% but mosquitoes from Bungoma and Emutete shows resistance to both ICON and deltamethrin with mortality rates ranging from 69%- 74%. Sprayed artificial walls shows lower mortality rates compare to sprayed filter papers. ICON had high mortality rates on the mosquitoes compared to Deltamethrin. Mosquitoes from Bungoma and Emutete showed resistance in Net bioassays with the mortality rates ranging between 60% -75%, but the control strain was highly susceptible to the nets with 100% mortality. HPLC results indicated that the nets still had a high concentration if insecticides ranging from 0.06 wt% - 0.19 wt%, the positive control net had the concentration of 0.14 wt%. The observed resistance to insecticides used for IRS and LLINs in An. gambiae Populations from western Kenya could affect the malaria vector control programmes in Kenya; therefore there is need urgent implementation of resistance management strategies and intergrated vector control intervention.

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**MECHANISM OF ARTELINIC ACID RESISTANCE IN PLASMODIUM FALCIPARUM IN VITRO**

Franka Teuscher1, Nanhua Chen2, Dennis E. Kyle2, Michelle L. Gatton3, Qín Cheng1  
1Australian Army Malaria Institute, Brisbane, Australia, 2University of South Florida, Tampa, FL, United States, 3Queensland Institute of Medical Research, Brisbane, Australia

The emergence of Plasmodium falciparum parasites with decreased in vivo sensitivity in several South East Asian countries has raised the urgent need to understand the underlining biological mechanism. We investigated the processes involved in the development of artelinic acid resistance using laboratory generated resistant P. falciparum lines in vitro. Our results demonstrate that resistance to artelinic acid has two major characteristics: 1) resistance affecting early asexual stage parasites demonstrated by the insensitivity of ring-stage parasites to the induction of dormancy, and a faster recovery from dormancy when it is induced with higher drug concentrations. 2) resistance of late stage parasites which allows continuous growth and multiplication of parasites under continued drug pressure. These results demonstrate that changes in the dormancy profile of parasites are part of the resistance phenotype and suggest that the development of artelinin resistance may involve two steps. The moleular events important in each step are currently being investigated to determine whether full artelinin resistance develops as a stepwise process or whether the two stages arise independently of each other.

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**PHARMACODYNAMICS OF ARTELINIC COMBINATIONS FOR DRUG SENSITIVE AND RESISTANT PLASMODIUM BERGhei IN VIVO**

Anuradha Srivastava1, Ericka Oliver1, Robert Boyle1, Fabian Saenz2, David Fidock3, Dennis E. Kyle1  
1University of South Florida, Tampa, FL, United States, 2Columbia University, New York, NY, United States

Artemisinin combination therapies (ACTs) combine a very potent, yet short-lived artemisinin (ART) derivative with a partner drug that usually has a longer half-life, yet established resistance in the field. This strategy enhances therapeutic efficacy and theoretically delays the emergence of ART resistant Plasmodium falciparum. Although ACTs are the first-line treatment globally, pharmacodynamic (PD) properties of the combinations are not well studied. In particular the impact of existing resistance to the partner drug on emerging resistance and reduced clinically efficacy is poorly understood. To examine the PD properties of ACTs we used drug sensitive and resistant P. berghei in standard mouse efficacy models. In these studies we examined the PD properties of artemether-lumefantrine (ATM-LMF), artesunate-mefloquine (AS-MFQ), AS-amodiaquine (AS-ADQ), dihydroartemisinin-piperaquine (DHA-PIP), and AS-pyreronidine (AS-PND). First we used drug-sensitive P. berghei and identified minimal curative drug concentrations that rapidly clear parasite infections, prevent parasite recrudescence, and are not antagonistic. Secondly we evaluated the impact of the following partner drugs by using the MFQ-resistant N1100, ADQ- resistant NAM, and PND resistant-NPN-10 lines of P. berghei. The data obtained thus far demonstrate that partner drug resistance significantly erodes the PD properties of the ACTs in current clinical use. For example with MFQ resistance, the minimal effective regimen of AS-MFA for sensitive parasites was poorly efficacious versus MFQ resistant parasites. These data demonstrate the utility of the rodent model to estimate PD properties of ACT combinations and to determine the most effective ACT regimens to delay emergence of ART resistance.

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**HIGH THROUGHPUT ANALYSIS OF IN VITRO ANTIMALARIAL SENSITIVITY DATA IN THE ERA OF ARTELINIC COMBINATION THERAPY: THE WWARN IN VITRO ANALYSIS AND REPORT TOOL (IVART)**

Charles J. Woodrow1, Sabina Dahlström1, Richard Cooksey1, Jennifer Flegg1, Hervé Le Nagard2, France Mentré2, Philippe Guérin1, Jacques Le Bras1  
1WorldWide Antimalarial Resistance Network, Oxford, United Kingdom, 2INSERM-Université Paris Diderot, Paris, France

In vivo assessment of antimalarial drug sensitivity remains an important tool in the era of artemisinin combination therapy, providing a way to assess parasite susceptibility to a range of drugs that is largely independent of clinical factors. In addition, investigation of molecular mechanisms of resistance via transfection depends absolutely on accurate and relevant in vitro phenotyping. WWARN and its collaborators have established a repository of raw, in vitro data derived from a wide range of locations and readout methods. Analysis of this large and varied dataset has now been undertaken using WWARN’s In Vitro Analysis and Report Tool (IVART), an application that performs high throughput data analysis with calculation of standard IC50 parameters via non-linear regression. Here we describe the development and validation of IVART, and report the evidence base for its design features, including methods for curve fitting and quality assessment that, until now, have relied on expert opinion.
The data also reveal differences in efficacy between artemisins and their partner drugs in several forms of assay readout, highlighting the importance of matching in vitro assay readouts to in vivo properties in areas of emerging drug resistance. Improvements in the standardization of in vitro assays are critically important and the development of a free, adapted software tool like IVART addresses the heterogeneity of analytical in vitro output. Such standardized in vitro outputs could play a major role in the validation of potential molecular markers of resistance to antimalarials including artemisinin.

**TRENDS OF THE FREQUENCY OF PLASMODIUM FALCIPARUM DRUG-RESISTANCE MOLECULAR MARKERS IN ISOLATES FROM PREGNANT WOMEN SIX YEARS AFTER INTRODUCTION OF INTERMITTENT PREVENTIVE TREATMENT WITH SULFADOXINE-PYRIMETHAMINE (IPTP-SP) IN GABON**

Marielle K. Bouyou-Akotet, Denise P. Mawili-Mboumba, Marie-Lou Tchibola, Gladys Tsombou-Bakana, Rosalie Nikiema, Maryvonne Kombila

Department of Parasitology Mycology Faculty of Medicine, Libreville, Gabon

Following WHO recommendations for malaria control, gabonese Ministry of Health adopted ACTs, insecticide-treated nets and intermittent preventive treatment with sulfadoxine-pyrimethamine (IPTP-SP) in 2003. Prevalence of triple dhfr and quintuple dhfr/dhps mutation were respectively of 86% and 22% in 2005. Six years after their implementation, the frequency of dhfr and dhps point mutations was assessed in Plasmodium falciparum isolates from Gabonese pregnant women according to the number of SP doses. Polymorphic codons of dhfr gene (51, 59, 108 and 164) and dhps gene (437, 540 and 581) were analysed using PCR-restriction fragment length polymorphism. Blood samples from 89 women were analyzed, 35 received 2 doses, 16 received 3 doses and 18 none dose of SP. Among patients with 3 SP doses, 11 had submicroscopic infection. None sample had a quadruple dhfr mutation but the frequency of triple mutation (51-59-108) was 98%. All parasites carried a wild-type allele at codon 164. The same was true for the codon 581 of dhps gene. These preliminary data indicate an increase in the frequency of multiple resistance markers to SP independently of the number of doses received during pregnancy. There is an urgent need to assess the in vitro susceptibility of Plasmodium falciparum isolates to SP, to study other factors associated with the presence of SP resistant parasites and to evaluate an alternative drug for IPTp for pregnant women.

**STUDIES ON AP2 ADAPTOR µ-CHAIN, A NEW CANDIDATE MOLECULAR MARKER FOR ARTEMISININ RESISTANCE IN PLASMODIUM FALCIPARUM**

Gisela C. Henriques, Khalid Beshir, Teun Bousema, Halidou Tinto, Umberto D’Alessandro, Paul Hunt, Pedro Cravo, Colin Sutherland, Rachel Hallett

1London School of Hygiene and Tropical Medicine, London, United Kingdom, 2Centre Muraz, Instituto de Recherche en Science de la Sante, Bobo Dioulasso, Burkina Faso, 3Medical Research Council, Banjul, Gambia, 4University of Edinburgh, Edinburgh, United Kingdom, 5Instituto de Patologia Tropical e Saude Publica, Instituto de Higiene e Medicina Tropical, Goiânia; Lisbon, Brazil

There is evidence of reduced susceptibility of the malaria parasite Plasmodium falciparum to artemisinin derivatives, expressed by delayed parasite clearance times in vivo. If artemisinin resistance spreads, it would threaten global malaria control. We lack validated molecular markers for monitoring these phenotypes. Using whole genome sequencing in the rodent malaria parasite Plasmodium chabaudi, we identified a mutation in the mu chain of the AP2 adaptor protein complex (pcap2-m) that arose along with the experimental evolution of artemisinin resistance. We screened several field isolates of P. falciparum from an ACT clinical trial in Burkina Faso, that were tested in vitro for their response to artemisinin derivatives and other drugs, and in pre- and post- treatment samples from an in vivo ACT trial carried out in Kenya, for genetic polymorphisms in the pcap2-µ orthologue. Genetic polymorphisms in pcap2-µ were analysed for association with several endpoints in both trials that might indicate a drug resistant parasite phenotype. Preliminary results indicate that polymorphisms in this adaptor protein subunit may be associated with in vitro and in vivo responses to artemisinin derivatives, quinine and lumefantrine. Further evaluation of pcap2-µ as a potential molecular marker of artemisinin resistance is now needed.

**DHFR AND DHPS SELECTIVE SWEEPS IN MALAWI AT A TIME OF HIGH SULFADOXINE-PYRIMETHAMINE USE**

Elena M. Artimovich, Ananias A. Escalante, Kristan Schneider, Terrie E. Taylor, James G. Kublin, Miriam K. Lauber, Christopher V. Plowe, Shannon Takala-Harrison

1Howard Hughes Medical Institute/Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, MD, United States, 2School of Life Sciences, Arizona State University, Tempe, AZ, United States, 3Department of MPI, University of Applied Sciences Mittweida, Mittweida, Germany, 4College of Osteopathic Medicine, Michigan State University, Lansing, MI, United States, 5Fred Hutchinson Cancer Research Center, Seattle, WA, United States

Malawi and other African nations have stopped using both chloroquine and sulfadoxine-pyrimethamine (SP) due to expansion of drug-resistant Plasmodium falciparum parasites. Directional selection of chloroquine resistance alleles in the form of a selective sweep has been shown, by analyzing variation in microsatellites flanking the chloroquine resistance gene, pfcrt. Similar selective sweeps of regions flanking dhfr and dhps, the genes that cause resistance to SP, have been identified in Africa, Southeast Asia, and South America. Here we report evidence of positive directional selection of dhfr and dhps resistance haplotypes and describe characteristics of the associated selective sweeps, at a time of high SP drug pressure in Malawi. Resistance alleles and flanking microsatellites were genotyped on 689 filter paper samples from children aged 6 months-12 years in Blantyre, Malawi from 1999-2001 when SP was the first-line treatment for malaria. All but one of the genotyped samples carried one or more SP resistance alleles. Dhfr triple-mutants conferring strong SP resistance predominated (51I/59R/108N), forming a quadruple mutant with dhps 540E. Dhfr/dhps quintuple mutants (dhfr 51I/59R/108N+dhps 437G/540E) were also observed. A reduction in microsatellite heterozygosity was identified in the regions flanking both dhfr and dhps. The sweep flanking dhfr extended from 10kb upstream to 20kb downstream of dhfr. The sweep flanking dhps extended from approximately 10kb upstream to at least 9kb downstream. Extended Haplotype Homozygosity was estimated, and showed increased linkage disequilibrium (LD) in regions flanking both dhfr and dhps. The selectivity of sweeps of resistant dhfr and dhps indicate that these alleles were under recent positive directional selection. The characteristics of the selective sweeps reported here, which were detected during a period of high SP drug pressure, will be compared to those detected after removal of SP as the first line therapy and in settings with different levels of malaria transmission.

**EVOLUTION OF DRUG RESISTANCE IN MALARIA PARASITES**

Mathieu Legros, Sebastian Bonhoeffer

ETH, Zurich, Switzerland

Efforts to relieve the burden caused by malaria rely critically on the availability of drugs targeting Plasmodium falciparum. The efficiency of these treatments is however seriously compromised by the appearance and spread of drug resistance. Resistance is observed today to some...
EXHAUSTED T CELLS

This expansion of PD-1, and double PD-1 and LAG-3 positive CD4 T cells, as well as single-positive LAG-3 positive exhausted CD4 T cells. We show that persistent Pf exposure drives expansion of both PD-1 and LAG-3 positive MBCs, and provide evidence suggesting that these MBC phenotypes differ with respect to malaria exposure. We confirm that current Pf exposure drives expansion of atypical MBCs, and provide evidence suggesting that these PF-associated atypical MBCs are expanded at the expense of naïve B cells. We show that persistent Pf exposure drives expansion of both PD-1 single, and PD-1 and Lag-3 double positive exhausted CD4 T cells, and to a lesser extent single-positive Lag-3 positive exhausted CD4 T cells. This expansion of PD-1, and double PD-1 and Lag-3 positive CD4 T cells is largely confined to CD45RA positive cells. The percentage of PD-1 and Lag-3 double positive CD45RA positive CD4 T cells correlated negatively with frequencies of activated and classical MBCs. Single PD-1, and double PD-1 and Lag-3 positive CD8 T cells were increased among the total, and T effector CD8 T cells, respectively, but only in the presence of asymptomatic parasitaemia. Together, these results suggest that Pf drives expansion of atypical lymphocytes.

This implication is that these cells may dampen inflammatory responses to malaria, thus reducing pathogenesis, but may also impede the generation of protective responses.

IDENTIFYING DRUG RESISTANCE GENOTYPES IN ECUADORIAN MALARIA PARASITES

Gabriela Valenzuela, Fabián E. Sáenz

Infectious Disease Research Center, Pontificia Universidad Católica del Ecuador, Quito, Ecuador

Approximately 40% of the world population lives in malaria-endemic areas and recent estimates indicate that there are several hundred million cases and about 1.2 million deaths each year caused by this disease. Severe disease and resistance to antimalarials has been documented for Plasmodium falciparum and P. vivax and efforts to control malaria have become more challenging in recent years due to widespread drug resistance. Today, the vast majority of P. falciparum isolates in Latin America are resistant to chloroquine (CQ) and other drugs and resistance to CQ has been reported for P. vivax. It is also widely recognized that drug resistance has played a role in the re-emergence of malaria in the Amazon basin at the end of the 20th century. The antimalarial resistance situation in Ecuador is not well known and genotypes for drug resistance from different parts of the country have not been studied. In order to identify and analyze genotypic markers for drug resistance in Ecuador we are doing PCR-RFLP from confirmed malaria blood samples spotted in filter paper using specific primers for Pfcrt, Pfdrhr and Pfdhps (P. falciparum) and Pvdhfr, Pvdhps (P. vivax). Our results so far show that the tested Ecuadorian P. falciparum isolates have a mutant Pfcrt. In addition, we will present P. falciparum and P. vivax genotype data from different resistance markers. The study of the prevalence of drug resistance in Ecuadorian P. falciparum and P. vivax will enhance our knowledge of drug resistance in Latin America, a necessary task to improve the way malaria is treated in this region of the world.

PERSISTENT PLASMODIUM FALCIPARUM INFECTIONS DRIVE EXPANSION OF ATYPICAL MEMORY B CELLS AS WELL AS EXHAUSTED T CELLS

Joseph Illingworth1, Noah S. Butler2, Peter D. Crompton3, Sophie Roetynck1, Susan K. Pierce1, John Harty4, Kevin Marsh1, Philip Bejon1, Francis M. Ndungu1

1Kenya Medical Research Institute, Centre for Geographical Medicine Research (Coast), Kilifi, Kenya, 2Department of Microbiology, University of Iowa, Iowa City, IA, United States, 3Laboratory of Immunogenetics, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States, 4Department of Microbiology, University of Iowa, Iowa, IA, United States

Recent epidemiological and animal studies suggest that Plasmodium parasites induce atypical/exhausted lymphocytes in their hosts, perhaps as an immune evasive strategy. Whilst these immunoregulatory lymphocytes may benefit the host by checking exaggerated immune responses and hence reducing immunopathology, they may also impede generation of protective immune responses. Thus such observations may explain in part: why naturally acquired immunity to malaria develops slowly, often requiring several years of repeated exposure to become effective, and why vaccines confirmed protective in animals and naïve volunteers fail to protect malaria-exposed individuals. Here, we compared frequencies of atypical memory B cells (MBC) and exhausted T cell phenotypes between well-characterised cohorts of children of similar genetic backgrounds and living in similar environmental conditions, but whose rate and history malaria exposure differs. We confirm that current malaria exposure drives expansion of atypical MBCs, and provide evidence suggesting that these Pf-associated atypical MBCs are expanded at the expense of naïve B cells. We show that persistent Pf exposure drives expansion of both PD-1 single, and PD-1 and Lag-3 double positive exhausted CD4 T cells, and to a lesser extent single-positive Lag-3 positive exhausted CD4 T cells. This expansion of PD-1, and double PD-1 and Lag-3 positive CD4 T cells is largely confined to CD45RA positive cells. The percentage of PD-1 and Lag-3 double positive CD45RA positive CD4 T cells correlated negatively with frequencies of activated and classical MBCs. Single PD-1, and double PD-1 and Lag-3 positive CD8 T cells were increased among the total, and T effector CD8 T cells, respectively, but only in the presence of asymptomatic parasitaemia. Together, these results suggest that Pf drives expansion of atypical lymphocytes.

THE EFFECT OF MATERNAL MALARIA AND HELMINTH INFECTIONS ON CHILDHOOD MALARIA: A BIRTH COHORT IN ENTEBBE, UGANDA

Juliet Ndirazza1, Emily L. Webb2, Swaib A. Lule1, Harriet Mpairwe1, Miriam Akello1, Gloria Odur1, Moses Kizza1, Helen Akurut1, Lawrence Muthingi2, Birgitte Vennervald4, Alison M. Elliott1

1Medical Research Council/Uganda Virus Research Institute, Entebbe, Uganda, 2London School of Hygiene and Tropical Medicine, London, United Kingdom, 3Entebbe Hospital, Entebbe, Uganda, 4DIBL-Centre for Health Research and Development, University of Copenhagen, Copenhagen, Denmark

Helmints and Plasmodium infections are common in the tropics, and positive associations have been observed between these parasitic infections in pregnancy. While malaria in pregnancy has been associated with adverse maternal and birth outcomes, knowledge on the effect of prenatal exposure to malaria and helminth infections on childhood malaria is still sparse. This study took place in Entebbe, Uganda. 2507 women were recruited in a trial on the effects of albendazole and praziquantel in pregnancy. Blood and stool samples were examined for helminth and P. falciparum infections. The offspring were followed up to age five years, and their malaria morbidity data collected prospectively. Clinical malaria was diagnosed as fever (≥37.5°C) with P. falciparum parasitaemia, and asymptomatic parasitaemia recorded annually at scheduled visits. In multivariate analyses we adjusted for risk factors associated with malaria and helminth infections. Common parasitic infections in pregnancy were hookworm (45%), Mansonella perstans (21%), Schistosoma mansoni (18%), and P. falciparum (11%). Of 2345 liveborn infants, 69% were infected with P. falciparum (21%), Mansonella perstans (45%) and their malaria morbidity data collected prospectively. Clinical malaria was diagnosed as fever (≥37.5°C) with P. falciparum parasitaemia, and asymptomatic parasitaemia recorded annually at scheduled visits. In multivariate analyses we adjusted for risk factors associated with malaria and helminth infections. Common parasitic infections in pregnancy were hookworm (45%), Mansonella perstans (21%), Schistosoma mansoni (18%), and P. falciparum (11%). Of 2345 liveborn infants, 69% were still under follow-up at age 5 years. The overall childhood malaria rate was 34 episodes per 100 child-years, and the cumulative prevalence of asymptomatic P. falciparum parasitaemia over the five years was 5%. Maternal hookworm and M. perstans infections were associated with an increased risk of childhood malaria (adjusted Hazard Ratio [aHR] 1.26, p<0.001 and 1.23, p=0.004 respectively), and increased cumulative prevalence of asymptomatic parasitaemia (adjusted Odds Ratio [aOR] 1.59, p=0.001 and 1.55, p=0.01 respectively). S. mansoni infection showed no such associations. Maternal P. falciparum infection was associated with an increased risk of childhood malaria (aHR 1.22, p=0.04) but not prevalence.
of asymptomatic malaria (aOR 1.21, p=0.4). This study shows that the effect of malaria in pregnancy on childhood malaria extends to age five years, and is the first report of an association between helminth infections in pregnancy and malaria in the offspring.

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VALIDITY OF SELF-REPORTED USE OF SULFADOXINE-PYRIMETHAMINE INTERMITTENT PRESCRIPTIVE TREATMENT DURING PREGNANCY (IPTP): A CROSS-SECTIONAL STUDY

Fatuma Namusoke1, Muhammad Ntale1, Mats Walhgren2, Fred Kironde3, Florence Mirembe1

1Makere University, Kampala, Uganda, 2Karolinska Institutet, Stockholm, Sweden

Malaria in pregnancy is a major health problem that can cause maternal anaemia, stillbirth, spontaneous abortion, low birth-weight and intra-uterine stunting. The WHO recommends use of Sulphadoxine-Pyrimethamine (SP) for Intermittent Preventive Treatment of malaria during pregnancy (IPTp) in endemic areas. Towards monitoring and assessing IPTp coverage in the population, the Roll Back Malaria program recommends use of self reported data. In this study, we assessed the validity of self reported use of IPTp by testing for sulfadoxine in maternal blood at delivery. Two hundred and four pregnant women were consented and enrolled in a cross-sectional study. We excluded participants who reported a history of taking sulfa containing drugs, those who were not sure of dates relating to last menstrual period or who took IPTp before 20 weeks of gestation. Data on demographic characteristics, obstetric history, and delivery outcome were collected. At delivery the baby, we took the mother’s venous blood, carried out blood smear microscopy for parasites and tested the plasma for sulfadoxine using High Performance Liquid Chromatography (HPLC). We found that 17.2% of participants reported to have used IPTp and indeed tested positive by HPLC while 30.4% reported not to have used IPTp and indeed tested negative for sulfadoxine. Participants possessing post primary education were more likely to have reported using IPTp. The low agreement between self report and actual presence of the drug in the blood casts doubt on the validity of self reported data in estimating IPTp coverage. We recommend further research of self reported data towards improving the accuracy of such information which is vital for guiding policy for malaria control in pregnancy since routine blood drug assays would be too expensive and impractical for population based studies.

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A SPATIO-TEMPORAL ANALYSIS OF PLASMODIUM FALCIPARUM AND P. VIVAX INFECTIONS AND TREATMENT SEEKING BEHAVIOR IN THA SONG YANG DISTRICT, TAK PROVINCE, THAILAND: 2008-2011

Daniel Parker1, Stephen Matthews1, Wanna Srisatjarak2, Peeryath Boonpan2, Rujira Lerdprom2, Ming-Chieh Lee3, Guiyun Yan2, Jetsumon Prachumstiri4, Liwang Cui5, Jereraph Sirichaisinthop6

1Pennsylvania State University, University Park, PA, United States, 2Vector Borne Disease Training Center, Pra Budhabat, Saraburi, Thailand, 3University of California, Irvine, CA, United States, 4Mahidol University, Bangkok, Thailand

Despite being relatively successful at controlling malaria in most of the country, the border areas surrounding Thailand continue to experience persistent, seasonal malaria. The heaviest malaria burden within Thailand is along the Thai-Myanmar border in Tha Song Yang District, Tak Province. This area is also a center of drug and multi-drug resistant malaria and recently decreased parasite sensitivity to artemisinin derivatives (largely considered the last defense against drug-resistant malaria) has been reported from Tak Province. Here we use exploratory spatial analysis and accelerated failure time models to evaluate spatio-temporal patterns in both Plasmodium falciparum and P. vivax case frequency and treatment seeking behavior. We are specifically interested in potential clustering of cases near the Thai-Myanmar border as well as the length of time between a patient’s reported onset of malaria symptoms and the time they actually visit a malaria clinic. Our temporal analysis is at the subdistrict level (within Tha Song Yang District) whereas our spatial analysis is at both the subdistrict and district levels (within Tak Province.) We find a general pattern of spatial decay, with general correspondence in both parasite species, and with the heaviest case-loads clustered in administrative units that touch the Thai-Myanmar border. However, this pattern isn’t a smooth gradient from the border towards central Thailand. Finally, our temporal analyses indicate an initial clustering of treatment seeking times around 2 to 3 days and several other clusters occurring after 8 days. For example, among those that seek treatment within 7 days after the onset of symptoms, Myanmar nationals are the quickest to seek treatment. Conversely, among those that wait until after a week of experiencing symptoms Myanmar nationals wait the longest to seek treatment. This discordance in treatment seeking behavior has important implications for public health and global health. Individuals who are carrying parasites in their blood for longer periods of time may increase the risk of infection for the populations surrounding them. These results are significant with regards to the increased potential of transmitting drug (potentially artemisinin) resistant malaria.

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UNDERSTANDING THE IMPACT OF SUBSIDIZING ARTEMISININ-BASED COMBINATION THERAPIES (ACTs) IN THE RETAIL SECTOR - RESULTS FROM FOCUS GROUP DISCUSSIONS IN RURAL KENYA

Sarah V. Kenedge1, Beth B. Kangwana1, Evelyn W. Waweru1, Andrew J. Nyandigisi2, Jayesh Pandit3, Simon J. Brooker4, Robert W. Snow5, Catherine A. Goodman6

1KEMRI-Wellcome Trust Research Programme, Nairobi, Kenya, 2Division of Malaria Control, Nairobi, Kenya, 3Pharmacy and Poisons Board, Nairobi, Kenya, 4London School of Hygiene and Tropical Medicine, London, United Kingdom, 5Centre for Tropical Medicine, Nuffield Department of Clinical Medicine, University of Oxford, CCVTM, Oxford, United Kingdom

There is considerable interest in the potential of private sector subsidies to increase availability and affordability of artemisinin-based combination therapies (ACTs) for malaria treatment. A cluster randomized trial of such subsidies was conducted in 3 districts in Kenya, comprising provision of subsidized packs of paediatric ACT to retail outlets, training of retail staff, and community awareness activities. The results demonstrated a substantial increase in ACT availability and coverage, though patient counselling and adherence were suboptimal. We conducted a qualitative study in order to understand why these successes and limitations occurred. Eighteen focus group discussions were conducted, 9 with retailers and 9 with caregivers, to document experiences with the intervention. Respondents were positive about intervention components, praising the focused retailer training, affordable pricing, strong promotional activities, dispensing job aids, and consumer friendly packaging, which are likely to have contributed to the positive access and coverage outcomes observed. However, many retailers still did not stock ACT, due to insufficient supplies, lack of capital and staff turnover. Advice to caregivers was poor due to insufficient time, and poor recall of instructions. Adherence by caregivers to dosing guidelines was sub-optimal, because of a wish to save tablets for other episodes, doses being required at night, stopping treatment when the child felt better, and the number and bitter taste of the tablets. Caregivers used a number of strategies to obtain paediatric ACT for older age groups. In conclusion, this study has highlighted that important components of a successful ACT subsidy intervention are regular retailer training, affordable pricing, a reliable supply chain and community mobilization emphasizing patient adherence and when to seek further care.
COMPARISON OF MALARIA RISK FACTORS AND PARASITEMIA AMONG CHILDREN LIVING EITHER WITH NON-PARENT GUARDIANS OR WITH BIOLOGICAL PARENTS: ANALYSIS OF 2009 UGANDA MALARIA INDICATOR SURVEY DATA

Samantha B. Dolan1, Carrie F. Nielsen2, Adam Wolkon3, Sussann Nasr4, Denis Rubahika5, Steven S. Yoon2, Kevin Sullivan1, Achuyt Bhattari2
1Emory University, Atlanta, GA, United States, 2Centers for Disease Control and Prevention, Atlanta, GA, United States, 3Centers for Disease Control and Prevention, Kampala, Uganda, 4Uganda Ministry of Health, National Malaria Control Program, Kampala, Uganda

As of 2009, approximately 2.7 million child orphans were living with one parent or non-parent guardians (NPG) in Uganda. These children may be at a higher risk of malaria than children living with their biological parents (BP) due to possible differences in access to malaria prevention measures and household characteristics. The 2009 Uganda Malaria Indicator Survey collected malaria prevention coverage and household data from 4,760 nationally representative households, and blood smear samples for malaria parasitemia from children under 5 years old (US) living in those households. Data were analyzed in SAS 9.2 (proc surveylogistic, surveyfreq). Odds ratios (OR) of main outcome measures and associated 95% confidence interval (CI) and p-values (p) were computed. Children under 5 years old were categorized as either living with NPG or BP based on their relationship to the head of the household. During the 2009 MIS, 707 (18%) out of 3933 US were living with NPG. NPG head of the household were likely to be older [median age; 54 years, IQR: 28-40 vs. 34, IQR: 47-63; p<0.01] and female [54% vs. 18%, p<0.01]. Fewer NPG households owned at least one insecticide treated net [76%, 95% CI: 71-81 vs. 80%, 95% CI: 77-84; p=0.33], and fewer NPG children slept under any bednet the night before the survey (bednet use) [58%, 95% CI: 50-67 vs. 75%, 95% CI: 72-79; p<0.01]. Adjusting for children's age and head of household's age, NPG children were less likely to use any bednet the night before the survey than BP children (OR: 0.6, 95% CI: 0.4-0.9; p<0.02). Adjusting for children's age, head of household's age and sex, household wealth quintile, and bednet use, the odds of malaria parasitemia was four times greater for NPG children than BP children (OR: 4.2, 95% CI: 1.8-9.7; p<0.01). The odds of parasitemia among NPG children were strongly modified by the interaction with age of head of household and bednet use (p for interactions<0.001). NPG children may be at a greater risk for malaria than BP children, and may warrant special targeting of malaria intervention efforts.

LOW PREVALENCE OF PLACENTAL MALARIA INFECTION AMONG PREGNANT WOMEN IN ZANZIBAR: POLICY IMPLICATIONS FOR IPTP

Marya Plotkin1, Khadija Said2, Natalie Hendler1, Asma R. Khamis1, Mwinyi I. Mselle3, Maryjane Lacoste1, Elaine Roman1, Veronica Ades1, Julie Gutman4, Raz Stevenson5, Peter McElroy6
1Jhpiego, Dar es Salaam, United Republic of Tanzania, 2Ministry of Health Zanzibar, Zanzibar, United Republic of Tanzania, 3Zanzibar Malaria Control Programme, Zanzibar, United Republic of Tanzania, 4Jhpiego, Baltimore, MD, United States, 5University of California San Francisco, San Francisco, CA, United States, 6Centers for Disease Control and Prevention and President’s Malaria Initiative, Atlanta, GA, United States

Efforts by the Zanzibar Ministry of Health to scale-up malaria prevention and treatment strategies, including intermittent preventive treatment for pregnant women (IPTp), have brought Zanzibar to the pre-elimination phase of malaria control. P. falciparum prevalence in the general population has been below 1% since 2008 and the diagnostic positivity rate among febrile patients was 1.2% in 2011. Zanzibar implemented IPTp using sulfadoxine-pyrimethamine (SP) in 2004 when malaria prevalence exceeded 20%. While coverage among pregnant women is low (47% received two doses SP), the value of this intervention in low transmission settings remains uncertain. Few countries in Africa have confronted policy questions regarding timing of IPTp scale-down. We designed a prospective observational study to estimate prevalence of placental malaria among pregnant women with no evidence of receiving any dose of SP for IPTp during pregnancy. From September 2011 to April 2012 we enrolled a convenience sample of pregnant women on day of delivery at six hospitals in Zanzibar (three in both Pemba and Unguja). Dried blood spots (DBS) on filter paper were prepared from placental blood specimens. DBS were analyzed via polymerase chain reaction indicating active Plasmodium infection (all species). To date, over 1,200 deliveries were enrolled at the six recruitment sites (approximately 12% of total, range: 8-26%). Two (0.19%; 95% CI, 0.05-0.69%) of 1,046 DBS specimens analyzed to date showed evidence of P. falciparum infection. Both were from HIV uninfected, multigravid women in Unguja. Birth weights for both deliveries were normal (>2500 g). Data collection will continue through the peak transmission season of May-July 2012. The very low prevalence of placental infection among women who received no IPTp raises policy questions regarding continuation of IPTp in Zanzibar. Alternative efforts to control malaria in pregnancy in Zanzibar, such as active case detection via regular screening and treatment during antenatal visits, should be evaluated.
spatial heterogeneity of transmission intensity and secular trends in transmission intensity. Our results indicate fine scale spatial variation in the epidemiology of malaria in a 150 sq km area that was comparable with the variation seen on more coarse spatial scales across regions or countries.

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ESTIMATING TRANSMISSION INTENSITY FROM PLASMODIUM FALCIPARUM SEROLOGICAL DATA USING ANTIBODY DENSITY MODELS
Emilie Pothin1, Neil Ferguson1, Chris Drakeley2, Azra Ghani3
1Imperial College London, London, United Kingdom, 2London School of Hygiene & Tropical Medicine, London, United Kingdom
Sero logical data are increasingly being used to monitor malaria transmission intensity and have been demonstrated to be particularly useful in areas of low transmission where traditional measures such as EIR and parasite prevalence are limited. The seroconversion rate is usually estimated using catalytic models in which the measured antibody levels are used to categorise individuals as seropositive or seronegative. One limitation of this approach is that the cut-off between positive and negative is arbitrary. Furthermore, the continuous variation in antibody levels is ignored thereby potentially reducing the precision of the estimate. To overcome these limitations we developed a series of age-specific density models which mimic antibody acquisition and loss. These were fitted to antibody titre data from multiple Plasmodium falciparum endemic settings to estimate the rate of acquisition of antibodies as an alternative measure of transmission intensity. Our results indicate that a model in which the boost in antibodies following exposure depends on the existing titre (with an exponential decline in the size of the antibody boost with higher levels of circulating antibodies) and that includes variation between individuals in the size of the response fits the data well. Furthermore our results show a consistent ordering of transmission intensities compared to those from a catalytic model. This approach, if validated across different epidemiological settings, could be a useful alternative model for measuring transmission intensity which avoids the need for an arbitrary cut-off value.

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PREVALENCE OF MALARIA AND ANEMIA AMONG PREGNANT WOMEN ATTENDING ANTENATAL CARE CLINICS IN THE EJISU-JUABEN AND SEKYERE-EAST DISTRICTS OF GHANA
Gifty D. Antwi1, Harry Tagborg1, Imelda Bates1
1Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, 2Liverpool School of Tropical Medicine, Liverpool, United Kingdom
Malaria and anaemia (haemoglobin concentration < 11g/dl) in pregnancy continue to be of public health importance in Ghana with malaria contributing to 9.4% of maternal deaths. Strategies are being implemented through the antenatal care system to reduce their occurrence but asymptomatic malaria parasitaemia and anaemia prevalence at term stand at 12.1% and 45.0% respectively. In preparation for a cluster randomised control trial to determine the effect of an enhanced antenatal care package on malaria and anaemia in pregnancy, a cross-sectional study was conducted from December 2011 to April 2012 among pregnant women with gestation ≥23 weeks and prior to delivery. Trained research assistants determined malaria parasitaemia and haemoglobin concentration levels using the malaria rapid diagnostic test and the HemoCue 301. An interviewer guided questionnaire was also administered to determine the demography, bed net use, IPTp administration and self-reported adherence to iron and folate supplementation among the pregnant women. The prevalence of malaria parasitaemia and anaemia was 15.5% and 42.6% respectively. Parasitaemia occurred in a significantly younger age group (25.1 (6.21) yrs vs 27.4 (6.29) yrs; p=0.007) and these had a significantly lower haemoglobin concentration (10.5 (1.37) g/dl vs 11.2 (1.29) g/dl; p<0.0001). Although 61.2% of the pregnant women owned bed nets, only 39.3% slept under one during the night before the survey. A total of 81.2% received two or more doses of SP and 50.5% reported high adherence to iron and folate supplementation however these were not significantly associated with the prevalence of parasitaemia or anaemia. Malaria parasitaemia and anaemia are still prevalent in the study area despite the implementation of current strategies including ITN use, SP-IPTp, iron and folate supplementation and prompt diagnosis and effective treatment of malaria. Probably new ways of delivering these strategies to make them more effective need to be explored.

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REVITALIZING ROUTINE HEALTH FACILITY DATA FOR MALARIA CONTROL
Jessica Butts, Melody Miles, Carrie Nielsen, Achuvt Bhattacharai, Steven Yoon
Centers for Disease Control and Prevention, Atlanta, GA, United States
Successful scale up of proven malaria control interventions across sub-Saharan Africa since 2000 has resulted in rapid changes in malaria epidemiology. Because of these changes, national malaria control programs and their partners need effective tools to adequately monitor malaria burden for surveillance and program planning. Existing tools, most notably national-level household surveys such as the Malaria Indicator Survey and Demographic and Health Survey, do not provide information on longitudinal changes in malaria burden. Cross-sectional data do not reflect seasonal fluctuations in malaria burden nor typically provide district- or sub-district-level data useful for program monitoring and planning. In many countries, routine health facility data are of unknown validity due to reporting of clinical diagnosis without laboratory confirmation and incomplete or late reporting. Recognizing the need for improved approaches to measure longitudinal changes in malaria burden, the authors have undertaken a comprehensive examination of routine health facility data and are optimistic about its potential to complement existing data for malaria control. Using models from Benin, Ethiopia, Madagascar, and Uganda, the authors have critically assessed the strengths and weaknesses of different health facility-based surveillance systems and created a framework to assist countries in developing robust data collection systems that will meet country-specific data needs while taking into account resource limitations. The framework guides stakeholders in the decision-making process and is comprehensive in that it considers scope, indicators, data collection tools, supervision, and data use. The anticipated outcomes of providing a framework for strengthening facility-based data collection systems for malaria include increasing the quality of routine system data, improving country capacity for planning, and sustaining the progress made in malaria control over the past decade.

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MOLECULAR EPIDEMIOLOGY OF PLASMODIUM VIVAX RELAPSES IN THE PERUVIAN AMAZON
Raul Chiquiquirai1, Pablo Peñataro2, Kimberly C. Brouwer1, Manuel Fasabi1, Maritza Calderon1, Sonia M. Torres1, Shira Abeles1, Robert Gilman1, Alejandro Llanos-Cuentas1, Margaret Kosek2, Joseph M. Vinetz1
1University of California at San Diego, San Diego, CA, United States, 2Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States
Plasmodium vivax accounts for 71-81% of malaria cases in the Americas. To determine the magnitude of P. vivax relapsing malaria in rural Amazonia, we carried out a study from 2005-2008 in four health posts in the Amazonian Region of Loreto in northwest Peru where the majority of nationally reported malaria cases originate. PCR-restriction fragment length polymorphism of PvMSP-3α (enzymes HhaI and AluI) and PCR of nine tandem repeat markers were compared for their ability to distinguish relapse vs. reinfection. Of 1507 subjects with P. vivax malaria, 354...
developed >1 episode during the study. 97/354 (27.4%) were defined as relapses using \( P_{vivax} \) alone. Adding tandem repeat polymorphism analysis significantly reduced the number of definitively-defined relapses to 26/354 (7.4%) (\( p<0.05 \)), allowing for more new infections to be identified. Odds of another episode of \( P_{vivax} \) malaria, whether due to relapse or reinfection, were 2.6 times higher in the more remote village of Mazan than in villages closer to Iquitos city (OR=2.6, 95% CI: 2.0,3.4). People in Mazan were 2.4 times more likely to develop a relapse (not reinfection) than people in other villages (OR=2.4, CI95%:1.1, 1.5; \( p=0.03 \)). The proportion of multiple genotype infections was 16.1% by TR, 4.5% by MSP-3a, and 18.8% using both. The use of highly resolving molecular markers of \( P_{vivax} \) allowed for finding an unexpectedly high proportion of multiple genotype infections, remarkable considering the current knowledge of transmission intensity and entomological inoculation rates in the region. Highly discriminatory molecular epidemiological tools will allow us to gain critical knowledge of the micro-geography of malaria transmission in this area of low transmission.

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TEMPORAL TRENDS IN SEVERE MALARIA IN CHITTAGONG, BANGLADESH

Richard J. Maude1, Mahtab U. Hasan2, Md. Amir Hossain3, Abdullah Abu Sayeed,2 Sanjib K. Paul,2 Waljir Rahman,2 Rapeepan R. Maude1, Nidhi Vaid1, Aniruddha Ghose3, Robed Amin1, Rasheda Samad4, Emran Bin Yunus2, M. Ridvanur Rahman2, A. M. Bengali5, M. Gofranul Hoque6, Nicholas P. Day1, Nicholas J. White1, Lisa J. White7, Arjen M. Dondorp2, M. Abul Faiz8

1Welcome Trust Mahidol-Oxford Tropical Medicine Research Unit, Bangkok, Thailand, 2Chittagong Medical College Hospital, Chittagong, Bangladesh, 3Dhaka Medical College, Dhaka, Bangladesh, 4Centre for Specialized Care and Research, Chittagong, Bangladesh, 5Shaheed Suvwarvadhy Medical College, Dhaka, Bangladesh, 6World Health Organization, Country Office, Dhaka, Bangladesh

Epidemiological data on malaria in Bangladesh are sparse, particularly on severe and fatal malaria. This hampers the allocation of healthcare provision in this resource-poor setting. Over 85% of the estimated 150,000-250,000 annual malaria cases in Bangladesh occur in Chittagong Division with 80% in the Chittagong Hill Tracts (CHT). Chittagong Medical College Hospital (CMCH) is the major tertiary referral hospital for severe malaria in Chittagong Division. Malaria screening data from 22,785 inpatients in CMCH from 1999-2011 were analysed to investigate the patterns of referral, temporal trends and geographical distribution of severe malaria in Chittagong Division. From 1999 till 2011, 2,394 malaria cases were admitted, of which 96% harboured \( P_{falciparum} \) (PF) and 4% \( P_{vivax} \) (PV). Infection was commonest in males (67%) between 15 and 34 years of age. Seasonality of malaria incidence was marked with a single peak in \( P_{falciparum} \) transmission from June to August coinciding with peak rainfall, whereas \( P_{vivax} \) showed an additional peak in February-March likely representing relapse infections. Since 2007 there has been a substantial decrease in the absolute number of admitted malaria cases. Case fatality in severe malaria was 18% from 2008-2011 remaining steady during this period. A travel history obtained in 220 malaria patients revealed only 34% had been to the CHT in the preceding 3 weeks. Of all admitted malaria patients, only 9% lived in the CHT, but none in the more remote malaria endemic regions near the Indian border. The overall decline in admitted malaria cases to CMCH suggests recent control measures are successful. However, there are no reliable data on the incidence of severe malaria in the CHT, the most endemic area of Bangladesh, and most of these patients do not reach tertiary health facilities. Improvement of early treatment and simple supportive care for severe malaria in remote areas and implementation of a referral system for cases requiring additional supportive care could be an important component of further reducing malaria-attributable disease and death in Bangladesh.

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SOCIO-DEMOGRAPHICS AND THE DEVELOPMENT OF MALARIA ELIMINATION STRATEGIES IN THE LOW TRANSMISSION SETTING

Raul Chiquiuyauri1, Maribel Paredes2, Pablo Peñataro2, Sonia M. Torres3, Silvia Marinì1, Alexander Tenorio1, Kimberly C. Brouwer4, Shira Abeles1, Alejandro Llanos-Cuentas2, Robert Gilman5, Margaret Kosek1, Joseph M. Vinetz1

1University of California at San Diego, San Diego, CA, United States, 2Asociacion Benefica Prisma, Lima, Peru, 3Vohns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, 4Universidad Peruana Cayetano Heredia, Lima, Peru

This analysis presents a comprehensive description of malaria burden and risk factors in Peruvian Amazon villages where malaria transmission is hyperendemic. More than 9000 subjects were studied in contrasting village settings within the Department of Loreto, Peru, where most malaria occurs in the country. \( Plasmodium vivax \) is responsible for more than 75% of malaria cases; severe disease from any form of malaria is uncommon and death rare. The association between lifetime malaria episodes and individual and household covariates was studied using polytomous logistic regression analysis, assessing effects on odds of some vs. no lifetime malaria episodes. Malaria morbidity during lifetime was strongly associated with age, logging, farming, travel history, and living with a logger or agriculturist. Select groups of adults, particularly loggers and agriculturists acquire multiple malaria infections in transmission settings outside of the main domicile, and may be mobile human reservoirs by which malaria parasites move within and between micro-regions within malaria endemic settings. For example, such individuals might well be reservoirs of transmission by introducing or reintroducing malaria into their home villages and their own households, depending on vector ecology and the local village setting. Therefore, socio-demographic studies can identify people with the epidemiological characteristic of transmission risk, and these individuals would be prime targets against which to deploy transmission blocking strategies along with insecticide treated bednets and chemoprophylaxis.

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FINE-SCALE SPATIAL HETEROGENEITY OF DRY SEASON PREVALENCE AND ENVIRONMENTAL RISK OF MALARIA AMONG CHILDREN IN RURAL MALAWI: A CASE-CONTROL STUDY

Lindsay R. Townes1, Dyson Mwandama2, Don P. Mathanga2, Mark L. Wilson1

1School of Public Health, University of Michigan, Ann Arbor, MI, United States, 2Malaria Alert Centre, University of Malawi College of Medicine, Blantyre, Malawi

Environmental risk factors for malaria during the dry season in rural Malawi remain relatively unstudied. We investigated the role of local environmental features, in particular active smallholder agricultural land, on malaria prevalence among children <5 years old living in villages in two neighboring rural Traditional Authority (TA) regions in southern Malawi during the dry season. Ten villages from TA Sitola and Msamala were randomly selected. All houses with children <5 were approached and informed consent was obtained from those who agreed to participate, after which the house location was recorded with GPS. At each participating house, a nurse administered a malaria rapid diagnostic test (RDT) to one child, and a questionnaire to parents. Environmental data were collected in and around each house, including land cover <50 meters. Environmental variables found to be significantly associated with RDT status (\( +/\) ) at \( p<0.10 \) in bivariate analysis (X2 or Student's t-test) were analyzed with multi-level multivariate logistic regression (MLLR). Geographic clustering of RDT status, environmental factors, and Pearson residuals from MLLR were calculated using the Getis-Ord GI* statistic. A total of 390 children were enrolled from six villages in Sitola (n=162)

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households) and four villages in Msamala (n=228), of whom 178 (45.6\%) tested malaria positive. MLLR was used to evaluate associations of RDT status and household proximity to agriculture (<25m radius), controlling for child sex and age (months), bed net ownership, elevation (meters), and random effects intercepts for village and TA-level unmeasured factors. Proximity to active agriculture was a significant predictor of being malaria positive (OR 2.80, 95\% CI 1.41-5.55). Mapping of Pearson residuals from MLLR showed significant clustering (G\*2 >2.58, p<0.01) predominantly within TA Sitola, with a somewhat different pattern in TA Msamala on the other side of the Shire River. Evidence shows significant spatial heterogeneity of malaria prevalence and risk factors at very fine scales in this rural Malawi setting, suggesting the need to focus intervention efforts.

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GENOTYPIC PATTERNS OF RELAPSING PLASMODIUM VIVAX INFECTIONS IN CAMBODIA

Jessica T. Lin1, Jonathan J. Juliano1, Oksana Kharabora1, William O. Rogers2, Sinuo Muth3, Chanusuda Wongsrichanalai2, Steven R. Meshnick4

1University of North Carolina School of Medicine, Chapel Hill, NC, United States, 2Naval Medical Research Unit #2, Phnom Penh, Cambodia, 3National Malaria Center, Phnom Penh, Cambodia, 4University of North Carolina Gillings School of Public Health, Chapel Hill, NC, United States

The propensity for Plasmodium vivax parasites to relapse is one of the major obstacles to malaria control and elimination in many regions of the world. Yet little is known about the nature of relapse. A key unanswered question is whether certain vivax variants are more likely to cause relapse, as many infections contain multiple variants. Using a newly developed P. vivax heteroduplex tracking assay (HTA) targeting P. vivax merozoite surface protein 1 (Pvmsp1), we genotyped 107 vivax infections in individuals from Chumkiri, Cambodia, 45 of whom developed recurrent parasitemia between day 28 and day 42 following chloroquine treatment without primaquine. The HTA, which is adept at uncovering minority variants, revealed multiple coinfecting genotypes in 83\% of individuals, with a mean multiplicity of infection (MOI) of 2.8 (IQR 2-4). Genotypes of paired initial and recurrent parasitemias were compared to look for genotypic patterns of relapse. Despite high allelic diversity in the overall cohort (Hs = 0.86), 86\% (38/44) of paired isolates were highly related, sharing at least half their variants. At the same time, novel variants appeared in 30\% (13/44) of recurrent isolates. When the genotypes from initial infections of 45 “relapers” and 62 “nonrelapers” (those who did and did not develop recurrent parasitemia within 42 days) were compared, two specific Pvmsp1 variants were associated with subsequent relapse. By accounting for the polyclonality of P. vivax in Cambodia, we find a complex scheme of relapse in which hypnozoites representing all or a subset of the multiple clones found in an initial infection can reactivate in concert to cause relapse. At the same time, the common appearance of novel variants supports the notion that latent hypnozoites may be reactivated at the time of relapse. Additionally, we have identified individual Pvmsp1 variants that demonstrate a greater propensity for early relapse, suggesting a genetic basis to relapse.

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REPRESENTATIVENESS, COMPLETENESS, TIMELINESS AND ACCURACY OF ZANZIBAR’S MALARIA EPIDEMIC EARLY DETECTION SYSTEM (MEEDS), 2008-2011

Abdul-wahid Al-mafazy1, David P. Ngilangwa2, Abdullah S. Ali1, Issa Garimo3, Fabrizio Molteni4, Mwinyi I. Msellim1, Peter McElroy2

1Zanzibar Malaria Control Program, Zanzibar, United Republic of Tanzania, 2Centers for Disease Control and Prevention/President’s Malaria Initiative, Dar es Salaam, United Republic of Tanzania, 3Research Triangle Institute International, Dar es Salaam, United Republic of Tanzania

Zanzibar’s recent population-based survey estimates of malaria prevalence (<1%) and diagnostic test positivity rates of parasitemia among febrile outpatients (<2%) are approaching pre-elimination levels. In 2008 Zanzibar developed and implemented a mobile phone-based malaria epidemic early detection system (MEEDS) at peripheral clinics to facilitate weekly reporting of confirmed malaria cases and help ensure prompt detection, confirmation, and response to sudden increases in Plasmodium falciparum transmission. Our objective was to analyze 2008-2011 MEEDS data and describe trends in several MEEDS attributes related to outbreak detection. System representativeness was the proportion of all public clinics in Zanzibar reporting data to MEEDS in a given year. Completeness of reporting was defined as submission of all weekly data elements submitted to the system, regardless of date. Timeliness of reporting was calculated as the proportion of expected reports received by the system by Monday of the following week. Finally, data accuracy was assessed through a manual count of weekly case totals from the routine health management information system (HMIS) registers compared to totals in MEEDS registers. Representativeness improved as MEEDS implementation moved forward from 10 (7\%) clinics in 2008 to 52 (37\%) in 2009, 69 (49\%) in 2010, 90 (63\%) in late 2010, and finally 142 (100\%) clinics by late 2011. Completeness of submitted data was 100\% each year except 2009 (84\%) when technical problems prevented data transmission from many clinics. Timeliness of weekly reports received by the following Monday increased from 19\% in 2009 to 43\% in 2011 (p<0.001). The MEEDS data accuracy as compared to routine HMIS increased from 89\% in 2009 to 97\% in the first-half of 2011 and fell to 93\% in the second-half of 2011. Despite accomplishments in reporting representativeness, completeness, and accuracy of the MEEDS over four years of implementation, additional efforts and resources are required to understand and address deficiencies in reporting timeliness, perhaps the most important attribute of an early epidemic detection system.

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DECREASE OF MALARIA INCIDENCE AMONG CONFIRMED CASES OF MALARIA IN MARY IMMACULATA CENTRE MUKURU KENYA IN 2007-2010

Moses Kiwou1, Johanshan Mawole1, John Mutuku Mulir1, Victor Namulanda1, Jirina Kafkova1, Mario Jancovic1, Dana Pechacova1, Jaroslava Sokolova1, Vladimir Krkmer1

1Mary Immaculata Centre, St. Elisabeth University Tropical Program, Mukuru, Nairobi, Kenya, 2Slovak Tropical Institute, St. Elisabeth University College of Health and Social Sciences, Bratislava, Slovakia, 3Department of Clinical Disciplines, School of Health Care and Social Work, Trnava University, Trnava, Slovakia, 4St. Elizabeth University of Health and Social Sciences, Bratislava, Slovakia

The aim of the study was to assess annual incidence of microscopically positive cases of malaria in urban area of Nairobi within last five years (2007-2011) and to compare malaria occurrence before and after intermittent preventive treatment (IPT) was initiated in this area. Mary Immaculata Centre is located in the slum area of Mukuru (Nairobi, Kenya) with about 40 000 inhabitants in height of 1900 meters above sea level. Traveling from Nairobi to down country of Rift Valley and back is usual 1-2 times per year. Two experienced lab technicians investigated daily 30-50 slides a day (15-25 per person). In 2011 also rapid diagnostic tests

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(RTD) were used to confirm positive malaria slide. Within five years (2007-2011), 56,688 samples were microscopically evaluated (8466 - 12333 per year) and 905 were positive for malaria (1.60%). Annual proportion decreased from 1.96% (2007), 2.54% (2008) and 2.11% (2009) to 1% in 2010 and 0.79% in 2011 (P<0.001). Severe cases of malaria were seen only exceptionally. Number of cerebral malaria cases was 1-5 patients/year and severe anaemia (<80 g/l) was also exceptional (15-30 cases/year). Decreasing proportion of microscopically positive malaria cases was probably due to major improvement in infrastructure (disinfection of surface water, canalization, waste water drainage) as well as due to IPT in all four schools in Mukuru since 2009/2010 in all children coming to first contact at the beginning of the year. Sewage water drains and canalizations of surface water in this area as well as IPT in school children and pregnant women may play a role in this trend within last five years.

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ESTIMATES OF MALARIA MORBIDITY BEFORE AND AFTER THE IMPLEMENTATION OF A SENTINEL SITE INPATIENT MALARIA SURVEILLANCE SYSTEM IN UGANDA

Arthur Mpimbaza1, Anne Gasasira2, Asadu Sserwanga3, Ruth Kigozi4, Stella Kakeeto5, Humphrey Wanizira6, Fred Kizito7, Denis Rubahika8, Sussann Nasr9, Melody Miles10, Steven S. Yoon11, Michelle Chang12, Sarah G. Staedke13, Moses Kamya14, Grant Dorsey15
1Child Health and Development Centre, College of Health Sciences, Makerere University, Kampala, Uganda, 2Institute of Health Metrics and Evaluation, University of Washington, Seattle, WA, United States, 3Infectious Diseases Research Collaboration, Kampala, Uganda, 4The National Malaria Control Program, Ministry of Health, Kampala, Uganda, 5Centers for Disease Control and Prevention, Atlanta, GA, United States, 6London School of Hygiene and Tropical Medicine, London, United Kingdom, 7Department of Medicine, College of Health Sciences, Makerere University, Kampala, Uganda, 8University of California, San Francisco, San Francisco, CA, United States

In Uganda, the National Malaria Control Program (NMCP) relies on Health Management Information System (HMIS) data for planning and monitoring trends in malaria burden yet majority of malaria cases reported are based on clinical case definition. For the past 2 years, we have implemented an in-patient malaria sentinel surveillance program at select district hospitals with emphasis on laboratory-based case definition. To better characterize the quality of HMIS-based malaria data, and understand the true burden of malaria in Uganda, we compare HMIS data to malaria sentinel surveillance site data at four public hospitals. These hospitals are situated in districts with varying malaria endemicity: Tororo and Apac (high transmission), Mubende (medium transmission) and Kambuga (low transmission). At the four sentinel hospitals, >95% of inpatient children less than 5 years were tested for malaria, and only those children with positive laboratory confirmation were recorded as malaria cases. Based on HMIS data, the proportion of hospitalized children under 5 with malaria was higher 12 months prior to start of the program as compared to 12 months after: Tororo (94% vs. 85%), Kambuga (83% vs. 52%), Mubende (71% vs. 55%) and Apac (67% vs. 40%). Actual comparison of HMIS data to surveillance program data, 12 month after its start, showed that HMIS data overestimates the burden of malaria when compared to surveillance program data: 27% higher in Kambuga (25% vs. 52%), 24% higher in Tororo, (61% vs. 85%), 18% higher in Mubende (55% vs. 37%) and 7 percentage points higher in Apac (42% vs. 35%). Improved precision of HMIS estimates of malaria adopted after start of the program may have contributed, in addition to other factors, to the observed differences in disease burden determined by HMIS before and after the start of the program. Even then, HMIS overestimated the burden of malaria among hospitalized children after start of the program. In order to improve the quality of HMIS malaria data, a case definition based on laboratory confirmation should be adopted.

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MALARIA AND RESPIRATORY TRACT INFECTIONS WERE THE COMMONEST TROPICAL DISEASES AND THE COMMONEST INFECTIONS IN AREA OF LOW HIV PREVALENCE IN SOUTH UGANDA: ANALYSIS OF 43,551 PATIENTS

Inocent Nkonwa1, Jozef Suvada1, Maria Bezekova1, Barbora Sihlarova1, Andrej Bebjak1, Petia Mikulasova1, Emilia Ceplova1, Renata Machalkova1, Nada Kulkova1, Jaroslava Sokolova1, Vladimir Krcmery1
1Tropical Program in Buikwe, St. Elizabeth University of Health and Social Sciences, Buikwe, Uganda, 2Department of Clinical Disciplines, School of Health Care and Social Work, Trnava University, Trnava, Slovakia, 3St. Elizabeth University of Health and Social Sciences, Bratislava, Slovakia

The aim of this study was assess reasons for hospitalization in rural hospital in South Uganda in area of low HIV prevalence among population (2.3% HIV positivity). Since January 2008 to December 2010 all 43,550 patients has been investigated, of them 6454 (14,8 %) inpatients and 37 096 (85,2 %) outpatients. Total 32 938 (75,6 %) were children under 5 years of age (2965 inpatients). Rapid diagnostic test plus microscopy has been used in the hospital laboratory with four florescent microscopes and six experienced laboratory technicians (analysis about 100-120 test/samples per day). Total 20 421 (46,9 %) of malaria cases within 3 years (2008-2010) were diagnosed. Of all malaria patients, 17 321 (84,8 %) were treated on outpatients and 3100 (15,2 %) on inpatients basis. Altogether 31 960 blood smears were microscopically investigated and 16 205 (50,7 %) of those were positive. Respiratory tract infections were diagnosed in 9255 cases (21,3 %), of them 3422 (36,9 %) had pneumonia and 5833 (63,1 %) lower respiratory tract infections. Otitis media (862 cases) was observed only in children. Tuberculosis was confirmed in 102 patients and 30 of them were HIV co-infected. Other frequently diagnosed infections were skin and soft tissue infections in 3383 patients (7,8 %), urogenital tract infections in 3145 (7,2 %), sexually transmitted infections 966 (2,2 %), of them 241 (24,9 %) laboratory confirmed cases of syphilis. Totally 2126 patients (4,9 %) have microscopically diagnosed geohelmints infections and 1387 (3,2 %) had diarrhea. Together 4513 patients were tested on HIV and 1188 (2,7 %) of these were positive. Another diseases with low prevalence were ocular infections in 793 (1,8 %), meningitis in 53 patients (0,1 %), measles in 14 (0,03%), schistosomiasis in 12 (0,03 %), sleeping sickness in 10 patients (0,02 %) and tetanus in 12 (0,03%) patients (4 of them neonates). In the area of Buikwe (Lugazi, Buikwe District, South Uganda), prevalence of HIV was surprisingly low (2,7%) as well as geohelmints infections (3,2 %) probably due to MDR with albendazol in all school children. Low HIV prevalence is probably result of outreach mobile HIV testing program and voluntary counseling testing program (since 2008) as well as high proportion of patients on HAART due to five years of governmental program in South-East Uganda since 2006.
MILITARY-TO-MILITARY ENGAGEMENT TO ENHANCE MALARIA PROGRAMS DURING PEACE TIME AND DEPLOYMENT IN EAST AFRICA

Refaat Hanna1, Christopher Arrumm2, Priya Baliga3, Adam Mwabulanga4, Juma Mwinula2, Zuberi Muvunyi5, Godfrey Bwire6, Marc Nimburanira7, Robert Miller1, Robert Holmes1, Annette Von Thun1

1United States Africa Command, Holzgerlingen, Germany, 2Kenya Defence Forces, Nairobi, Kenya, 3Armed Forces Health Surveillance Center, Silver Spring, MD, United States, 4The Tanzania People’s Defence Force, Dar es Salaam, United Republic of Tanzania, 5The Rwanda Defence Force, Kigali, Rwanda, 6The Uganda People’s Defence Force, Kampala, Uganda, 7Burundian National Defense Force, Bujumbura, Burundi

Occurrence of malaria is decreasing with higher military engagement during peacetime and military-to-military engagement to enhance malaria programs. During the Malaria Symposium hosted by the United States Africa Command in April 2011, representatives from several African military nations discussed the need to coordinate common military malaria programmatic challenges. After assessing countries’ current malaria activities, willingness to participate, perceived needs and expected outcomes, five nations of the East Africa Community (Burundi, Kenya, Rwanda, Tanzania and Uganda) united to hold the first East Africa Malaria Task Force (E-AMTF) meeting in December 2011. The mission of the E-AMTF is to strengthen and expand effective malaria programs and provide support for military personnel, their families and communities. The E-AMTF intends to assist national and regional malaria programs in harnessing the full potential of the armed forces as behavioral and social change agents. In preparation for the second E-AMTF meeting in Tanzania, gap analyses of the various malaria program components (Prevention, Diagnosis, Treatment, Surveillance and Human Resources/Capacity Building) during both peacetime and deployment were conducted. The process of critically evaluating their programs helped identify, document, and evaluate program requirements against current capabilities. Based on the urgency and impact on partner nations’ military malaria programs, components were prioritized. Partner nations will take their accountability roadmaps and have a follow-up review meeting with key stakeholders to review, endorse and validate the roadmaps and define clear roles and responsibilities. This regional multi-lateral cooperation between the militaries of partner African nations, leveraging data-driven programmatic assessments of their malaria program needs, allow for the collaboration with US agencies’ assets to enhance and develop malaria programs.

OCCURRENCE OF MALARIA IS DECREASING WITH HIGHER ATTITUDE IN BURUNDI HIGHLANDS

Eva Misikova1, Renata Machalkova2, Jozef Marada2, Lucia Paskova1, Jan Dubec3, Eva Uderzo3, Nada Kulkova2, Vladimir Krcmery1

1Tropical Program in Murogo, St. Elizabeth University College of Health and Social Sciences, Murago, Burundi, 2Tropical Program in Rutovu, St. Elizabeth University College of Health and Social Sciences, Rutovu, Burundi, 3Tropical Program in Gasura, St. Elizabeth University College of Health and Social Sciences, Gasura, Burundi, 4Department of Clinical Disciplines, School of Health Care and Social Work, Trnava University, Trnava, Slovakia, 5St. Elizabeth University of Health and Social Sciences, Bratislava, Slovakia

Altitude above sea can influence the spectrum of infective diseases, especially those like dengue fever or malaria and other mosquito-vector transmitted diseases. We have monitored occurrence of malaria and other tropical diseases in 4 rural Burundian hospitals working within the St. Elisabeth Tropical Program. Buranrio hospital is the lowest localized one in average height of 1280 m, Gasura health center is in 1550 m above sea level, Rutovu hospital is placed in 2065 m and Murago in 2663 m above sea. Overall size of all hospital is similar, those counting for 80 - 120 beds. In all four hospitals, the overall number of health consultation and gynecological consultation, number of malaria and other tropical cases are registered using form-filling. We evaluated incidence of malaria during December 2011 among all hospitals. During the December 2011, 9524 health consultations and 1465 hospitalizations were carried out in those hospitals. Lowest proportion of malaria during December 2011 was detected in Murago (606 cases per month, 47,6%) and highest it was in Gasura (1559 cases, 91,3%), then in Rutovu (732 cases, 81,2%) and Buraniro (4436 cases, 78,6%). Comparing to other types of consultations (gynecological, AIDS, other tropical diseases), malaria was the most frequent disease, even though some patients received more than one type of consultation. In this study we showed, that occurrence of malaria negatively correlates with altitude above sea and was lowest in Murango hospital placed above 2500 m (P < 0,05), where we have noted 606 malaria cases of which 499 (83,2%) were microscopically confirmed. Proportion of AIDS-consultations was lowest in remote hospitals of Rutovu and Murango where only few people are travelling to large cities or crowded places (such as Great Lake Tanganyika).

AN ASSESSMENT OF THE MALARIA-RELATED KNOWLEDGE AND PRACTICES OF TANZANIA’S DRUG RETAILERS: EXPLORING THE IMPACT OF DRUG STORE ACCREDITATION

Boniface Johannes1, Rebecca Thomson2, Charles Festo1, Admirabilis Kalolella1, Mark Taylor1, Katia Bruxvoort1, Sarah Tougher1, Yazoume Ye1, Andrea Mann2, Ruilin Ren1, Barbara Willey3, Fred Arnold3, Kara Hanson3, Catherine Goodman2

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

In Tanzania drugs can be purchased from 2 types of retail outlets: Part I pharmacies and drug stores. Since 2005 Tanzania has been upgrading the approximately 7,000 drug stores to Accredited Drug Dispensing Outlets (ADDOs), involving dispenser training, introduction of record keeping and enhanced regulation. ADDOs are permitted to stock 49 prescription only medicines, including artemisinin-based combination therapies. Non-ADDO drug stores can officially stock over the counter medicines only, although many stock prescription only antimalarials. By the end of 2011 ADDO conversion was complete in 14 out of 21 regions, but limited information is available on their performance. Here we examine the malaria-related knowledge and practices of Tanzania’s drug retailers, exploring variation between the different types of drug retailers. The data were collected as part of the AMFM Phase 1 Independent Evaluation, commissioned by the Global Fund to Fight AIDS, Tuberculosis and Malaria, which draws on methods developed by the ACTwatch group. We conducted a nationally representative survey of antimalarial retail outlets in Oct-Dec 2011. We randomly selected 49 wards, and interviewed all outlets stocking antimalarials. As Part I pharmacies were relatively rare these were oversampled by including all pharmacies in the districts - larger administrative units in which the selected wards were located. Interviews were conducted in 334 Part 1 pharmacies, 148 drug stores in ADDO regions, and 261 drug stores in other regions. We will present findings on outlet characteristics (number of staff, staff education and qualifications); staff knowledge (of first line antimalarial drug and its dosing); and antimalarials and malaria diagnostics (availability, retail prices, markups, sales volumes and wholesale sources). ADDO conversion is frequently cited as a model for improving retail sector drug provision but there is concern that the impact may be constrained by staff turnover and inadequate regulatory supervision. This study will provide important information to inform future policy on drug retailers in Tanzania and elsewhere in the region.

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MALARIA RISK FACTORS IN UNDER-FIVES CHILDREN IN OUELESSEBOUGOU, MALI

Patrick E. Duffy1, Yahia Dicko2, Amadou Barry2, Souleymane S. Diarra2, Youssoufa Sidibe2, Almahamoudou Mahamar2, Oumar Attaher2, Abdoulbaki I. Diallo3, Moussa B. Kanoute4, Bakary Diarra2, Kodijia Cisse2, Nianwanlou Dara2, Michel Fried1, Alassane Dicko2

1Laboratory of Malaria Immunology and Vaccinology, National Institute of Allergy and Infectious Diseases/National Institutes of Health, Rockville, MD, United States, 2Immuno-Epidemiology Program (IMEPP) of Malaria Research and Training Center, Faculty of Medicine and Dentistry, University of Bamako, Bamako, Mali

Childhood malaria is a major cause of mortality particularly in sub-Saharan Africa, and we do not understand the intrinsic or acquired mechanisms of resistance. We have undertaken intensive longitudinal cohort studies to assess malaria risk factors and acquired protective immunity. A cohort of children aged 0-3 years of age was enrolled starting in September 2010. During the 2011 malaria transmission season (July to December), thick and thin smears were performed every two weeks in under-ones children, every four weeks in older toddlers, and also at the time of any illness. Clinical malaria was defined as the presence of asexual stages of *P. falciparum* on blood smear with signs or symptoms of malaria. Poisson regression was used to assess the relationship between host factors and clinical malaria risk. During the season, 486 malaria episodes occurred in 265/479 (55.3%) enrolled children (incidence rate of 1.03 episodes per child per season), with some children experiencing up to six clinical episodes in the season. The risk of clinical malaria was lower in children less than 2 years of age compared to those of 2-4 years (0.84 versus 1.2 episodes/child/season; adjusted incidence rate ratio (IRR) = 0.70; 95% CI 0.58 - 0.84; p < 0.001). The risk was also significantly lower in children with hemoglobin S, (0.68 versus 1.07 episodes/child/season; IRR = 0.63, 95% CI 0.42 - 0.94, p = 0.02). No significant association was found with blood group (ABO, Rh) or Fulani ethnicity. Preliminary analysis of this single season data indicates that hemoglobin S and age are associated with resistance to clinical malaria. Additional factors, such as iron status, are being assessed, and will provide a detailed definition of which subsets of children are or are not susceptible to malaria. This context will allow us to undertake detailed immunologic studies in the susceptible children, and define the targets and mechanisms by which children become resistant to clinical malaria.
PHILIPPE BRASSEUR, MALICK BADIANE, MOSTAFA CISSE, MICHEL VAILENT, PIERO L. OLLIARO

1 Institut de Recherche pour le Développement (IRD), UMR 198 Dakar, Senegal, 2 District Médical d’Oussouye, Oussouye, Senegal, 3 Programme National de Lutte contre le Paludisme (PNLP), Ministère de la Santé et de la Prévention, Dakar, Senegal, 4 Unité d’Épidémiologie Clinique et de Santé P Centre d’Études en Santé, CRP-Santé, Luxembourg, Luxembourg, 5 World Health Organization, Geneva, Switzerland

During 2000-2011, the staggered introduction of ACT (artesunate-amodiaquine, ASAQ) and RDT in Mlomp (~6000 inhabitants), southwestern Senegal coincided with profound changes in health providers’ behaviour and malaria epidemiology. Through 2006 ASAQ and microscopy were rolled-out on experimental basis, from 2007 ASAQ+RDT were policy and free of charge. Injectable quinine has been available throughout. The dispensary is the only health provider in the village. The dispensary registries recorded 67,015 consultations, of which 35,169 (52%) for fever. Fevers accounted for 62% of consultations in 2000 vs. 33% in 2011; fevers dropped by >90% in 2000 to 112 in 2011 (from 1365 in 2000 to 112 in 2011) (P0). Of these, 23,481 (90%) received an antimalarial treatment (-36% in 2011 vs. 2000), of which 6893 (29%) were for parasitologically-proven malaria (P+), 10,122 (43%) for parasitologically-negative fevers (P-), and 6466 (28%) without a parasitologic diagnosis (P0). Overall, 18,859 malaria (P+), 10,122 (43%) for parasitologically-negative fevers (P-), and 6466 (28%) without a parasitologic diagnosis (P0).

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Plasmodium falciparum malaria is essential for effective disease management. WHO recommends the use of malaria Rapid Diagnostic Tests (RDTs) in settings where microscopy services are not available. Following improved global malaria control and regional elimination efforts, there is a critical need for novel surveillance tools and strategies. Used RDTs have shown to be a reliable source of parasite DNA. Together with highly sensitive molecular assays, wide scale collection of used RDTs may serve as a modern tool for improved malaria case detection and drug resistance surveillance. The aim of this study was to compare and evaluate different methods of DNA extraction from RDTs and to test the field applicability for the purpose of molecular epidemiological investigations. DNA was extracted from two RDT devices (Paracheck-Pf and SD Bioline Malaria Pf/Pan), seeded in vitro with ten-fold dilutions of cultured 3D7 Pl. falciparum parasites diluted in malaria negative whole blood. The level of Pl. falciparum detection was determined for each extraction method and RDT device with multiple nested-PCR and qPCR assays. The field applicability was tested on 875 paired RDT (Paracheck-Pf) and filter paper (Whatman 3MM) blood samples collected from febrile patients in Zanzibar 2010. Preliminary in vitro results show that DNA extraction efficiency varied with extraction method and RDT device. The method of Pl. falciparum detection influenced the detection limit by 1-2 log units. No apparent difference in quality of DNA extracted from RDTs and filter papers was observed, in terms of PCR results from both in vitro and field samples. The results support the field applicability of RDT-DNA extraction for the purpose of improved molecular surveillance of antimalarial drug resistance, malaria case detection and RDT quality control.

PLASMODIUM FALCIPARUM EXPOSURE SINCE BIRTH AND RISK OF SEvere MALARIA: A NESTED CASE-CONTROL STUDY ON THE COAST OF KENYA

KLARA LUNDDBLOM, LINDA MURUNGI, VICTORIA NYAGA, DANIEL OLSSON, JOSÉA RONÖ, FAITH OSIER, EDNA OGADA, SCOTT MONTGOMERY, ANTHONY SCOTT, KEVIN MARSH, ANNA FÄRNET

1 Karolinska Institute, Stockholm, Sweden, 2 Kenya Medical Research Institute, Kilifi, Kenya

Severe malaria affects mainly young children in Plasmodium falciparum endemic areas. The mechanisms by which immunity to severe malaria develops remain largely unclear, as does the number of infections needed to acquire protection. The aim of this study was to establish how exposure to Pl. falciparum infections during the first years of life affects the risk of severe malaria. A cohort of 5949 children born 2001-2008 in Kilifi District on the Kenyan Coast was followed with three-monthly visits from birth until 2 years of age. Infection patterns in children who subsequently developed severe malaria (according to strict criteria) were compared to three-monthly profiles of age-matched community controls in a 1:3 nested case-control design. Detection of Pl. falciparum by microscopy or PCR in at least one sample from birth conferred an increased risk of severe malaria and particularly if a multiclonal infection, as defined by genotyping of the polymorphic merozoite surface protein 2 gene, was ever detected. Antibodies to Pl. falciparum schizont extract were similarly prevalent in cases and controls, indicating the overall same level of exposure. In this area of moderate-low malaria transmission, parasite positivity and diversity since birth confer an increased risk of developing severe malaria. This study demonstrates for the first time with parasitological data differences in previous exposure between children who developed severe malaria and community matched controls.

RAPID DIAGNOSTIC TESTS AS A TOOL FOR MOLECULAR SURVEILLANCE OF PLASMODIUM FALCIPARUM MALARIA

ULRICA MORRIS, BERIT AYDIN-SCHMIDT, PEDRO FERREIA, LOUISE JÖRHAGEN, ABDULLAH S. ALI, DELER SHAKELY, MIWiny I. MSELLEM, ANDREAS MÅRTENSSON, J. PEDRO GIL, ANDERS BJÖRKMAN

1 Malaria Research Laboratory, Department of Medicine Solna, Karolinska Institutet, Stockholm, Sweden, 2 Department of Protozoology, Institute of Tropical Medicine, Nagasaki University, Nagasaki, Japan, 3 Zanzibar Malaria Control Program, Zanzibar, United Republic of Tanzania, 4 Drug Resistance Unit, Division of Pharmacogenetics, Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden

Prompt and accurate parasitological confirmation of Plasmodium falciparum malaria is essential for effective disease management. WHO
MEASURING PLASMODIUM FALCIPARUM TRANSMISSION IN LOW-ENDEMIC SETTINGS USING A COMBINATION OF COMMUNITY PREVALENCE AND HEALTH FACILITY DATA

Joshua O. Yukich1, Olivier Briët2, Michael Bretscher2, Adam Bennett1, Seklewengel Lemma1, Yemane Berhan1, Thomas Eisele1, Joseph Keating1, Tom Smith2

1Tulane University School of Public Health and Tropical Medicine, New Orleans, LA, United States, 2Swiss Tropical and Public Health Institute, Basel, Switzerland, 3London School of Tropical Medicine and Hygiene, London, United Kingdom, 4Addis Continental Institute of Public Health, Addis Ababa, Ethiopia

As some malaria control programs shift focus from disease control to transmission reduction, there is a need for transmission data to monitor progress. At lower levels of transmission, this becomes increasingly difficult to measure precisely, whether through entomological or other studies. Many programs conduct regular cross sectional parasite prevalence surveys, and have access to malaria treatment data routinely collected by ministries of health, often in health management information systems. By themselves, these data are poor measures of transmission. We propose an approach for combining annual parasite incidence and treatment data with cross-sectional parasite prevalence and treatment seeking survey data to estimate the incidence of new infections in the human population, also known as the force of infection, with limited supplementary data. The approach is based on extension of a reversible catalytic model. The accuracy of the estimates from this model appears to be highly dependent on levels of detectability and treatment in the community, indicating the importance of information on private sector treatment seeking and access to effective treatment.

SPATIAL AND TEMPORAL TRENDS IN MALARIA TRANSMISSION CAN BE CAPTURED BY THE DIAGNOSTIC POSITIVITY RATE REPORTED FROM SUMMARIES OF QUALITY ASSURED HEALTH FACILITY RECORDS RELAYED THROUGH MOBILE PHONES

Busiku Hamainza1, Akilu Seyoum2, Gerry Killeen3

1National Malaria Control Center, Lusaka, Zambia, 2Liverpool School of Tropical Medicine, Vector Group, Liverpool, United Kingdom, 3Ifakara Health Institute, Biomedical and Environmental Thematic Group, Dar es Salaam, United Republic of Tanzania

Measurement of malaria incidence among humans is central to monitoring malaria control program implementation. Quality-assurance and rapid reporting systems are required to reliably measure malaria transmission through passive reporting systems as current health management information system reports are not sufficiently rapid or reliable. Weekly summaries of malaria Rapid Diagnostic Test (mRDT) results from 14 health facilities (HFs) in Luangwa and Nyimba districts of central and eastern Zambia were reported via mobile phone text message. Diagnostic positivity rates reported by this passive monitoring system were compared with both the detailed data from the facility patient registers and a longitudinal incidence cohort comprising clusters of approximately 1,000 residents in the immediate catchment areas of each facility. While passive HF-based surveillance reported fewer cases of malaria (10345 versus 12267, P=0.0001), particularly non-febrile cases (33 versus 8311, P=0.0001), the diagnostic positivity rates obtained correlated well with geographic (P=0.002) and temporal (P=0.000) heterogeneity in rigorously measured incidence rates. The HF surveillance system described adequately captured malaria transmission trends in local HF catchment populations and offers a cost-effective method for fine-scale program monitoring that can be applied on large scales. In conclusion, rapid, accurate reporting of quality-assured HF records of mRDT diagnostic positivity could enable population-wide, continuous longitudinal monitoring of malaria transmission so that integrated vector management programmes can be effectively managed, optimized by both local and national malaria control programmes.

MAINTENANCE OF UNIVERSAL COVERAGE OF LONG-LASTING INSECTICIDE TREATED BEDNETS (LLINS) IN RWANDA: PRELIMINARY RESULTS OF LONGITUDINAL LLIN DURABILITY AND EFFICACY STUDY

Emmanuel Hakizimana1, Beatus Cyubahiro1, Alphonse Rukundo2, Allan Kabayiza1, Michael Green1, Raymond Beach3, Jon Eric Tongren4, Roopal Patel5, Corine Karema2

1National Malaria Control Program/Malaria and Other Parasitic Diseases Division, Kigali, Rwanda, 2National Malaria Control Program/Malaria and other Parasitic Diseases Division, Kigali, Rwanda, 3Entomology Branch, Division of Parasitic Diseases and Malaria, Centers for Disease Control and Prevention, Atlanta, GA, United States, 4Malaria Branch, Division of Parasitic Diseases and Malaria, Centers for Disease Control and Prevention/ U.S. President’s Malaria Initiative in Kigali, Rwanda

The use of long lasting insecticidal nets (LLINS) is a proven effective malaria control intervention. While LLINS are expected to last for 3-5 years or 20 washes, the reality of net effective life in Rwanda could be different. Rwanda achieved universal bednet coverage (1 net per 2 people) in February 2011 after distributing 6.1 million LLINS since 2009. In December 2016, the national programme conducted a national survey to determine the current status of net coverage and the net’s durability and efficacy.

SEROLOGY CONFIRMS MODELED RISK FOR TRANSFUSION MALARIA FROM BLOOD DONORS WITH TRAVEL TO MEXICO AND AFRICA

Megan Nguyen1, Bryan R. Spencer2, Tami Goff3, P. Dayand Borge3, David A. Leiby1

1American Red Cross, Rockville, MD, United States, 2American Red Cross, Dedham, MA, United States, 3American Red Cross, Baltimore, MD, United States

There have been only 7 cases of transfusion-transmitted malaria (TTM) reported in the US since 1998, most attributable to former African residents. The apparent efficacy of current US malaria policy in preventing TTM is counterbalanced by annual deferral of ~160,000 US blood donors for travel-associated malaria risk. Most deferred travelers have visited low risk areas, especially Mexico (66,000yrs), which recent models suggest presents malaria risk 1000x lower than Africa. We compared estimates of modeled risk with measured malaria risk based on antibody (Ab) testing of donors deferred for travel to Africa and Mexico. Blood donors deferred for malaria risk (travel/residency in an endemic country or past history of malaria) were recruited, consented and enrolled. Study subjects provided 2 EDTA tubes of blood and completed a risk-factor questionnaire. Samples were tested for Plasmodium Abs by EIA (Lab21 Healthcare); repeat reactive (RR) samples were considered positive and tested by real-time PCR. Since 2006, 6,077 deferred donors were tested by EIA, including 5,879 deferred for travel. Overall, 91 (1.5%) subjects were RR, with 49 (54%) reporting a history of malaria infection; none were PCR positive. Only two (0.2%) of 1,223 travelers to Mexico were RR, with both reporting prior infections acquired elsewhere (Turkey, 1976 & Ghana, 2005). Among 275 donors tested for travel to Africa, 9 (3.3%) were EIA positive, 6 reported a history of malaria; all 9 were infected in Africa. Travel to Mexico accounts for a large percentage of US donors deferred for malaria risk, but most visit low risk areas. Testing of travel deferred donors identified no cases of malaria acquired in Mexico, supporting modeled estimates of exquisitely low risk associated with travel to Mexico. In contrast, few donors are deferred for travel to Africa yet acquire infection at much greater rates from travel to or residence in Africa. A more effective approach to preventing TTM would be to defer donors reporting a past history of malaria or significant exposure in high risk areas (i.e., Africa).
2010, Rwanda initiated a 3-year longitudinal study to track net efficacy in 3,000 LLINs (1500 polyethylene/permethrin; 1500 polyester/deltamethrin) at 6 sites. At one month post-distribution, and every 6-monthly interval, 10 LLINs are sampled from each site and tested for bio-efficacy (insecticidal effect) using WHO cone bioassay. A colorimetric field test (CFT) is used to assess surface deltamethrin levels. LLIN durability is assessed using a probability hole index (PHI) and theoretical cutoff values to identify the percentage of LLINs in good or serviceable condition. Preliminary results show that at one month, 6 months, and 12 months following distribution 8.5%, 12.9%, and 17.8% of LLINs were missing. LLIN cone bio-efficacy decreased to an average of 84.3% (84.0-84.7) at 6 months and 83.8% (83.3-84.3) at 12 months. Deltamethrin surface levels show 50-80% depletion of insecticidal after 6 months with little change at 12 months compared to the baseline. LLINs remain viable with effective insecticide surface concentration at least equivalent to 10% of the baseline. The durability assessment indicates that in 5 out of 6 sites <10% (pH=768 threshold; polyethylene: 3-10%; polyester: 7-30%) of LLINs would require replacement after 6 months and 32% after 12 months (pH>768 threshold; polyethylene: 37%; polyester: 13-50%). The data suggest that LLINs remain effective after one year of use in Rwanda. However, projected CFT and durability trends indicate that approximately 50% may become ineffective in the next 6 months. These observations highlight the need to conduct LLIN efficacy and durability studies to guide strategies for LLIN replacement and ensure effective universal coverage.

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STUDY ON PERSISTENCE OF INSECTICIDES (BIOASSAY TEST) IMPREGNATED NET-JACKETS FOR MALARIA PREVENTION IN RUBBER TAPPER GROUP AT SURATHANI PROVINCE

Suteera Poolthin, Boonserm Aumaung
Bureau of Vector Born Diseases, Nonthaburi, Thailand

This study aimed to find out persistence of difference insecticides that were taken to impregnate net-jackets and to compare persistence of used and unused impregnated net-jackets in laboratory and field trial. The net-jackets were impregnated by insecticides namely Permethrin 10% EC, Deltamethrin 1% SC and Alphacypermethrin 10% SC at dosage 300 mg/m², 30 mg/m² and 30mg/m², respectively. Impregnated net-jackets were tested at laboratory room temperature and some were given to rubber tapper volunteer group at Surathani Province in field trial. This volunteer group usually daily wore impregnated net-jackets while they had worked at night. Evaluation was conducted by bioassay test method that Anopheles dirus (laboratory stain) was tested to determine insecticide persistence. In laboratory trial, impregnated net-jackets were bioassay tested after impregnation 4, 8, 18 and 24 weeks. The result of persistence of three insecticides showed mortality rate of An. dirus that were more than 80% significantly at 24 weeks or 6 months. In field trial, impregnated net-jackets were bioassay tested after impregnation 2, 4 and 8 weeks. The result of impregnated net-jackets could kill An. dirus effectively that were less than 4 weeks. Deltamethrin and Alphacypermethrin were higher effectiveness than Permethrin. Thus, persistence of unused impregnated net-jackets (Permethrin 300 mg/m², Deltamethrin 30 mg/m² and Alphacypermethrin 30 mg/m²) were more than 6 months at temperature room. The used impregnated net-jackets would have persistence at less than 4 weeks.

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STRENGTHENING COMMUNITY SYSTEM COMPONENTS FOR MALARIA CONTROL: FIVE YEARS INTERVENTIONS IN PASTORALIST COMMUNITIES IN AFAR REGION-EThIOpIA

Medhanit Getachew¹, Kassahun Negash¹, Sylia Thiam²
¹AMREF Ethiopia, Addis Ababa, Ethiopia, ²AMREF HQ, Nairobi, Kenya

A community based malaria prevention and control programme has been implementing in Afar Region since 2005. The goal of the programme was to contribute to the reduction of malaria related morbidity and mortality among pastoralist population in Afar region, by specifically targeting children under five years and pregnant women. Interventions focused on improving case management of malaria, increasing ITN coverage at community level, and enhancing behaviour and social change in support of all interventions. A baseline survey was done followed by a midline survey in 2007 and a final evaluation in 2010. Both quantitative and qualitative data collection methods were employed to collect data from respondents to assess the impact of this five years programme by comparing the key indicators before and after intervention. The proportion of community members who correctly identified the transmission methods of malaria had increased from 27.4% in 2005 to 88% in 2010. ITN coverage of at least one had significantly changed from 7.5% of base line year to 76% in year 2010. ITN usage of pregnant women and children under five who slept under ITN had also considerably increased from 27% to 79% and 17% to 82% respectively between the two periods. Furthermore, treatment seeking behavior was also improved and the percentage of children under five with fever who took antimalarial drugs within 24 hours increased from 9% at baseline to 53.4% at end of the evaluation period. Mortality rate at health facility level decreased dramatically from 25% in 2005 to 2% at the end of year 2010. The results indicate that strengthening community system in pastoralist populations and linking them to the health system improve the capacity of the community to own their health and contribute to reduce malaria mortality.

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MAN, ECOSYSTEM HEALTH AND MALARIA ON RUSINGA ISLAND, WESTERN KENYA

Evelyn A. Olanga, Wolfgang R. Mukabana
International Centre of Insect Physiology and Ecology, Nairobi, Kenya

Malaria is a leading cause of morbidity and mortality in Kenya. Existing evidence indicates that prevalence of the disease is greatly influenced by human and ecological factors. While it is well known that certain alterations on natural ecosystems aggravate malaria there is a dearth of information about the relationship between malaria and livelihoods. This study seeks to find the relationship between livelihoods, malaria and ecosystem health. The underlying objective is to generate an evidence base for reviewing malaria health and control policy. The study will be conducted on Rusinga Island, Lake Victoria, western Kenya, where local residents rely on fishing and small-scale farming to support their livelihoods. By carrying out fishing activities outdoors and at night Rusinga fishermen are exposed to a higher risk of malaria because (i) transmitting mosquitoes mainly bite at night, and (ii) currently deployed vector control tools are designed for indoor mosquito control. The apparently high risk of malaria posed by fishery and certain small-scale farming activities will be investigated. This will be accomplished by determining malaria prevalence and screening for mosquito saliva antigens among individuals associated with different livelihood practices. The specific livelihood-related groups of people that will be recruited for studies include subsistence farmers, fish traders, boat owners, fishing crew and stakeholders involved in fishery activities namely net/boat makers/repairers, transporters, and fish bait miners/traders. We will also attempt to explain how actions directed towards supporting livelihoods modify ecosystems in ways that may aggravate malaria. Communication tools will be developed to share knowledge generated from these activities among the local residents. An outcome mapping model will be developed to measure changes in behaviour among collaborating boundary partners namely Kibisom women group and the Mbita district public health office (Mbita-OPHO).
SIMULATED COMMUNITY-LEVEL EFFECTS OF COMBINING LONG LASTING INSECTICIDAL NETS WITH INDOOR RESIDUAL SPRAYING FOR MALARIA CONTROL IN AFRICA

Fredros O. Okumu, Gerry F. Killeen, Sarah J. Moore
Ifakara Health Institute, Ifakara, Morogoro Region, United Republic of Tanzania

It is common practice to combine indoor residual spraying (IRS) with long-lasting insecticide nets (LLINs) in highly endemic communities, but there is limited evidence to suggest that the strategy confers greater protection against malaria than either intervention alone. Experimental hut trials have demonstrated improved personal and household protection with certain LLIN/IRS combinations, but it remains unclear whether there are proportionately greater benefits at community level. A deterministic mathematical model of mosquito life cycle processes was adapted and used to estimate how malaria transmission might be affected if LLINs are combined with IRS, relative to use of either method alone. The model was modified to use data derived directly from experimental hut evaluations where untreated bed nets are used as experimental controls. We simulated a closed community where residents own cattle, and the main malaria vector is Anopheles arabiensis, an increasingly important vector species in Africa, which remains a major challenge even with high LLINs and IRS coverage. Considering situations with either LLINs or IRS as the pre-existing intervention, we calculated relative improvement in transmission control each time a complementary intervention was introduced. Transmission control is improved when the common pyrethroid based LLINs are added onto toxic IRS treatments such as pirimiphos-methyl and lambda cyhalothrin, but not DDT, which is known to be less toxic against mosquitoes. On the other hand, the outcome remains unchanged when IRS with lambda cyhalothrin or DDT is added to communities already using LLINs. Addition of pirimiphos-methyl IRS provided the greatest improvement relative to the LLINs alone. This in-silico assessment shows that whereas introduction of LLINs into communities with pre-existing IRS will generally result in improved control of malaria transmission, introduction of IRS into communities with pre-existing LLIN use will most likely be redundant unless the IRS is highly toxic to malaria mosquitoes.

EVALUATING PROGRESS OF INTENSE IMPORTED MALARIA TRANSMISSION IN SOUTH AFRICAN PROVINCES: RETROSPECTIVE ANALYSIS

Infanta M. Spence-Lewis, Ernest A. Alema-Mensah
Morehouse School of Medicine, Atlanta, GA, United States

For the past 50 years, because of national and global malaria strategies put in place vector transmission was low in South Africa. Successful early malaria control policies and strategies developed non-immunity to malaria amongst most South Africans. The results were an increased risk of complicated and severe infections from Plasmodium falciparum and other untreated vector species. Southern African populations consistently visit or migrate to and from malariaous areas, including countries bordering South Africa. Exposure to mobile populations with malaria infections contributes to the burden of disease in South African Provinces. The most vulnerable are children under five, pregnant women and those with co-morbidities such as HIV and TB. Imported malaria is identified as a major concern within endemic and non endemic Provinces of South Africa in regional mapping and by the Republic of South Africa’s National Malaria Programme Performance Review-2009. A geographical focus is used to identify high transmission areas in South African Provinces in low lying North East areas of: Limpopo, KwaZulu-Natal and Mpumalanga where malaria is endemic and seasonal. A literature review synthesized previous research from 1982-2012. The Study also analyzed trends in the understanding and knowledge of imported malaria in Southern Africa. Qualitative indicators are used to build on existing malaria control measures in South and Southern Africa while evaluating the progress of the intense burden of imported malaria in South Africa. This was achieved by analyzing: sentinel surveillance measures, malaria control interventions, and transmission rates based on data from mosquito breeding sites and climate. The Study emphasizes sustainable capacity building for: surveillance, quantification and local community participation. Cross border malaria initiatives from five countries bordering the Provinces of: Limpopo, KwaZulu-Natal and Mpumalanga were analyzed based on the quantitative indicators described. Studying imported malaria in South Africa is a regional and global contribution to: improving surveillance, human interaction with ecological systems that are breeding sites for mosquitoes, economic development, health outcomes and public health policies associated with malaria as a debilitating and potentially fatal infection.

IMPACT OF TREATING PLASMODIUM FALCIPARUM ASYMPTOMATIC CARRIERS WITH ARTEMETHER-LUMEFANTRINE ON MALARIA TRANSMISSION

Wamdaogo M. Guelbeogo1, Alphonse Traore1, Antoine Sanou1, John Lucas1, Sagnon N’Fale1
1Centre National de Recherche et de Formation sur le Paludisme, Ouagadougou, Burkina Faso, 2Sumitomo Chemical Company, Vector Control Division, London, United Kingdom

Asymptomatic Plasmodium falciparum carriers serve as parasite reservoirs for malaria transmission; and community screening and treatment of these asymptomatic carriers with artemether-lumefantrine (AL) may reduce the pool of infectious gametocytes and potentially malaria transmission in that area. To test this hypothesis, entomological parameters were assessed before and after AL treatment in a longitudinal entomological survey. Mosquitoes were collected once per week using the indoor spray catch method in 5 villages where mass screening and treatment of asymptomatic carriers was implemented, and in 5 control villages. Data collected were used to infer the human-biting rates. Mosquitoes collected were processed by ELISA assay to estimate sporozoite index and entomological inoculation rate (EIR) in each site and susceptibility of malaria vectors to insecticide was also determined. The population was provided with long-lasting insecticide treated nets (ITNs). A total of 15768 mosquitoes were collected during the survey. After morphological identification, 90% were confirmed as Anopheles gambiae s.l. The remaining catch were not Anopheles. The temporal analysis shows that the density of mosquitoes increases from January to August, which is mainly due to the rainy season creating an abundance of breeding sites. The comparison of vector composition, human-biting rates, EIR and insecticide susceptibility between the control and the intervention zones is done to measure the impact of the intervention on the malaria transmission. Results will be presented at the conference.

DEVELOPMENT OF LABORATORY TESTS FOR THE PHYSICAL DURABILITY OF LONG-LASTING INSECTICIDAL BEDNETS

Stephen C. Smith1, Jan P. Ballard2, Teresa J. White2
1Centers for Disease Control and Prevention, Atlanta, GA, United States, 2North Carolina State University, College of Textiles, Raleigh, NC, United States

A key performance attribute of long-lasting insecticidal nets (LLINs) is durability. According to the WHO, $3.8 billion could be saved between 2011 and 2020 if LLIN longevity can be increased from 3 years to 5 years. Although technical advances in LLIN longevity have focused on insecticide retention, there is growing evidence that the net fabric can also deteriorate rapidly in many settings. As insecticide resistance becomes a greater concern, the ability of LLINs to maintain physical integrity during years of use becomes increasingly important. Despite this, no laboratory test method yet exists to evaluate the physical durability of LLINs and researchers must conduct multi-year comparison studies of LLINs in the
field to acquire durability data. The objective of the present work is to develop one or more laboratory tests that can be used to evaluate how well LLINs withstand realistic physical challenges, using standard textile testing equipment. The focus has been on measuring the susceptibility of fabrics to deterioration after suffering initial damage by rodents or hot surfaces. Modifications of standard bursting strength, tensile strength, tear resistance, and abrasion resistance test methods have been evaluated for reproducibility and consistency with the results of field studies. This investigation has also provided insight into the mechanisms of LLIN deterioration and possible strategies for improving durability.

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MODELING THE EFFECTS OF VECTOR CONTROL INTERVENTIONS IN REDUCING MALARIA TRANSMISSION AND DISEASE BURDEN

Nakul Chitnis, Olivier Briet, Thomas Smith

Swiss Tropical and Public Health Institute, Basel, Switzerland

Malaria interventions are usually prioritized using efficacy estimates from intervention trials, without considering the context of existing intervention packages or long term dynamics. Currently, long lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) form the mainstay of most malaria control programs. However, in the face of emerging resistance in mosquitoes and a push to elimination, extensions and new combinations of these interventions are being considered, along with the development of novel interventions, such as outdoor traps, and a revival of older interventions such as larval source management. We use numerical simulations of an ensemble of mathematical models of malaria in humans and mosquitoes to provide robust quantitative predictions of the effectiveness and cost-effectiveness of combinations of these interventions, in reducing transmission, morbidity and mortality. We estimate reductions in entomological inoculation rate, prevalence, clinical cases, and malaria deaths from simulations of different coverage levels of LLINs, IRS, larval control, and outdoor traps. We simulate scenarios with various vector distributions, and transmission and health system settings. Our results suggest that sustained coverage of one or two vector control interventions reduces malaria prevalence through the first two or three years, and that vector control interventions reduce ed transmission; however, larval control or outdoor traps are necessary for sustained control.

Our results suggest that high coverage of both LLINs and IRS can lead to interruption of transmission, however, larval control or outdoor traps are necessary when a separate population of mostly outdoor biting mosquitoes exists. We can simultaneously capture in mathematical models the dynamics of mosquito ecology, malaria epidemiology, human demography, health systems effects, and control interventions. Fitting an ensemble of models to data leads to plausible quantitative predictions, with accompanying uncertainty ranges, of the effects of a comprehensive set of different interventions in reducing and potentially interrupting transmission.

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MARKET COMPETITION AND CUSTOMER DEMAND DETERMINE STOCKING PATTERNS AND RETAIL PRICES OF ACTS IN PRIVATE DRUG SHOPS IN TANZANIA

Peter S. Larson1, Prashant Yadav1, Jessica Cohen2, Sarah Alphs1, Jean Arkedis1, Julius Massaga*

1University of Michigan, Ann Arbor, MI, United States, 2Harvard University, Cambridge, MA, United States

Many sub-Saharan African households utilize the private sector as a primary source of treatment for malaria episodes. Cost, however, is reported to be a major impediment to shop level stocking of effective anti-malarials such as ACTs. The Affordable Medicines Facility - malaria (AMFm), an innovative financing mechanism hosted by the Global Fund, is a unique supply side subsidy designed to increase availability of ACTs in eight pilot countries including Tanzania. A series of shop level surveys of private drug retailers in two regions of Tanzania aimed to discover supply and demand side factors which determine availability and reported retail sale price of subsidized ACTs in private shops. Accredited Drug Dispensing Outlets (ADDOs) in the Rukwa and Mtwa regions were surveyed between February 2010 and May 2012. Surveyors noted whether subsidized ACTs were being sold and recorded retail prices. Shop attendants were asked a battery of questions including malaria knowledge, source of drug supply, and participation in training programs. Exit interviews with customers at the ADDOs provided data on the type and price of the antimalarial purchased. Trends and extent of ACT stocking over time and space were described using statistical and spatial methods. Supply side determinants of ACT stocking were assessed using distance to reported medicine supplier. Competition from surrounding providers was assessed using the location of retail shops and public reproductive health clinics (RCH). GIS layers for surrounding population were used to estimate the size of catchment populations. Remoteness and distance to wholesale sources of medicines were not found to be associated with ACT stocking patterns. Shop size and population were found to be highly associated with ACT stocking. Proximity to other shops stocking ACTs (p=.04) and increased number of competing shops (p=.006) were statistically significantly associated with an increased likelihood of stocking ACTs in Rukwa. Similarly, presence of RCH clinics determined stocking. High numbers of proximal shops was also associated with increased prices charged for ACTs. Stocking of ACTs and prices charged were both higher in areas along Lake Rukwa than in other areas of similar distance from urban areas and population size. The AMFm program appears to have resulted in increased availability of ACTs, though shop level factors also influence stocking and prices charged.

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INDUCIBLE INSULIN-LIKE PEPTIDE SYNTHESIS IN ANOPHELES STEPHENSI: A MECHANISM FOR PLASMODIUM MEDIATED IMMUNOSUPPRESSION

Jose E. Pietri, Shirley Luckhart

University of California, Davis, Davis, CA, United States

The insulin-like peptides (ILPs) and their respective signaling and regulatory pathways are highly conserved across diverse phyla. Previously, we reported that infection with the human malaria parasite, Plasmodium falciparum, induces ILP transcription in the midgut of Anopheles stephensi, suggesting that the ILPs are produced in response to infection-associated signals and modulate some aspects of sporogonic development. In particular, our data revealed that soluble factors derived from P. falciparum, but not from bacteria or fungi, can induce ILP transcription and secretion in An. stephensi cells. This induction was dependent on insulin/insulin-like growth factor signaling (IIS) through MEK-ERK and PI3K-AKT activation. Additionally, knockdown of an infection-induced ILP in vivo resulted in enhanced immune effector gene expression and decreased parasite survival in P. falciparum infected mosquitoes. Together, these data suggest that Plasmodium-specific factors signal through IIS to induce immunosuppressive ILPs in the midgut, a critical tissue for parasite development. The ILPs should be considered, therefore, important targets in future efforts to engineer Plasmodium-resistant mosquitoes.
AGE AND MALARIA RISK DETERMINE INSECTICIDE TREATED NET USE NEAR LAKE VICTORIA, MBITA DISTRICT, KENYA

Peter S. Larson¹, Noboru Minakawa², Gabriel O. Dida³, Mark L. Wilson¹

¹University of Michigan, Ann Arbor, MI, United States, ²Nagasaki University, Nagasaki, Japan, ³Kenya Medical Research Institute, Nairobi, Kenya

Despite scaled-up coverage of insecticide treated nets (ITNs) in malarious areas of sub-Saharan Africa, proper and regular ITN use remains inadequate. An understanding of what determines ITN use could help improve effectiveness. In early 2011, a household-level, questionnaire-based survey of ITN practices was conducted following a mass distribution program. The goal was to assess post-intervention Plasmodium infection, and whether households used ITNs to protect target groups such as pregnant women and children. Following a complete enumeration of all households (~3,340), each one was censused for all residents and surveyed. Pre-school children were tested for presence of parasites using PCR methods. Questions of household heads involved who slept under what ITN the previous night, as well as age and sex. Data analysis involved spatial methods and regression models tailored to account for non-linear patterns in age-related ITN use. GIS methodologies were used to determine spatial patterns of ITN use and malaria cases. Information on 12,095 individuals aged 90 years old was gathered, of which ~25% were <5 years of age. More than half (56%) of people reported not sleeping under an ITN the previous night. Age was an important determinant of ITN use. Adults over 30 and infants sleep under ITNs more than children and young adults. The distribution of age and ITN use followed a significant (p < 0.001) nonlinear pattern, decreasing from birth to age 18, increasing to and remaining constant after age 30. This pattern was significant even when accounting for confounding factors. Differences in gender were not significant for any age group, but women between the ages of 15 and 30 tended to use ITNs more than males. Household-level clusters of Plasmodium infections were associated with fewer children sleeping under nets, and were geographically located in wet, low lying areas closer to the lake, despite high levels of net use and possession. Though ITNs were found to be effective in reducing Plasmodium infections, spatially, evidence suggests that net possession and use were highest in areas prone to nuisance mosquitoes and possible perception of high malaria risk. Results suggest that ITN use may be high among some members of higher-risk groups, however there is inadequate coverage among young and school-age children. Efforts to further scale up ITN possession and programs to focus messages regarding proper use remain necessary.

SHIFTING FROM BLANKET TO TARGETED INDOOR RESIDUAL SPRAYING FOR MALARIA CONTROL IN ZANZIBAR: A NOVEL APPROACH FOR INTEGRATED MANAGEMENT OF MALARIA VECTORS

Shabbir Lalji¹, Abdullah R. Salum¹, Abdullah A. Suleiman¹, Abdul-wahid H. Al-mafazy², Rosemary Lusinde¹, Peter McElroy³, Mahdi Ramsan¹, Uche Ekenna¹, Jessica M. Kafuko¹, Fabrizio Molteni¹

¹RTI International, Dar-es-Salaam, United Republic of Tanzania, ²Zanzibar Malaria Control Programme, Zanzibar, United Republic of Tanzania, ³President’s Malaria Initiative, Dar-es-Salaam, United Republic of Tanzania

Zanzibar (1.2 million population) has significantly reduced Plasmodium falciparum prevalence to less than 1% over six years through scale-up of multiple malaria interventions, including indoor residual spraying (IRS) and long-lasting insecticidal nets (LLINs). Between 2006 and 2011 six rounds of blanket IRS with lambda-cyhalothrin were applied to 210,000 structures. A policy to transition from blanket to targeted IRS was agreed to by Ministry of Health and partners in 2009 pending universal coverage of LLINs, establishment of a weekly surveillance system for diagnostically confirmed malaria cases, and an insecticide resistance mitigation plan (IRMP). In 2012 universal coverage of LLINs was achieved, weekly malaria surveillance was scaled-up to all 142 public health facilities with complete (100%) and timely (77% by Friday) reporting, and an IRMP was introduced. Malaria incidence was calculated for each health facility catchment area as the number of confirmed P. falciparum cases per 1000 population per year and used as the primary indicator for selecting locations for targeted IRS. Incidence for all of Zanzibar in 2011 was 2.4/1000/yr (95% CI, 2.3-2.5/1000/yr). The peak transmission period incidence was 5.6/1000/yr (95% CI, 5.4-5.8/1000/yr) during May to August compared to 0.7 and 0.9/1000/yr during January to April and September to December, respectively. We defined three risk strata: A) <0.3 cases/1000/yr; B) 0.3-15 cases/1000/yr; and C) >15 cases/1000/yr. Category A received no targeted IRS, category B one round, and category C two rounds. Based on the seasonal incidence data, the first targeted round of IRS with bendiocarb was completed in March 2012 for 120,000 structures (category B and C). A second round will target 15,000 of these same structures (category C) in September 2012. Zanzibar has met policy prerequisites to transition from blanket to targeted IRS. Weekly surveillance data will be monitored to assess whether targeted IRS can further reduce malaria transmission. These findings will help inform other malaria control programs considering a scale-down IRS after universal LLIN coverage is achieved.

STRENGTHENING COMMUNITY SYSTEM FOR MALARIA CONTROL: THE CONTEXT OF GLOBAL FUND GRANT IN SENEGAL

Sylla Thiam¹, Mamadou Lamine Diouf², Moussa Thor³, Mame Birame Diouf³, Cheikh Thiam³, Ouleye Tall Beye³, Ibrahima Diallo³, Fatou Ba Fall³, Medoune Ndopi³, Moustapha Cisse³, Cheikh Tacko Diop³

¹AMREF HQ, Nairobi, Kenya, ²Programme National de Lutte contre le Paludisme, Dakar, Senegal, ³Ministere de la Sante, Dakar, Senegal, ⁴Intrahealth, Dakar, Senegal

Community involvement in health programming aims to achieve improved outcomes of interventions to deal with major health challenges such as HIV, tuberculosis and malaria. This is vital for making progress towards universal access to health care and meeting the Millennium Development Goals. Support for community-level and NGO programming is a key component of Global Fund grants. However, there remains lack of evidence and lessons learned about how Community System Strengthening (CSS) can be developed, effectively implemented and linked to the formal health system in a malaria endemic country. This paper reports Senegal’s National Malaria Control Programme experience on CSS using Global Fund opportunity. Available information generated between 2005 and 2010 from the program database, annual reports, reports to the Global fund, partners reports, program performance review reports, surveys and published articles were reviewed. The Global Fund framework for CSS was used to analyze the malaria program contribution. Sixty nine District Health Teams (DHTs)- through District Community Networks Against Malaria- and 16 NGOs were involved as sub recipients to implement community based interventions. A total of 34,628 community volunteers were trained to carry out sensitization and awareness campaigns, distribute nets, and destroy breeding sites. A further 3,176 community health workers (in the health huts) and 861 Home Care Providers (HCPs) in remote areas were involved in malaria case management using RDT and ACT. In this process, local capacity was built and stakeholders involved in the entire process from planning to assessment. ITN coverage increased among children under five from 9.7% in 2005 to 45% in 2010. Between 2009 -2010, 12,582 suspected malaria cases were managed by HCPs, 93% of whom were tested with an RDT. Among those tested, 37% had a positive RDT, 97% of whom were treated and got cured. CSS by building capacity of local communities and actively involving them in improving their own health is a key means to control malaria and sustain gains in resource poor countries.

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RANDOMIZED STUDY IN SUB-SAHARAN AFRICA
AND GAMETOCYTE CARRIAGE: A 12-MONTH, CLUSTER-
RANDOMIZED STUDY IN SUB-SAHARAN AFRICA

Alfred B. Tiono1, Alphonse Ouédraogo2, Bernhards Ogutu3, Amidou Diarra4, Sam Coulibaly5, Marc Cousin6, Christine Remy7, Amitta Mukhopadhyay8, Issiaka Soulama9, Sodiomon B. Sirima9, Kamal Hamed10
1Centre National de Recherche et de Formation sur le Paludisme, Ouagadougou, Burkina Faso, 2Walter Reed Project-Centre for Clinical Research, Kenya Medical Research Institute, Nairobi, Kenya, 3Novartis Pharma AG, Basel, Switzerland, 4Novartis Healthcare Private Limited, Hyderabad, India, 5Novartis Pharmaceuticals Corporation, East Hanover, NJ, United States

Human to mosquito transmission of Plasmodium falciparum depends on the presence of sexual stage parasites, gametocytes, in the peripheral blood. Interventions in asymptomatic carriers (ACs) aiming to reduce disease transmission should also be effective against gametocytes. This 12-month, controlled, parallel, cluster-randomized (18 clusters: 9 intervention, 9 control) study was conducted in Burkina Faso to evaluate the impact at the community level of systematic screening and artemether-lumefantrine (AL)/AL dispersible treatment of RDT-detected ACs during three community screening campaigns (CSCs 1-3). CSCs 1-3 occurred before the rainy season and CSC4 occurred after, marking the end of the study. Symptomatic malaria episodes were treated with AL or an alternative in both arms during the study. The prevalence of microscopy-confirmed ACs in the intervention and control arms was 42.8% vs. 47.5%; 4.1% vs. 35.7%; 2.8% vs. 32.2% and 34.4% vs. 37.8% at CSC1, 2, 3 and 4, respectively. The proportion of gametocyte carriers (GCs) was evaluated by microscopy in all subjects at CSCs 4 in the intervention arm and in a randomly selected 40% subset of the control arm, and by qRT-PCR at CSC4 in 1,999 randomly selected subjects across both arms. The overall proportion of GCs in the intervention and control arms was 9.5% vs. 10.2%, 0.6% vs. 5.5%, 0.4% vs. 5.8% and 4.8% vs. 5.1% at CSC1, 2, 3 and 4, respectively. The prevalence (least square mean (SE)) of microscopy-confirmed GCs at CSC4 in the intervention arm was 4.9 (0.41) vs. 5.1 (0.41) in the control arm (p=0.7208). Prevalence of GCs at CSC4 as assessed by qRT-PCR was around 8 times greater in both arms compared to microscopy (49.7% vs. 6.0% intervention; 47.3% vs. 5.4% control). In this community-setting study, the intervention arm showed greater reductions in the prevalence of ACs and GCs than the control arm at CSCs 2 and 3, relative to CSC1 (p<0.0001). However, AC and GC prevalence rose thereafter in the intervention arm to reach a level similar to the control arm at CSC4 (p=NS).

ONLINE INTERACTIVE PLATFORM FOR MAPPING REPORTS OF INSECTICIDE RESISTANCE IN MALARIA VECTORS

Tessa B. Knox1, Helen Pates Jamet2
1Vestergaard Frandsen (East Africa), Nairobi, Kenya, 2Vestergaard Frandsen SA, Lausanne, Switzerland

Insecticide-based interventions including indoor residual spraying and treated bed nets have led to significant reductions in malaria morbidity and mortality. However, the emerging and rapid spread of resistance to all available classes of public health insecticides threatens current malaria vector control efforts. The Global Plan for Insecticide Resistance Management released by the WHO in May 2012 contained guidance on the rationale and implementation of strategies for preserving the efficacy of current tools, which included utilization of insecticide resistance data for informing vector control decisions. There has long been a need for a comprehensive global resistance database to aggregate data currently scattered across many sources in order to facilitate a coordinated response across the malaria-stakeholder community. IR Mapper was developed to address this need (www.irmapper.com); this free online resource consolidates published information from WHO susceptibility tests on Anopheles malaria vectors from 1959 to date. Information is provided via a user-friendly interface that allows users to project data on maps based on selected vector species, insecticide classes and types. Susceptibility data are viewable based on old WHO susceptibility categories or using the new categories as recommended from May 2012. Resistance mechanisms data are similarly presented, with links to original data sources provided along with other key study information. The utility of this resource will be demonstrated using examples from two large-scale malaria control programs in Africa.

DETECTION OF EASTERN EQUINE ENCEPHALOMYELITIS VIRUS RNA IN NORTH AMERICAN SNAKES

Andrea M. Bingham1, Sean P. Graham1, Nathan D. Burkett-Cadena1, Gregory S. White1, Thomas R. Unnasch1
1University of South Florida, Tampa, FL, United States, 2Auburn University, Auburn, AL, United States

The role of non-avian vertebrates in the ecology of Eastern Equine Encephalitis virus (EEEV) is unresolved, but mounting evidence supports a potential role for snakes in the EEEV transmission cycle, especially as over wintering hosts. To determine rates of exposure and infection, we examined serum samples from wild snakes at a focus of EEEV in Alabama for viral RNA using RT-PCR. Two species of vipers, the Copperhead...
A LABORATORY CONFIRMED CASE OF JAMESTOWN CANYON VIRUS ENCEPHALITIS IN A QUEBEC RESIDENT WITH TRAVEL HISTORY TO MAINE AND NEW HAMPSHIRE

Michael A. Drebott, Kristina Dimitrova, Maya Andonova, Stephen Turner, Bouchra Serhir, Michel Couillard, Cecile L. Tremblay

1National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, MB, Canada, 2Hospital Fleury, Montreal, QC, Canada, 3Laboatoire de sante publique du Quebec - Institut national de sante publique du Quebec, Montreal, QC, Canada

Jamestown Canyon virus (JCV) is a mosquito-borne arbovirus belonging to the California serogroup (CSG) of bunyaviruses. JCV is widely distributed throughout North America, however, reports of human JCV infection with associated febrile and neurological disease are rare. We report a recent laboratory confirmed case of JCV encephalitis in a Montreal, Quebec resident with travel history to Maine (ME) and New Hampshire (NH). The patient was a 53 year old male who presented with symptoms of fever, headache and chills 10 days after returning from a camping trip in ME and NH in mid August, 2011. Several days later he was hospitalized and his illness progressed to an altered mental state comprising of confusion and difficulty speaking suggestive of encephalitis. He had trouble breathing and was intubated. He was hospitalized for approximately a month during which blood was collected and lumbar punctures performed. Cerebrospinal fluid (CSF) testing indicated normal protein and glucose with the presence of a low leukocyte count. Serological testing of acute and convalescent serum collected three weeks apart gave a 4-fold rise in specific neutralizing antibody to JCV (640 to 2560). Using a CDC-based IgM ELISA the acute and convalescent serum samples were positive for JCV IgM. Testing of acute and convalescent CSF for JCV antibodies also indicated a positive IgM result for JCV and a seroconversion by neutralization testing (range 0 -16). Significantly lower or negative cross reacting titre were observed for related CSG viruses such as snowshoe hare and La Crosse viruses and no antibodies were detected to other arboviruses such as West Nile, eastern equine encephalitis, or Powassan virus. Based on several laboratory case definition criteria our results indicate that the patient's febrile and neurological symptoms were associated with an infection of JCV. The incubation period of JCV is believed to range from 3 to 14 days and the travel history of the patient is consistent with exposure to JCV infected mosquitoes in ME or NH. It is also possible that he may have been infected in Quebec since symptom onset did occur several days after his return to Canada. Based on available CDC CSG virus case data no confirmed cases of JCV illness have been documented in ME or NH previously. Our findings underscore that JCV can cause serious neurinvasive disease such as encephalitis and should be considered when an arbovirus infection is suspected in this region of North America.

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contribute to neutralization of infection, and how they relate to protein function. We expect that this approach will help define the range of immunodominant structures on CHIKV Env and identify novel neutralizing antibody epitopes that can be used for the development of improved therapeutics, diagnostics, and vaccine candidates.

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DEVELOPMENT OF AN IRES-BASED VACCINE FOR WESTERN EQUINE ENCEPHALITIS VIRUS
Nicholas A. Bergren, Rodion V. Gorchakov, Shannan L. Rossi, Kenneth Plante, Scott C. Weaver

The development of an effective and safer vaccine is crucial for controlling WEEV. Two different live-attenuated WEEV vaccines were engineered via the introduction of an internal ribosomal entry sequence (IRES) from encephalomyocarditis virus (EMCV), to control translation of the structural (WEEV/IRESv1) or capsid (WEEV/IRESv2) protein(s). Previous research shows the IRES element from EMCV cannot initiate efficient translation in arthropod cells, making this vaccine unable to be propagated in its natural transmission cycle. Serial passageing in Vero cells showed no reversion to a wild-type-like phenotype; however, several mutations were observed in the structural genes that provided for higher titers in cell culture. WEEV/IRESv1 and WEEV/IRESv2 were tested for immunogenicity and attenuation in relevant murine models. Our results suggest that our IRES-dependent live-attenuated vaccine for WEEV merits further study and this vaccine could be used for the development of an emergency vaccine that can be used during a natural outbreak, bioterrorist attack, or accidental lab exposure.

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DISTRIBUTION OF KILLER CELL IMMUNOGLOBULIN-LIKE RECEPTORS (KIR) GENES IN AN ADMIXED PERUVIAN POPULATION
Sandra S. Morales1, Daniel Clark2, Elsa Gonzalez1, Eduardo Gotuzzo1, Michael J. Talledo1

1Institute of Tropical Medicine Alexander von Humboldt Universidad Peruana Cayetano Heredia, Lima, Peru; 2Laboratorios de Investigación y Desarrollo (LID), Universidad Peruana Cayetano Heredia, Lima, Peru

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: a) 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 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 definition and the effects of population stratification, and therefore should be used in order to avoid false results when searching for gene-disease associations in admixed populations.

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NOROVIRUS INFECTION IN PERU
Maria E. Silva, Giannina Luna, Carlos A. Figueroa, Erik J. Reaves, Juan F. Sanchez, Kimberly A. Edge, Matthew R. Kasper, Drake H. Tilley, Paul C. Graf, Andres G. Lescano, Daniel G. Bausch

Naval Medical Research Unit - 6, Bellavista, Peru

Norovirus (NoV) is one of the most frequent causes of outbreaks and sporadic cases of gastroenteritis worldwide. Although rarely fatal, NoV transmission has important economic repercussions, including loss of work days and incurrence of costly medical care. The incidence of NoV gastroenteritis is usually highest in adults. Although well-studied in industrialized countries, few data are available on NoV in the developing world. We report NoV surveillance data from 3 distinct regions and populations in Peru. The first two surveillance populations and areas were healthy children <5 years old in rural communities near the town of Pisco (coastal desert) in 2009 and in Loreto District (Amazon forest) in 2010. The third target population and surveillance area was from a 9-year prospective cohort study of diarrheal illness among Peruvian military recruits at the Vargas-Guerra Army Training Base in the city of Iquitos, also in the Amazon. At each site, fecal samples were collected and sent to the U.S. Naval Medical Research Unit-6 laboratory in Lima for testing for NoV by real-time PCR. From Pisco, NoV was found in 27 (9%) of 294 samples. Five (19%) were genotype I and 22 (81%) II. From Loreto, 32 (11%) of 290 samples were positive. 10 (31%) genotype I and 22 (69%) genotype II. From the Vargas-Guerra Training base, 49 (25%) of 200 samples were positive. 6 (12%) for genotype I, 38 (78%) for genotype II, and 5 (10%) co-infection with both genotypes. Our results indicate that NoV circulates in both pediatric and adult populations in Peru and that genotype II predominates. Interestingly, infection was common even in the healthy children. Epidemiologic studies are underway to explore the significance of the NoV infections in children and the ultimate incidence of disease. In addition, we are undertaking further molecular characterization and phylogenetic analysis of NoV strains in Peru.

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SEROPREVALENCE OF ALPHAVIRUSES AND FLAVIVIRUSES IN FREE-RANGING GAME ANIMALS AND NON-HUMAN PRIMATES IN THE CONGO BASIN
Rebekah J. Crockett1, Erin Borland1, Mike Cranfield2, Ann Powers1

1Centers for Disease Control and Prevention, Fort Collins, CO, United States; 2Mountain Gorilla Veterinary Project, Baltimore, MD, United States

Vector-borne and zoonotic pathogens have comprised a significant proportion of the emerging infectious diseases in humans in recent decades. The role of many wildlife species as reservoirs for arthropod-borne viral pathogens is poorly understood. We aimed to investigate the exposure history of various African wildlife species from the Congo Basin to mosquito-borne flaviviruses (Flaviviridae: Flavivirus) and alphaviruses (Togaviridae: Alphavirus) by testing previously-archived serum samples. In total, sera from 24 African forest buffalo (Syncerus caffer nanus), 34 African elephants (Loxodonta africana), 40 duikers (Cephalophus and Philantomba species), 25 mandrills (Mandrillus sphinx), 32 mountain gorillas (Gorilla beringei beringei), five Grauer’s gorillas (Gorilla beringei graueri), two L’hoest’s monkeys (Cercopithecus lhoesti), two golden monkeys (Cercopithecus kandti), and three chimpanzees (Pan troglodytes) sampled between 1991 and 2009 in the Congo basin were tested for antibodies against chikungunya virus (CHIKV) (Togaviridae: Alphavirus),...
SOUTHEAST ASIA

HERD IMMUNITY AND POTENTIAL VACCINE IMPACT ON OUTBREAKS OF HAND FOOT AND MOUTH DISEASE IN SOUTHEAST ASIA

Tiffany L. Bogich1, Sebastien Ballesteros2, Jonathan L. Zelner1, Hoang Quoc Cuong3, Jeremy Farrar1, Eddie Holmes3, Tran Than Hien1, Alex Cook3, Cameron Simmons3, Ottar Bjornstad4, C. Jessica E. Metcalf6, Nguyen van Vinh Chau5, Bryan T. Grenfell1, H. Rogier van Doorn5

1National Institutes of Health Fogarty International/Princeton University, Princeton, NJ, United States, 2Princeton University, Princeton, NJ, United States, 3Oxford University Clinical Research Unit, Ho Chi Minh City, Vietnam, 4Pennsylvania State University, University Park, PA, United States, 5National University of Singapore, Singapore, Singapore, 6Oxford University, Oxford, United Kingdom

Hand Foot and Mouth Disease (HFMD) is typically a mild and self-limiting childhood infection caused by any number of viruses in the Enterovirus genus of the Picornaviridae family, with the most commonly implicated
pathogens being Coxackievirus A16 (CA16) and Enterovirus 71 (EV71). Increasingly, outbreaks of HFMD, particularly those caused by EV71, have garnered the attention of the public, clinicians and national and international health agencies. Recently, there has been an alarming increase in the number of patients and an increase in the number of cases complicated by central nervous system and cardiopulmonary involvement and deaths in young children in countries across South East Asia. Epidemic patterns are complex, though large outbreaks tend to be cyclical, occurring every 2 to 3 years. With vaccine in development, the question remains whether HFMD caused by CA16 or EV71 is an immunizing infection and whether infection by one pathogen confers protection from infection by the other. Using over a decade of weekly infection case data from Japan and Singapore, we find that the case data are consistent with the pathogen acting as an immunizing infection with a strong signature of herd immunity. Preliminary simulations indicate that vaccinations could succeed at limiting epidemics. We validate these findings with multianual infection case data from other countries in the region. Our finding that HFMD acts qualitatively as an immunizing infection is promising for the success of future vaccination efforts for the control of this disease. Further surveillance and regional cooperation including analysis of epidemiological, clinical, and virological data sets for Hand Foot and Mouth Disease would help guide future control strategies and inform policy.

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HANTAVIRUS RODENT RESERVOIRS IN BULGARIA

Mustafa A. Aboualy1, Iva Christova2, Hristo Dimitrov2, Emad W. Mohareb3

1NAMRU-3, Cairo, Egypt, 2National Centers of Infectious and Parasitic Diseases, Sofia, Bulgaria, 3University of Plovdiv, Plovdiv, Bulgaria

Hantaviruses are a group of RNA viruses belonging to the Bunyaviridae family, genus Hantavirus, and their natural reservoirs are wild rodents. Hantaviruses are the global emerging diseases, with evolving strains detected throughout the world. Different serotypes as Seol (SEO), Dobrava (DOB), Pumuula (PUU) and Hantaan (HTN) were detected in the Balkan region. Human disease causes from DOBV in Bulgaria were recently confirmed. In a previous study on hospital-based acute febrile illness cases, we detected PUUV IgM using ELISA (Progen) and DOBV neutralizing antibody by PRNT. In this study we conducted rodent surveillance in areas with documented human cases to identify the circulating strains of Hantaviruses. Rodents were trapped starting May 2011 through March 2012 for two successive night patterns per month. Temperature and humidity were monitored at specific GPS coordinates recorded for trapping sites. A total of 705 rodents from 2 different sites (Plovdiv and Burgas) were trapped. Fourteen different rodent species were collected during the study period. Serum samples and organs (lung, spleen and kidneys) were collected. An organ pool, preserved with RNA-later, was homogenized under BSL3 conditions, followed by RNA extraction using Qiagen products. Real-time RT-PCR for DOBV and PUUV testing was performed on rodent collected in May, June and October (n=224), of which three DOBV and no PUUV position samples were detected. All DOBV was found in male Apodemus flavicollis species (susceptible to DOBV) collected in June: two from Plovdiv and one from Burgas. Screening of the remaining rodent collection is still in progress, and analysis of temporal conditions and abundance of species may provide a potential outbreak prediction model.

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EVALUATION OF VIRUS STRAINS AND THE EFFECT OF E1 MUTATIONS ON THE EXPRESSION OF CHIKUNGUNYA VIRUS-LIKE PARTICLES

John W. Balliet, Ryan Swoyer, James M. Wagner, Jennifer Girard, Jessica A. Flynn, Sha Ha, Jian He, Shyamsundar Subramanian, Danilo R. Casimiro

Merck & Co., Inc., West Point, PA, United States

A challenge to the development and manufacture of affordable vaccines for a global market is reducing the cost of goods required for vaccine production. Due to the inverse relationship between the productivity of antigen expression and cost, increasing the efficiency of antigen production is one strategy used to reduce cost. In collaboration with the NIH, we are developing a chikungunya virus (CHIKV) vaccine based on transient expression of ORF2 in human cells to produce CHIKV virus-like particles (VLPs), as reported previously. In order to minimize the cost of goods, we have tested two strategies to increase the levels of CHIKV VLPs in culture supernatants. First, we evaluated the levels of CHIKV VLPs among 10 different CHIKV strains. Expression of strain 37997 ORF2 yielded the highest levels of CHIKV VLPs in the culture media, which was consistent with the study by Akahata, et al. Six strains including LR2006 OPY-1 and S27 exhibited low levels of VLPs, while two strains, ALSA-1 and Nagpur, failed to produce any detectable VLPs. Western blotting demonstrated a defect in p62 processing and low levels of E1 compared to strain 37997. As an alternate strategy for increasing VLP productivity, we tested the hypothesis that increasing the stability of the E2-E1 heterodimer by reducing the threshold pH for conformational changes in E1 that lead to membrane fusion may result in increased CHIKV VLP production. To test this hypothesis, we introduced 3 different E1 mutations that were shown to decrease the pH of fusion of another alphavirus. We found that those mutations increased the levels of VLPs in culture media after transient transfection. Moreover, additional substitutions for each of those 3 residues have identified several other mutants with enhanced VLP productivity. Current efforts are underway to determine if combining the mutations will further increase CHIKV VLP productivity. Work is also in progress to better understand the effect of these mutations on the stability of the E2-E1 heterodimer.

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MOLECULAR CHARACTERIZATION OF ANTIVIRAL SUSCEPTIBILITY OF INFLUENZA A ISOLATES OBTAINED IN KENYA FROM 2008 TO 2011

Meshack Wadegu1, Wallace Bulimo1, Rachel Achilla1, Janet Majanja1, Silvanos Mukunzi1, Finnye Osuna2, James Njiri1, Janet Nyambura1, Julia Wangui1, Benjamin Opot1, Steven Ocholla1, Rose Nyawira1, Eyako Wurapa1

1USAMRU-Kenya, Nairobi, Kenya, 2USAMRU-Kenya/KEMRI, Nairobi, Kenya

Presently, there are two main classes of antivirals in use which function by inhibiting specific steps within the virus replication cycle: M2 inhibitors block the uncoating of the virus through acidification of the interior of the virion. In neuraminidase inhibition, inhibitor molecules mimic NAs natural substrate and binds to the active site, preventing NA from cleaving host cell receptors and releasing new virus. The study characterized antiviral susceptibility of the 2008-2011 influenza A strains using known molecular markers in neuraminidase (NA) protein. In the 2008-2009, 2009-2010 and 2010-2011 influenza seasons, a total of 836 viruses were isolated. 344 (41%) were influenza A/H3N2, 144 (17%) seasonal influenza A/H1N1 and 348 (42%) belonged to the pandemic influenza A/H1N1 strain. A total of 108 (13%) isolates were analyzed for susceptibility to NA inhibitors. In the year 2008, 33 influenza A/H3N2 and 11 seasonal influenza A/H1N1 were included in the genotypic characterization assay for neuraminidase inhibitor resistant mutations. Sequence assembly and alignment revealed absences of molecular markers of neuraminidase inhibitor drug resistance (Y226S) in influenza A/H3N2. 64% (7) of the 2008 seasonal influenza A/
APPLICATION OF IN VIVO IMAGING IN THE CHARACTERIZATION OF OLFACTORY INFECTION OF MICE WITH WEEV

Aaron T. Phillips, Charles B. Staut, Tawfiq A. Aboellail, Ann M. Powers, Ken E. Olson

Isolates of western equine encephalitis virus (WEEV) can cause severe disease in both humans and animals, and may serve as a model for other neurovirulent alphaviruses. Infection of the McMillan strain (McM) of WEEV leads to high mortality in an outbred CD-1 mouse model. An infectious recombinant WEEV/McM expressing firefly luciferase (FLUC) was developed to characterize CNS infection after intranasal exposure. Correlative relationships were determined between bioluminescence and both viral titer and immunological markers of WEEV/McM/FLUC. Histopathological examination of tissue was guided by corresponding images and revealed that neuroinvasion occurred primarily through the olfactory tract. Olfactory bulb neurons were initial targets and both viral titer and immunological markers of WEEV/McM/FLUC.

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SEVERITY OF ACUTE RESPIRATORY INFECTIONS ASSOCIATED WITH RESPIRATORY SYNCYTIAL VIRUS, GUATEMALA, 2008-2012

John P. McCracken, Wences Arvelo, Maria Renée López, María Luisa Müller, Chris Bernhart, Antonio Paredes, Fabiola Moscoso, Jennifer Gray, Alejandra Estévez, Juan Carlos Moir, José Ortiz, Kim Lindblade

Respiratory syncytial virus (RSV) is a major cause of acute respiratory infections. The epidemiology of RSV in all age groups has not been well described in Central America, particularly regarding disease severity. We aimed to address these knowledge gaps with surveillance data from Guatemala. We conducted active surveillance of ambulatory visits due to influenza-like illness (ILI: cough or sore throat and measured fever >38°C) and hospitalizations due to acute respiratory infections (ARI: sign of infection and a respiratory sign or symptom) in Santa Rosa (Nov 2007-Mar 2012), Quetzaltenango (Feb 2009-Mar 2012), and Guatemala City (Nov 2009-Apr 2011). Nasopharyngeal swab specimens were tested for RSV using real-time reverse-transcription polymerase chain reaction. Among ARI cases, we measured associations between RSV-positivity and indicators of severity using linear and logistic regression, adjusted for age, gender, surveillance site, and year. To test for effect modification by age, we added an interaction term for RSV-positivity and age <5 years to the models. We enrolled and tested for RSV 7919 patients; 5626 met the ARI and 2292 the ILI case definitions. In persons <5 years of age (n=5009), the proportion of cases RSV-positive was higher among ARI (34%) than ILI (17%); in person ≥5 years of age (n=2910), the proportions were similar for ARI and ILI (7%). Among ARI, RSV-positivity was associated with lower oxygen saturation (>-0.9; 95% CI: -0.4, -1.4) and lower odds of admission to intensive care unit (OR=0.70; 95% CI: 0.54, 0.91), and death in hospital (OR=0.69; 95% CI: 0.48, 1.00). We found a lower OR for death associated with RSV in persons <5 years of age (p=0.017). RSV infection is more common among hospitalized ARI compared to ILI cases in young children and ARI patients present with lower oxygen saturation if they are RSV-positive, both which suggest RSV is associated with more severe disease. However, other findings suggest RSV-positive cases are less severe. Further analysis is required to understand whether RSV infection causes more severe disease than other pathogens.

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TACKLING A GLOBAL CHALLENGE ON DOMESTIC GROUND: GEOGRAPHIC AND DEMOGRAPHIC ANALYSES OF TB IN ORANGE COUNTY, CA

Amruta Dixit, Curtis Condon, Ryan Ramos

Orange County, California carries one of the country’s highest burdens of tuberculosis (TB) at a rate of 6.4 cases per 100,000 population in 2009. In this generally affluent county marked by pockets of poverty, the sociodemographic makeup of the populace was discerned through the use of GIS technology. We carried out a retrospective cohort study of all TB cases diagnosed in Orange County, CA from 2005 to 2009 and of all TB hospitalizations from 2005 to 2008 and performed geographic and demographic analyses on the data. Based on global trends, we expected the burden to be highest in the poorest portions of the county as well as in those with high immigrant populations. We found the highest incidences of both TB cases and TB hospitalizations in the city.
of Santa Ana. As the poorest city in the county, Santa Ana (per capita income $16,891), had a case rate of 9.65 per 100,000 population. The highest case rate by city was found in Westminster (23.76 per 100,000 population). Foreign-born patients treated by the county represented 85.4% of all TB cases, placing the burden largely on the immigrant population of the county. There were clear relationships between relative risks & case rates and per capita income of the city (R= 0.423 and R= 0.434, respectively). Relative risk ratios indicated that males [1.49], Asians [8.55], and seniors (65 yrs+) [3.34] were at greatest risk for a TB infection. The relative risk for a TB infection in an Asian male aged 65 years or older was 21.6. At greatest risk for hospitalization for a TB infection: males [1.43], Asians [4.17], and seniors [4.52]. The relative risk of an Asian male aged 65 years or older in the county being hospitalized due to TB was 17.4. More than 71% of all TB hospitalizations were government-funded with total charges exceeding $29.4 million over 4 years. These data suggest that the burden of TB in Orange County warrants continued attention and additional resources and also demands a change in policy with regards to the domestic handling of global health issues.

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INDIVIDUAL LEVEL RISK FACTORS FOR SECONDARY TRANSMISSION OF INFLUENZA-LIKE ILLNESS: SECONDARY DATA ANALYSIS FROM THE BANGLADESH INTERRUPTION OF SECONDARY TRANSMISSION OF INFLUENZA STUDY (BISTIS)

Pavani Kalluri Ram1, Manoshi Islam2, Kaniz Jannat2, Margaret DiVita3, Emily Cercone1, Kimberly Rook1, Eduardo Azizz-Baumgartner3, Badrul M. Sohel2, W. Abdullah Brooks2, Jihnhee Yu1, Alicia M. Fry2, Stephen P. Luby4

1University at Buffalo, Buffalo, NY, United States, 2International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh, 3Centers for Disease Control and Prevention, Atlanta, GA, United States, *International Centre for Diarrhoeal Disease Research, Bangladesh and Centers for Disease Control and Prevention, Dhaka, Bangladesh

Respiratory infections are a leading cause of mortality worldwide. Understanding risk factors for secondary transmission to close contacts will facilitate development of interventions to prevent respiratory pathogen transmission. We describe index case-patient and susceptible contact risk factors for secondary transmission of influenza-like illness (ILI) in the control arm of a randomized controlled trial evaluating the impact of handwashing promotion on ILI in Bangladesh. We identified index case-patients with ILI (fever in persons < 5 years old, and fever with cough or sore throat in persons ≥5 years old). Susceptible contacts were persons without respiratory symptoms at enrollment living in household compounds of index case-patients. Compounds included index case-patient households and 1 secondary households. We recorded demographics and behaviors among all contacts, and frequency of interaction with index case-patient in a subset. We conducted daily ILI surveillance from the day after enrollment to 10 days after resolution of index case-patient symptoms. We used logistic regression to evaluate risk factors for ILI, adjusting standard errors for clustering of illness in households and compounds. In compounds of 185 index case-patients, 1477 (91%) of 1615 contacts enumerated were susceptible and took part in surveillance. We detected 111 (8%) secondary cases of ILI. Index case-patient demographics were not associated with ILI in contacts. Contacts with ILI were more frequently < 2 years old (OR=7.5, 95% CI=3.6 – 15.9), and 2 to < 5 years old (OR=4.7, 95% CI=2.6 – 8.6) than contacts without ILI. After age group adjustment, reported frequent daily interaction with the index case-patient was the only significant individual-level risk factor: OR=1.9, 95% CI=1.1 – 3.5. Sex, parental relationship, living in same household or sleeping in same room as index case-patient, smoking, and time spent in cooking space were not associated with ILI overall or in age group-stratified analyses. In this low-resource setting, young age and frequent interaction with ill persons were significant risk factors for susceptibility to household ILI transmission. These data underscore the need to distance young children from persons ill with respiratory symptoms. Studies should assess feasibility and efficacy of distancing between household members as a strategy to minimize transmission of respiratory infections to the most vulnerable.

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A COMMUNITY RANDOMIZED CONTROLLED TRIAL OF AN INTEGRATED HOME-BASED INTERVENTION IMPROVING HOUSEHOLD-AIR POLLUTION, DRINKING WATER QUALITY AND HYGIENE IN RURAL PERU

Stella M. Hartinger1, Claudio F. Lanata2, Jan Hattendorf2, Ani GiP5, Hector Verastegui2, Daniel Mausezahl1

1Swiss Tropical and Public Health Institute, University of Basel, Basel, Switzerland, 2Instituto de Investigación Nutricional, Lima, Peru

Diarrhoea and acute lower respiratory infections are leading causes of childhood mortality. Simple low-cost interventions have proven efficient in reducing diarrhoea and severe pneumonia; however, an integrated package provides opportunities for synergism. We conducted a community-randomised controlled trial in 51 rural communities in Peru to evaluate an environmental home-based intervention package (HIP) in reducing acute lower respiratory infections, diarrhoeal disease and preventing malnutrition in children under 36 months of age. All homes used open fires and 80% had access to piped, untreated water supplies. Ecol was found in drinking water in 66% of the households. The proportion of stunted children was 55%. In the intervention arm an improved stove (OPTIMA) was installed and members were trained in the correct use and proper maintenances; a solar disinfection of drinking water (SODIS) method was established; and a water faucet with a kitchen sink was installed and handwashing practices were promoted. Diarrhoea, respiratory (weekly) and anthropometric (every two months) surveillance was done at home during a 12 months period. To reduce potential impact of non-blindness bias, the control arm received a psychomotor stimulation programme, We randomized 51 communities and enrolled 534 children. Baseline characteristics were balanced between study arms: The rate of diarrhoeal episodes in children in the intervention was 2.8 episodes per child per year as compared to 3.1 episodes in the control arm. The relative rate was 0.78 (95%CI: 0.58-1.05). Similarly, care takes in the intervention group reported fewer days of diarrhoea (mean 4.9 vs. 6.4 days per year; OR: 0.71, 95% CI: 0.47-1.06). No effect on acute lower respiratory infections or child's growth rates was observed. In conclusion we found no evidence for synergistic effects associated with the intervention package. Introducing several interventions and messages simultaneously may have overwhelmed the households and compromised use, operation and maintenance of all components.

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ACTIVE HOUSEHOLD-BASED SURVEILLANCE AND REGIONAL VARIATION IN INCIDENCE OF INFLUENZA IN PERU

Yeny O. Tinoco1, Hugo Rázuri1, Ernesto Ortiz1, Candice Romero1, Maria L. Morales1, Patricia Breña1, Abel Estela1, Erik J. Reaves1, Erick S. Halsey1, Alberto Laguna-Torres1, Jorge Gomez2, Marc-Alain Widdowson3, Eduardo Azizz-Baumgartner3, Timothy M. Uyekii1, Robert H. Gilman4, Daniel G. Bausch1, Joel M. Montgomery2

1U.S. Naval Medical Research Unit Six, Lima, Peru, 2General Directorate of Epidemiology, Lima, Peru, 3Influenza Division, Centers for Disease Control and Prevention, Atlanta, GA, United States, 4Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, 5Division of Global Disease Detection, Centers for Disease Control and Prevention, Nairobi, Kenya

Most estimates of the disease burden of influenza rely on passive sentinel surveillance at health clinics and hospitals. These estimates lack population denominators necessary for calculations of incidence, especially of milder disease, potentially leading to underestimates of the true burden. In 2009 we implemented active community-based household surveillance in 4 ecologically distinct regions of Peru: coastal desert (Lima), dry

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INCIDENCE OF HUMAN METAPNEUMOVIRUS IN RURAL AND URBAN POPULATIONS IN KENYA, 2006-2011

Victor Omballa 1, Leonard Cosmas1, Allan Audi1, Gilbert Kikwai1, Juliette Ongus2, Dean Erdmann3, Joe Oundo3, Godfrey Bigogo1, Daniel Feikin4, Barry Fields1, Robert Breiman1, Kariuki Njenga1, Leonard Cosmas1, Allan Audi1, Gilbert Kikwai1, Juliette Ongus2, Dean Erdmann3, Joe Oundo3, Godfrey Bigogo1, Daniel Feikin4, Barry Fields1, Robert Breiman1, Kariuki Njenga1

1Centers for Disease Control and Prevention-Kenya, Nairobi, Kenya,
2Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya,
3Centers for Disease Control and Prevention, Atlanta, GA, United States,
4Centers for Disease Control and Prevention-Kenya, Nairobi, Kenya

Human metapneumovirus (hMPV) is a suspected cause of acute respiratory tract infections, mostly in young children, the elderly and immunocompromised patients. We investigated the incidence of hMPV in Kenya using longitudinal, population-based surveillance in two sites; two villages (total population = 28,000) in Kibera urban slum located in Nairobi City together comprising one site and in thirty-three villages in a rural Asembo community (total population = 26,000) in western Kenya as the second site. Between 1st October 2006 and 24th March 2011, nasopharyngeal and oropharyngeal swabs collected from consenting patients meeting the case definition for either hospitalized severe acute respiratory infection or outpatient influenza like illness were tested for hMPV by real time Reverse Transcription polymerase chain reaction (RT-PCR). Incidence rates were calculated as the number of hMPV cases per person-years of observation (pyo) per site with adjustments for patients meeting the case definition at study clinics who were not swabbed and for participants who sought medical attention at non-study clinics. The HIV status was included in analysis for patients aged >18 years. Seventeen (n = 17) hMPV isolates were sequenced at the 347bp F-gene fragment for subtyping. Of 9000 cases tested from both sites, 614 (6.8%) were positive for hMPV, consisting of 345/4284 (8.1%) in Kibera and 269/4716 (5.7 %) in Asembo. In Kibera, the adjusted rates were highest in children < 12 months [99.6/1000 pyo (95% CI 80.9 - 122.6)] and lowest in those >50 years: [0.7/1000 pyo (95%CI 0.1 - 5.3)]; in Asembo, the adjusted rates were highest in children aged 12 - 23 months [62.7/1000 pyo (95%CI 62.6 - 62.8)] and lowest in those aged in 18 - 34 years [14.6/1000 pyo (95%CI 14.5 - 14.7)]. In Kibera, 33% (14/443) of the hMPV-positive patients ≥ 18 years were also positive for HIV whereas in Asembo, 40% (12/30) of hMPV positive cases were positive for HIV. The clinical symptoms associated with hMPV included fever, cough, and runny nose. On genetic analysis, 5 of 17 (29 %) Kenya viruses belonged to subgroup A, and 12 (71%) viruses to subgroup B. No A1 subgroup viruses were detected. Thus hMPV incidence was higher in children aged ≤5 years in both study sites and incidence rates decreased with increasing age.
RANDOMIZED, DOUBLE-BLINDDED, PHASE 2 TRIAL OF WR 279,396 (PAROMOMYCIN AND GENTAMICIN) FOR THE TREATMENT OF CUTANEOUS LEISHMANIASIS CAUSED BY LEISHMANIA PANAMENSIS

Nestor Sosa1, Captitán Zeuz Captitán1, Javier Nieto1, Melissa Nieto1, José Calzada1, Hector Paz1, Mara Kreishman-Deitrick2, Karen Kopydlofois2, Diane Ullman3, William McCarthy2, Janet Ransom3, Chuck Scott3, William Ravis3, Max Grogl3

1Gorgas Institute, Panama City, Panama, 2U.S. Army Medical Materiel Development Activity, Fort Detrick, MD, United States, 3Fast-Track Drugs and Biologics, North Potomac, MD, United States, 4Auburn University, Auburn, AL, United States, 5Walter Reed Army Institute of Research, Silver Spring, MD, United States

This randomized double-blinded Phase 2 trial included 30 patients (17 adults and 13 children ages 5 to 17 years) with Leishmania panamensis cutaneous leishmaniasis (CL). Patients were randomly allocated (1:1) to receive once daily topical treatment with WR 279,396 (15% paromomycin + 0.5% gentamicin) or Paromomycin Alone (15% paromomycin) for 20 days. Patients were followed for pharmacokinetics (PK), safety and efficacy for six months. Blood for paromomycin and gentamicin PK parameters was collected from adult subjects after the first days' and last days' drug application. The primary efficacy endpoint was cure of a parasitologically confirmed index lesion, defined as at least 50% reepithelialization by Day 100 with no relapse. The index lesion cure rate after 6 months follow-up was 13/15 (87%) for WR 279,396 and was 9/15 (60%) for Paromomycin Alone (p = 0.099). When all treated lesions were evaluated for cure, the final cure rate for WR 279,396-treated patients was the same, but the final cure rate for Paromomycin Alone-treated patients was lower at 8/15 (53.3%; p = 0.046). Both creams were well tolerated with mild application site reactions including erythema (20%), edema (13.3%), and pain (6.7%) being the most frequent adverse event in the WR 279,396 group, which were slightly higher in the Paromomycin Alone group. PK data showed that there is limited paromomycin and gentamicin systemic absorption thus avoiding drug accumulation and toxicity. The increased final cure rate in the WR 279,396 group in this small Phase 2 study suggests that the combination product may provide greater clinical benefit than paromomycin monotherapy against L. panamensis CL. The excellent tolerability and low systemic drug exposure suggests that WR 279,396 may offer an alternative to more toxic systemic therapies for CL.

A PROSPECTIVE REFERRAL HOSPITAL STUDY OF SEVERE PLASMODIUM KNOWLESI MALARIA IN SABAH, MALAYSIA: HIGH INCIDENCE BUT NO MORTALITY WITH EARLY REFERRAL AND ARTESUNATE THERAPY

Bridge E. Barber1, Timothy William1, Matthew J. Grigg1, Jayaram Menon2, Nicholas M. Anstey3, Tsin W. Yeo3

1Menzies School of Health Research, Darwin, Northern Territory, Australia, 2Infectious Diseases Department, Queen Elizabeth Hospital, Kota Kinabalu, Sabah, Malaysia, 3Department of Medicine, Queen Elizabeth Hospital, Kota Kinabalu, Sabah, Malaysia

The simian parasite Plasmodium knowlesi is a common cause of severe malaria in Malaysian Borneo, and high case-fatality rates have been reported with chloroquine and/or quinine treatment. We compared risk, spectrum and outcome of severe disease from Pknowlesi, Pfalciparum and P vivax following the introduction of early referral and intravenous artesunate for all severe malaria. From September 2010-October 2011 we prospectively recorded clinical and laboratory features of non-pregnant patients ≥12 years-old admitted to Queen Elizabeth Hospital (QEH), Sabah, with PCR-confirmed malaria mono-infection. Standardised referral (4+ parasite-density and/or any severity-criterion) and intravenous artesunate was instituted at district hospitals. Severe malaria (modified-WHO 2010 criteria) occurred in 38/130 (29%) patients with P knowlesi, 15/122 (12%) with P falciparum and 7/43 (16%) with P vivax. Severity criteria in knowlesi malaria included hyperparasitaemia (>100,000 parasites/μL, N=18), respiratory distress (N=14), jaundice (N=20), acute renal failure (N=9), hypotension (N=13), metabolic acidosis (N=4), anaemia (N=2) and abnormal bleeding (N=2). Severe knowlesi malaria occurred in 27/57 (47%) patients ≥50 years old compared to 11/73 (15%) <50 years. However using logistic regression, only parasite density independently predicted severe malaria, excluding hyperparasitaemia as a sole severity-criterion (OR [log-increase in density count] = 2.01, p<0.0001). Nearly all (92%) patients with knowlesi malaria received artesinin therapy; 36/38 (95%) and 39/92 (42%) patients with severe and non-severe disease respectively received ≥1 dose of intravenous artesunate. Median parasite clearance-time was 2 days and no deaths occurred from any species. Pknowlesi is the commonest cause of severe malaria at QEH. parasite density and schizontemia >10% were the only independent risk-factors for severity. Early treatment with artesunate was highly effective and associated with zero mortality.

EFFECTIVENESS OF INTERMITTENT PREVENTIVE TREATMENT WITH SULFADOXINE-PYRIMETHAMINE IN PREGNANT WOMEN IN WESTERN KENYA: RESULTS OF AN OBSERVATIONAL STUDY

Peter O. Okuma

Centers for Disease Control and Prevention/KEMRI, Kisumu, Kenya

Intermittent preventive treatment with sulfadoxine pyrimethamine (IPTp) remains a key strategy for malaria prevention in pregnant women living in malaria endemic regions. However, increasing SP resistance threatens IPTp effectiveness. We assessed the effectiveness of IPTp in an area of western Kenya where Plasmidium falciparum resistance to SP is high. From August 2008 to June 2009, women presenting to deliver at two district hospitals were enrolled in a cross-sectional survey. We collected information on obstetric history, use of IPTp, insecticide treated nets, and antimalarial treatment during pregnancy. At delivery, we measured the prevalence of maternal anemia (Hb< 8g/dl), peripheral parasitaemia, placental parasitaemia (impression smear) and low birth weight (LBW) by number of IPTp doses administered by self-report or as recorded in the antenatal card. Overall 977 HIV-negative women were enrolled. Of these 637 (65%) were eligible for IPTp. Of these, 540 (84.5%) received at least one dose of IPTp. We followed women from the first trimester to delivery. We found no reduction in LBW by IPTp doses administered (p=0.73). Among multigravidae, significant trends by number of SP doses received were not observed for anemia, or peripheral or placental parasitemia, but were associated with reduced LBW (p=0.02). Among gravidae 1 or 2, but not multigravidae, having received more doses of IPTp was significantly associated with lower prevalence of maternal anemia, peripheral parasitemia and placental parasitemia. In multigravidae, IPTp resulted in reduced prevalence of LBW. During this time period, IPTp remained beneficial in this area of western Kenya, despite high SP resistance.
INVASIVE STAPHYLOCOCCUS AUREUS INFECTIONS IN CHILDREN IN THE TROPICAL TOP END OF AUSTRALIA: CLINICAL AND MICROBIOLOGICAL EPIDEMIOLOGY

Daniel Engelman1, Alexandra Hofer1, Joshua Davis2, Jonathan R. Carapetis3, Steven Y. Tong4

1Royal Darwin Hospital, Darwin, Australia, 2Menzies School of Health Research, Darwin, Australia

Previous studies report very high incidence of Staphylococcus aureus bacteremia in adult Aboriginal populations in tropical northern Australia. There are few studies describing incidence or outcomes in children. We aimed to describe the clinical and microbiological epidemiology of invasive S. aureus infections in children. We conducted a retrospective review for all cases of bacteremia and sterile site infection, for children under 15 years, in the Top End of Australia over a four year period (2007–2010). Forty-four cases (9 neonatal, 35 paediatric) were identified. The annual incidence of invasive S. aureus was 27.9 cases per 100,000 population and was significantly higher in the Aboriginal population (incidence rate ratio [IRR] compared to non-Aboriginal population: 5.3 [95%CI 2.5-12.6]). Among non-neonatal cases, the annual incidence was 22.2 per 100,000 population (Aboriginal 46.6, non-Aboriginal 4.4, IRR: 10.6 [95%CI 3.8-41.4]). There were significant differences between the neonatal and paediatric groups. Neonatal cases were all born prematurely, typically with significant comorbidities and episodes were often intravascular catheter related and nosocomially acquired. There was one death. Of the 35 paediatric (non-neonatal) cases, 17% had pre-existing comorbidities, 14% were malnourished and 11% were nosocomial. Major foci of infection were malnourished and 11% were nosocomial. There were deaths. There were 9 cases (26%) due to community-associated MRSA strains. Molecular genotyping results will be presented. There was no difference in severity or outcome between infections due to MRSA and MSSA. In conclusion, the annual incidence of invasive S. aureus infection in this study is the highest described in any paediatric population. Almost 1 in 2000 Aboriginal children develop invasive disease each year. Late onset sepsis in premature infants is the main neonatal cause. Most paediatric cases were community acquired and severe, yet rates of mortality, endocarditis and readmission were low.

TRYPANOSOMA CRUZI SURVIVAL FOLLOWING STORAGE: IMPLICATIONS FOR TISSUE TRANSPLANTATION-DERIVED TRANSMISSION

Diana Martin1, Brook Goodhew2, Shawn Hunter2, Jan Zajdowicz4, Scott Brubaker4

1Centers for Disease Control and Prevention, Atlanta, GA, United States, 2Community Tissue Services, Kettering, OH, United States, 3Rocky Mountain Tissue Bank, Aurora, CO, United States, 4American Association of Tissue Banks, McLean, VA, United States

Trypanosoma cruzi, the causative agent of human Chagas disease, is typically transmitted via the feces of a reduvid bug vector following a blood meal. Infection can also occur through infected blood and tissue products. An estimated 300,000 individuals in the United States are infected with T. cruzi and six cases of transmission through organ donation have been reported. The potential for transmission by tissue transplantation is considered herein. Tissue recovered for transplantation, in contrast to solid organ transplants, undergoes a range of storage and processing procedures depending on the tissue type. At the outset, tissues do not have to be recovered from a beating-heart or live donor, but instead may be removed from the donor up to 24 hours after asystole. Once removed, tissues are often stored and eventually undergo some degree of processing, which can include lyophilization, irradiation, and various sterilization techniques. As an example, heart valves are cryopreserved and undergo minimal processing, whereas other tissue may be stored as is at -80 C for as long as five years. The ability of infected tissue to transmit T. cruzi is likely related to the ability of T. cruzi to survive these processing and storage conditions. We examined the viability of T. cruzi parasites after room temperature and cold storage conditions. T. cruzi trypomastigotes were unaffected by 24 hours at room temperature, both in cell cultures and when spiked into whole blood to mimic decomposition following asystole. Parasite-infected cells stored up to 5 days at 4 C proved 100% viable after re-culture, whereas only 2 of 8 cultures stored 28 days at 4 C became culture-positive. A significant decrease in parasite viability was observed in samples stored up to 120 days at -80 C in the absence of cryopreservation, yet some live, infective parasites were recovered. These data demonstrate the heartiness of T. cruzi following cold storage. Studies are underway to examine the effects of more rigorous tissue processing procedures on T. cruzi viability.

A SYSTEMATIC REVIEW AND META-ANALYSIS OF MALARIA AND SEXUALLY TRANSMITTED AND REPRODUCTIVE TRACT INFECTIONS IN PREGNANCY IN SUB-SAHARAN AFRICA: OPPORTUNITIES FOR ANTENATAL INTERVENTION

R. Matthew Chico, Philippe Mayaud, Cono Ariti, David Mabey, Carine Ronsmans, Daniel Chandramohan

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

Malaria and sexually transmitted infections/reproductive tract infections (STIs/RTIs) in pregnancy are direct and indirect causes of stillbirth, prematurity, low birth weight, and maternal and neonatal morbidity and mortality. Novel use of diagnostic tools and/or drugs may improve birth outcomes with the impact depending on the prevalence of malaria and STI/RTI. PubMed, MEDLINE, EMBASE, the World Health Organization International Clinical Trials Registry, and reference lists were searched for studies reporting malaria, syphilis, Neisseria gonorrhoeae, Chlamydia trachomatis, Trichomonas vaginalis, or bacterial vaginosis among pregnant women attending antenatal care facilities in sub-Saharan Africa. Included studies were conducted in 1990-2011 with open enrollment. Studies from South Africa, where malaria is no longer endemic, were excluded. Point prevalence estimates were corrected for diagnostic precision. A random-effects model meta-analysis was then applied to produce pooled prevalence estimates. A total of 171 studies met inclusion criteria, providing 307 point prevalence estimates for malaria or STIs/RTIs. The pooled prevalence estimates (95% confidence intervals; n=positive diagnoses) among studies in 1990-2011 in East and Southern Africa were as follows: syphilis 4.5% (3.9, 5.1; n=835), N. gonorrhoeae 3.7% (2.8, 4.6; n=626), C. trachomatis 6.9% (5.1, 8.6; n=350), T. vaginalis 29.1% (20.9, 37.2; n=5,502), bacterial vaginosis 50.8% (43.3, 58.4; n=4,280), peripheral malaria 32.0% (25.9, 38.0; n=11,688) and placental malaria 25.8% (19.7, 31.9; n=1,388). West and Central Africa prevalence estimates were as follows: syphilis 3.5% (1.8, 5.2; n=851), N. gonorrhoeae 2.7% (1.7, 3.7; n=73), C. trachomatis 6.1% (4.0, 8.3; n=357), T. vaginalis 17.8% (12.4, 23.1; n=822), bacterial vaginosis 37.6% (18.0, 57.7; n=1,208), peripheral malaria 38.2% (32.3, 44.1; n=12,242) and placental malaria 39.9% (34.2, 45.7; n=4,658). In conclusion, the dual prevalence of malaria and STIs/RTIs in pregnancy among women attending antenatal care facilities in sub-Saharan Africa is considerable, with the combined prevalence of curable STIs/RTIs being equal to, if not greater than, malaria.
**ANSWERING THE MAIL: USING A CASE-BASED MODEL TO TEACH TELECONSULTATION SKILLS TO INFECTIOUS DISEASE FELLOWS**

Andrew Letizia, Tim Whitman, Michael Zapor, David Byers, Greg Martin, Rose Ressner, Glenn Wortman, Josh Hartzell

Walter Reed National Military Medical Center, Bethesda, MD, United States

The United States Military has offered a vast array of teleconsultative services to assist health care providers (HCP) deployed in remote areas of the world via e-mail. The Infectious Diseases Teleconsult system (IDTS) has been an effective tool in optimizing care for Service Members overseas. Despite the continuing development of telemedicine in both civilian and military communities, we are unaware of any standardized education given to HCP prior to participating in this method of consultation. Giving medical recommendations via a teleconsult system requires unique skills not used in the standard clinical setting. Consultants provide advice without questioning or examining the patient and must understand system constraints unique to the patient’s location (i.e. isolated mountain top in Afghanistan or remote village in West Africa). Cases referred to the IDTS often involve diseases unique to tropical climates and the developing world that inexperienced providers may not recognize. To address this gap in medical education, the Infectious Diseases (ID) fellowship program at Walter Reed National Military Medical Center implemented a training program in 2011 that utilized a series of simulated patients based upon real teleconsults from the IDTS system. Ten cases were chosen to highlight classic infectious diseases that have been common consults such as cutaneous Leishmaniasis, Q fever and malaria. The simulated cases are administered via e-mail to an ID fellow who then has one hour to appropriately answer the teleconsult. The fellow is then given feedback on his/her ability to generate an accurate assessment and plan for the simulated case and the practicality of their advice considering the patient care setting. An overview of the case-based model, selected examples, and evaluation criteria will be reviewed. This educational process is an effective way to prepare ID fellows for the real-world experience of offering advice via a teleconsult service and has improved their knowledge base of infectious diseases in the developing world.

**MATERNAL HIV EXPOSURE OR MOTHER TO CHILD TRANSMISSION OF HIV AND RISK OF TUBERCULOSIS IN INFANTS**

Grace Kaguthi1, Videfilm N. Nduba1, Anja Van’t Hoog2, Kayla Laserson2

1Kenya Medical Research Institute (KEMRI)/Centers for Disease Control and Prevention, Kismu, Kenya, 2University of Amsterdam, Academic Medical Centre, Amsterdam, The Netherlands, 3Centers for Disease Control and Prevention, Center for Global Health/KEMRI, Atlanta, GA, United States

Infants born to HIV infected mothers who test HIV negative (seroexposed) and those that become HIV infected (seroreactive) are at an increased risk of progression from latent tuberculosis (TB) infection to disease. However, TB diagnosis in this group is challenging due to low utility of scoring systems and low tuberculin skin test (TST) sensitivity. We sought to document the incidence rates of TB disease among seroexposed and seroreactive babies, among an infant cohort under follow up to determine TB incidence. Infants aged 0-6 weeks were recruited from Western Kenya. HIV DNA PCR testing was offered at six weeks to all infants. Through follow up and sick visits, TB suspects were identified based on signs and symptoms and suspected for TB through gastric aspirates, sputum induction, chest radiographs, HIV tests and TST. Definite cases were culture confirmed and probable cases had Keith Edward(KE) Scores of ≥7 and/or suggestive chest radiographs. Of 2900 infants enrolled, 435 (15%) were seroexposed, including 75 (2.6%) seroreactive. Of 923 (32%) TB suspects, 174 (19%) out of 923 TB suspects were seroexposed, including 43(25%) seroreactive. The mean TST induration among sero exposed and non exposed suspects was 5mm(SD=6) and 4mm (SD=5) p<0.01 respectively. Additionally, mean KE score was 2.3 (SD2.4) and 1.6 (SD2.0) p<0.01 for seroexposed and non-exposed respectively. There were 48 TB cases, (incidence rate 1.44 per 100 person years) [95% CI (1.09, 1.91)], sixteen of these (33%) were among seroexposed infants RR 2.1 [95% CI(1,1.4,0)]. Out of ten definite TB cases, five (50%) were seroexposed (p=0.05). HIV exposure and infection was associated with increased risk of being diagnosed with tuberculosis. HIV infected mothers have increased risk of developing TB and exposing their babies to TB infection. Rigorous antenatal screening for tuberculosis in HIV infected mothers is recommended to protect their infants from early latent infection and progression to TB disease.

**INCREASED RATES OF RESPIRATORY AND DIARRHEAL ILLNESSES AMONG HIV-NEGATIVE INDIVIDUALS ≥5 LIVING WITH HIV-INFECTED INDIVIDUALS IN AN URBAN SLUM (KIBERA) OF NAIROBI, KENYA**

Joshua M. Wong1, Dhillion Nyachieo2, John M. Williamson3, Leonard Cosmas4, Beatrice Olack5, George Okoth6, Henry Njuguna1, Lucy Nganga-Wanjiku6, Rebecca Bunnell7, Warren Dalal8, Daniel R. Feikin9, Heathie Burke9, Katherine A. Robinson9, Nancy Knight7, Joel M. Montgomery1, Robert F. Breiman4

1Centers for Disease Control and Prevention, Nairobi, Kenya, 2Kenyan Medical Research Institute, Centers for Disease Control and Prevention, International Emerging Infections Program, Nairobi, Kenya, 3Kenyan Medical Research Institute, Centers for Disease Control and Prevention, Nairobi, Kenya, 4Centers for Disease Control and Prevention, International Emerging Infections Program, Nairobi, Kenya, 5Centers for Disease Control and Prevention, Influenza Program, Nairobi, Kenya, 6Centers for Disease Control and Prevention, Division of Global HIV/AIDS, Nairobi, Kenya, 7Centers for Disease Control and Prevention, Atlanta, GA, United States, 8Centers for Disease Control and Prevention, Division of Global Migration and Quarantine, Atlanta, GA, United States

For some acute illnesses in HIV-infected patients, prolonged pathogen shedding, symptom duration, and other socioeconomic factors potentially contribute to increased incidence. Using data from a prospective, population-based infectious disease surveillance system of acute illnesses and a cross-sectional HIV serosurvey conducted in the urban slum of Kibera, Kenya, we calculated incidence of influenza-like illness (ILI), diarrhea, and non-specific febrile illness among 2,105 HIV-negative household contacts of HIV-infected participants and 13,747 participants living within exclusively HIV-negative households during 2008. Of the 4,285 household in which a test was performed, 83.8% had only HIV-negative tests, 13.5% had 1 HIV-positive test, and 2.7% had more than 1 HIV-positive test; untested adults were not included in the analysis. We stratified household contacts by number of HIV-infected individuals in households and modeled it as a continuous variable to determine a dose-dependent relationship. For children and adults ≥5 years old, incidence was significantly increased for ILI (incident rate ratio [IRR], 1.47; 95% confidence interval [CI], 1.07:1.99; p<0.05), and diarrhea (IRR, 1.41; CI, 1.11:1.77; p<0.05) in HIV-negative households contacts of HIV-infected participants and 13,747 participants living within exclusively HIV-negative households during 2008. Of the 4,285 household in which a test was performed, 83.8% had only HIV-negative tests, 13.5% had 1 HIV-positive test, and 2.7% had more than 1 HIV-positive test; untested adults were not included in the analysis. We stratified household contacts by number of HIV-infected individuals in households and modeled it as a continuous variable to determine a dose-dependent relationship. For children and adults ≥5 years old, incidence was significantly increased for ILI (incident rate ratio [IRR], 1.47; 95% confidence interval [CI], 1.07:1.99; p<0.05), and diarrhea (IRR, 1.41; CI, 1.11:1.77; p<0.05) in HIV-negative households contacts of HIV-infected residents when compared with rates in HIV-negative persons living in households without an HIV-infected resident. The risk for illness among HIV-positive persons was directly proportional to the number of HIV-infected people living in the home for ILI (IRR, 1.40; CI, 1.03:1.88; p<0.05), diarrhea (IRR, 1.36; CI, 1.11:1.67; p<0.01), and non-specific febrile illness (IRR, 1.15; CI, 1.02:1.29; p<0.05). There was no evidence for a similar pattern of increased incidence in a parallel analysis among children <5 years old. Since treatment and support for HIV-infected individuals reduces their incidence of infections, future interventions ensuring that HIV-infected persons are receiving appropriate care may help to reduce infections for all household members.
RUSH TO JUDGMENT: SOURCES OF CONFOUNDING IN STI-HIV PREVENTION TRIALS

Larry Sawers¹, Eileen Stillwagon²
1American University, Washington, DC, United States, 2Gettysburg College, Gettysburg, PA, United States

Substantial evidence indicates that sexually transmitted infections (STIs) promote HIV transmission and acquisition by producing genital ulcers, inflammation, and viral shedding. Ten randomized controlled trials in sub-Saharan Africa (SSA) examined effects of STI control on HIV incidence. One produced statistically significant results. Consequently, support for STI treatment for HIV prevention has faded. We conducted an intensive review of methods and outcomes of the 10 STI-control trials in SSA and subsequent analyses. All 10 trials reveal potentially serious confounding from multiple untreated genital morbidities. Some trials studied the impact of treating bacterial STIs on HIV incidence; others studied treatment of viral STIs. None studied both. None examined treatment of genital morbidity from other causes that could enhance HIV transmission or acquisition. The trials excluded consideration of fungal STIs. None considered genital ulceration and inflammation from non-sexually transmitted pathogens, such as Schistosomiasis hematobium (highly prevalent in SSA), and from ulcers caused by abrasions infected with streptococci or staphylococci, also common. Treating one type of genital morbidity may have little effect on HIV incidence when there is untreated genital morbidity from multiple sources. Furthermore, 8 trials reported the same or lower levels of risky sexual behavior in the control arm as in the treatment arm or reported the same or larger reductions in risky behaviors among controls. (Two trials did not report on sexual behavior.) This could have resulted from the trials’ successful interventions in the control arm or from spontaneous reductions in risky behavior prompted by the trial. Confounding by genital morbidity of multiple etiologies, behavioral change, and other factors in the 9 trials lacking statistically significant results renders those trials unable to inform HIV-prevention policy. Given abundant evidence that STIs promote HIV spread, STI treatment should be considered an important method for reducing HIV incidence in SSA and elsewhere.

HIV STIGMA AS A BARRIER TO RECEIVING HIV CARE AT A GENERAL HOSPITAL IN LIMA, PERU: A CASE-CONTROL STUDY

Carla V. Valenzuela¹, Aaron Kipp², Cesar Ugarte², Jorge Paz³, Juan Echevarria⁴, Eduardo Gotuzzo⁵
1Vanderbilt School of Medicine, Nashville, TN, United States, 2Vanderbilt Institute for Global Health, Nashville, TN, United States, 3Universidad Peruana Cayetano Heredia, Lima, Peru

Poor retention in care may increase the risk of morbidity, mortality, and community HIV transmission. The role of HIV stigma in poor retention has not been well studied. The objective of this case-control study was to evaluate the association between HIV stigma and retention in care among HIV patients in Lima, Peru. We evaluated HIV-positive patients who were diagnosed and/or initiated care at a general hospital between 2005-2010, with inclusion based on status of care by March 31, 2011. Those retained in care (n=150) had ≥ 2 documented medical care visits per year and were approached and interviewed privately in clinic during their appointment. Those not retained in care (n=55) had no documented visits for ≥ 1 year and home visits were used to locate them and conduct interviews. The Berger HIV stigma scale was used to quantify the 4 domains of stigma: enacted stigma (ES), disclosure concerns (DC), negative self-image (NSI), and concern with public attitudes (CPA). Each domain had 5 items with higher scores indicating higher stigma (score range 0-15). Multivariable logistic regression was used to calculate adjusted odds ratios (OR) and 95% confidence intervals (CI) for being out of care. Stigma was modeled as a continuous variable and linearity assumptions were assessed. Mean stigma scores were low for ES (6.1) and NSI (5.3) but high for DC (9.6) and CPA (9.0). ES and NSI had U-shaped associations with retention (odds of not being retained increased then decreased at higher stigma levels). DC and CPA showed linear associations. Patients who agreed to all items (score of 10) were more likely to not be retained than patients who disagreed to all items (score of 5) for ES (OR=2.36; 95% CI: 0.98, 5.67), DC (OR=2.72; 95% CI: 1.11, 6.67), NSI (OR=1.82; 95% CI: 0.50, 6.60), and CPA (OR=3.30; 95% CI: 1.37, 7.92). This study suggests that all aspects of HIV stigma, particularly concern with public attitudes, play a role in being out of care.

DO PEOPLE LIVING WITH HUMAN IMMUNODEFICIENCY VIRUS (PLHIVS) HAVE MORE RISKY SEXUAL BEHAVIORS THAN UNINFECTED PEOPLE IN BURKINA FASO? A CASE-CONTROL STUDY

Adama Baguiya¹, Bertrand Méda¹, Adja Mariam Ouédraogo¹, Aristide Romaric Bado¹, Damien De Walque², Seni Kouanda¹
1Research Institute for Health Sciences, Ouagadougou, Burkina Faso, 2World Bank, Washington, DC, United States

Antiretroviral therapy has reduced mortality and morbidity due to HIV/AIDS. Consequently, it is widely believed that PLHIV could keep new infections low because of their more risky sexual behaviors. This study aimed to compare PLHIV’s sexual behaviors with those who are infected, and to determine factors that affect their condom use. We conducted a case-control study involving all the PLHIVs and not infected people of the second round in Burkina Faso’s Treatment Accelerating Program assessment, from July to December 2009. This assessment involved PLHIVs (with their households) and households without PLHIV. Chi-square test was used to compare proportions with p<0.05 as statistically significant. Logistic regression was employed to determine associated factors of condom use. Two hundred ninety one PLHIVs and as many controls were interviewed in the 590 households. Seventy four percent of PLHIVs versus 58.1% of controls were women (p<0.001). Among men, 78.7% of PLHIV and 84% of controls had sex in the last twelve months (p<0.05). Among women, we had 48.1% versus 56.4% of PLHIV (p<0.05). Three percent of PLHIV versus 13% of controls were not aware of their last sexual partner’s HIV status (p<0.05). Among PLHIV, 27.1% versus 67% of the controls had not used condom during their last sexual intercourse (p<0.001). Both men’s and women’s group had presented the same difference (p<0.001). ART status was not associated with condom use (OR=1.2, 95%CI=0.6-2.5). But, voluntary testing (OR=3.4, 95%CI=1.1-10.7) and knowing that the partner has had sex with another person during the last twelve months (OR =5.2, 95%CI=1.6-16.7) were associated with PLHIV’s condoms use. PLHIV use condom more than uninfected and in general, they don’t have more risky sexual behaviors. Besides, condom use is not significantly affected by ART, so their availability doesn’t increase risky behaviors and therefore must be improved. This argument could be considered to strengthen public awareness campaigns against stigma and discrimination against PLHIV in Burkina Faso.
We and others have previously shown that Plasmodium falciparum-derived hemoglobin (PHz) promotes dysregulation of CCR5 and CXCR4, and their cognate ligands in monocytes and CD4+ T cells, resulting in increased HIV-1 replication. To further explore these molecular interactions, we examined the relative expression of CCR5 and CXCR4 on CD14+ monocytes and CD4+ T cells through flow cytometric analyses on cells collected from children (age, 2.6-33.6 mos; n=67) from western Kenya categorized into the following groups: 1) P. falciparum negative and HIV-1 negative (mal(-)/HIV-1(-), n=11), 2) P. falciparum positive and HIV-1 negative (mal(+)/HIV-1(+), n=33); 3) P. falciparum positive and HIV-1 exposed (mal(+)/HIV-1exp, n=20); and 4) P. falciparum positive and HIV-1 positive (mal(+)/HIV-1(+), n=3). Proportions of CD14+CXCR4+ cells were elevated in mal(+)/HIV-1(+)+ children compared to the mal(-)/HIV-1(-) group (P=0.048). However, proportions of CD14+CXCR4+ cells were reduced in the mal(+)/HIV-1(-) children relative to mal(+)/HIV-1(-) (P=0.039) and mal(+)/HIV-1exp (P=0.077) groups. In addition, expression of CD3+CXCR4+ and CD3+CD4+CCR5+ cell subsets were lower in the mal(+)/HIV-1(+)+ group compared to mal(+)/HIV-1(+)- children (P<0.05 for both). Further analyses in the combined population of malaria-infected children revealed that CD3+CXCR4+ cells were inversely correlated with the percentage of monocytes containing PHz (r=-0.883, P=0.020). Taken together, the preliminary results presented here suggest that CXCR4 and CCR5 are dysregulated in children co-infected with malaria and HIV-1, and that altered expression may be driven, at least in part, through acquisition of PHz by monocytes.
In the year 2010, the Far North Region of Cameroon experienced its most severe cholera outbreaks in the last four decades with about 9,400 reported cases and 600 deaths. This study describes the spatiotemporal dynamics of cholera epidemics from 1996 to 2010 using epidemiological data from this region, including reported cholera cases from 28 health districts, and environmental parameters such as temperature, relative humidity, rainfall and access to clean water. The data were entered into a geographical information system for further analysis. Regression analysis methods were used to analyze the data. The spatiotemporal patterns of the incidence rate were analyzed and associated with environmental factors to explore the determinant factors of the dynamics of cholera epidemics. The results revealed that there were three major epidemic periods and specific hotspots during the last 15 years. The annual dynamics showed a seasonal pattern coinciding with the wet seasons and significant differences in both incidence and timing by health districts. The spatial pattern revealed higher incidence rate in health districts in proximity with water bodies and in periurban areas. The study also revealed a connection with outbreaks in the neighboring countries of Chad and Nigeria. This study presents information related to the epidemiology and spatiotemporal pattern of cholera epidemics that can be used to help public health services plan prevention and control strategies against the spread of this disease.

**THE RELATIONSHIP BETWEEN DISTANCE TO HOUSEHOLD WATER SOURCE AND MODERATE-TO-SEVERE DIARRHEA IN YOUNG CHILDREN IN THE GLOBAL ENTERICS MULTI-CENTER STUDY (GEMS), KENYA, 2009-2011**

Benjamin L. Nygren1, Ciara E. O’Reilly1, Anangu Rajasingham3, Richard Omore4, Maurice Ombo4, Alex O. Awuor5, Penny Moke4, John Vulu6, Kayla Laserson7, Tamer H. Farag8, Dilruba Nasrin9, Sandra Panchalingam10, James P. Nataro4, Karen L. Kotloff5, Myron L. Levine2, Jeffrey Switchenko1, Tracy Ayers1, Gordana Derado1, Eric D. Mintz1, Robert F. Breiman6

1, Centers for Disease Control and Prevention, Atlanta, GA, United States, 2KEMRI/Centers for Disease Control and Prevention, Kisumu, Kenya, 3Center for Global Health Research, KEMRI, Kisumu, Kenya, 4Centers for Disease Control and Prevention, K SUMU, Kenya, 5University of Maryland, Baltimore, MD, United States, 6Centers for Disease Control and Prevention, Nairobi, Kenya

Water sources for rural households in the developing world are often located away from the home. Fetching water can be a substantial burden that can negatively affect household water quantity and quality, thereby increasing diarrheal disease risk. We visited households of 127 randomly selected pairs of case and age-, gender-, and village-matched controls enrolled in the GEMS study of moderate-to-severe diarrhea (MSD) in Kenya. We asked households to guide field teams from their home to their water source, and this path was captured as spatial data using GPS units. If no guide was available, we used GPS coordinates of the home and the water source to estimate distances. We compared GPS-sourced data to self-reported data in GEMS about round trip times spent to collect water, and evaluated each type of measurement as a predictor of MSD using conditional logistic regression. The paths recorded were a median of 1.18 (range 1.00 – 2.49) times the length of the straight line distances between their start and endpoints. Self-reported collection times were significantly correlated with the log-transformed distance measurements via GPS (Spearman correlation coefficient = 0.80, p < 0.01). The median recorded distance to water source was 196m (range: 1 - 1775m); 89 (35%) households were within 50m, all of which also reported the source to be in the household area. Collection times of 30 - 59 minutes were reported by households of 24 cases (median distance 561m, range 100 - 1775m) and 8 controls (median distance 562m, range 197 - 981m), and the odds of MSD were significantly higher vs. those with no travel (p <0.01). Collection times longer than 1 hour were reported for 12 cases (median distance 744m, range 148 - 1466m) and 3 controls (median distance 530m, range 460 - 1048m) and were significantly associated with MSD (p=0.02). These data suggest that distances traveled by households in rural Kenya to fetch water varied widely, that self-reported water collection times are correlated with measured distances, and that these may be useful in multivariate analyses of risk factors for MSD.

**FOOD PREPARATION PROCESSES AND HYGIENE PRACTICES IN RURAL BANGLADESH: OPPORTUNITIES TO IMPROVE HANDWASHING INTERVENTIONS**

Md. Fosiul A. Nizame1, Md. Nuruzzaman1, Notan C. Dutta1, Elii Leontsini2, Pavani K. Ram1, Peter J. Winch2, Shahana Parveen1, Stephen. P. Luby1, Leanne Unicomb1

1International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh, 2Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, 3University at Buffalo, State University of New York, Buffalo, NY, United States

In rural Bangladesh handwashing with soap during food preparation is associated with reduced child diarrhea, but we have limited data characterizing food preparation and related handwashing to inform behavior change interventions. This study explored the steps of food preparation, related handwashing opportunities, community perceptions and current practices regarding handwashing during food preparation. In 3 rural Bangladeshi villages, we conducted 12 in-depth interviews, 12 unstructured observations and 12 video observations with caregivers, and 3 focus group discussions with household heads on handwashing related to food preparation. Eating, preparing and serving food with bare
hands was common in the study communities. Varieties of mashed foods, salads and mixed dried foods that involved direct hand contact, which are not further cooked, were popular in rural Bangladesh. Mashed foods are prepared by boiling vegetables or dried fish, then peeling, mashing and mixing with raw ingredients using bare hands. Salad preparation involved cutting raw vegetables and mixing them by hand. For mixed dried foods, puffed rice and dried snacks are hand-mixed with raw ingredients.

Participants perceived that handwashing with soap was only necessary if hands were covered with visible dirt. Most respondents reported that they wash their hands with water during food preparation, but we observed that out of 54 opportunities to wash hands, participants washed hands with soap 2 times, with water alone 9 times, rinsed hands or hands came into contact with water 26 times, and did not wash hands 17 times. Food preparation was often interrupted by other tasks that could contaminate the preparer's hands, after which they continued food preparation without washing hands. Participants cited that absence of soap in appropriate place is a potential barrier to wash hands with soap. Caregivers do not usually wash their hands with soap during food preparation in rural Bangladesh. Food preparation is a complex, multi-step, often interrupted process where villagers do not recognize moments of high risk of environmental contamination as a time to wash hands with soap. Identifying the highest risk food preparation steps and prioritizing those when handwashing with soap is important will help focus handwashing interventions. Bringing soap and water together in the food preparation area may make it easier to wash hands with soap during such high risk moments.

CONTINUOUS ENVIRONMENTAL FECAL CONTAMINATION FOLLOWING IMPLEMENTATION OF SANITATION HARDWARE

Sania Ashraf1, Faruque Hussain1, Elli Leontsini2, Leanne Unicomb1, Stephen P. Luby3

1International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh, 2Johns Hopkins, Baltimore, MD, United States, 3Centers for Disease Control and Prevention, Atlanta, GA, United States

Even with latrines available, child and animal feces are often found in and around households in rural Bangladesh. These feces may contain important gastrointestinal pathogens. We piloted child potties and a customized handlike tool (sani scoop) to measure their impact on environmental fecal contamination. We distributed child potties in 104 rural households with children <3 years of age. All households in study household compounds were given sani scoops to encourage hygienic disposal of child feces in latrines and animal feces in designated pits. Local health promoters encouraged using scoops and potties in these households through nurture and disgust themed messages. Field workers administered a 3 month follow-up survey that included participant reported frequency of use, and field worker observation of the presence and condition of hardware and presence of animal and human feces around the household. We used in-depth interviews and focus group discussions with mothers from these communities to identify the barriers to using the hardware. Reported use of potties (67%) and sani scoops (89%) for child defecation events were high. Little difference in the presence of human feces was detected 3 months after receiving the intervention (19/96; 20%) compared to baseline (16/104; 16%), and to control households (22%). Similarly animal feces were found in 87/104 (84%) of intervention households at 3 months compared to 92/96 (96%) at baseline and 99% in control compounds. During in-depth interviews participants reported incomplete potty training, inconsistent potty use and delays in disposing feces because of their many other household tasks. Ubiquitous poultry and other domestic animals regularly produce fresh feces. The utility of cow dung as biofuel led to conflict over ownership of feces and appropriate handling. Perceptions of child and some animal feces as harmless limited household's motivation to dispose of feces. Though potties and sani scoops had high acceptability and self-reported use, most households maintained high levels of observable feces at follow-up. Although improving disposal of child feces is often mentioned as part of sanitary interventions, additional research is needed to develop practical strategies to reduce contamination in a child's household environment.

LOOKING BEYOND KDR: THE EMERGENCE OF A NEW MUTATION, N1575Y, IN THE SODIUM CHANNEL OF ANOPHELES GAMBIAE AND ITS ROLE IN INSECTICIDE RESISTANCE

Christopher M. Jones1, Fiacre R. Agossa2, Toé K. Hyacinthe1, David Weetman1, Hilary Ranson1, Martin James Donnelly1, Craig S. Wilding1

1Liverpool School of Tropical Medicine, Liverpool, United Kingdom, 2Centre de Recherche Entomologique de Cotonou, Cotonou, Benin

Insecticide resistance is a major threat to malaria vector control. In Anopheles gambiae, resistance to pyrethroid and DDT insecticides is strongly associated with the mutations L1014F and L1014S within the para voltage-gated sodium channel (VGSC). Across much of West Africa, 1014F frequency approaches fixation. Here, we document the emergence of a mutation, N1575Y, within the linker between domains III-IV of the VGSC. In data extending over 40 kbp of the VGSC, 1575Y occurs on only a single long-range haplotype, also bearing 1014F. The 1014F-1575Y haplotype was found in both M and S molecular forms of An. gambiae in West/Central African sample sites separated by up to 2,000 km. In Burkina Faso M form, 1575Y allele frequency rose significantly from 0.053 to 0.172 between 2008 and 2010. Extended haplotype homozygosity
analysis of the wild-type 1575N allele showed rapid decay of linkage disequilibrium (LD), in sharp contrast to the extended LD exhibited by 1575Y. A haplotype with long-range LD and high/increasing frequency is a classical sign of strong positive selection acting on a recent mutant. 1575Y occurs ubiquitously on a 1014F haplotype background, suggesting that the N1575Y mutation compensates for deleterious fitness effects of 1014F and/or confers additional resistance to insecticides. Haplotype tests of association suggest the latter: The 1014F-1575Y haplotype confers a significant additive benefit above 1014F-1575N for survival to DDT (M form P = 0.03) and permethrin (S form P = 0.003). DNA-based diagnostics are supplementing phenotypic bioassays as a proactive means of detecting resistance alleles at low frequency. The discovery of N1575Y at an early stage highlights the importance of continual monitoring for novel resistance mutations and its spread should be monitored closely.

THE EVOLUTION OF RESISTANCE TO CARBAMATES AND ORGANOPHOSPHATE INSECTICIDES IN ANOPHELES GAMBIAE

Luc S. Djogbé,1 David Weetman,1 Roch Dabiré,1 Guillaume Ketö1, Fabrice Chandre,2 Mylène Weill,1 Martin Donnelly2
1Institut Régional de Santé Publique/Université d’Abomey-Calavi, Ouidah, Benin, 2Liverpool School of Tropical Medicine and Hygiene, Liverpool, United Kingdom.

RESISTANCE TO PYRETHROID AND CARBAMATE THREATENS VECTOR CONTROL IN WEST OF TANZANIA

Natacha Protopopoff,1 Robert Malima,1,2 Alex Wright1, Reginald Kavishe1, Johnson Matowo1, Philippa West1, Franklin W. Mosha1, Immo Kleinschmidt1, Mark Rowland1
1London School of Hygiene and Tropical Medicine, London, United Kingdom, 1National Institute for Medical Research, Muheza, United Republic of Tanzania, 1Kilimanjaro Christian Medical College, Moshi, United Republic of Tanzania.

Kagera region on the western side of Lake Victoria had the highest malaria burden in Tanzania according to the 2007 Malaria Indicator Survey. To reduce malaria transmission an annual round of indoor residual spraying (IRS) has been conducted since 2007 initially with lambda chops (pyrethroid) and more recently with bendiocarb (carbamate). A campaign of universal coverage of long lasting insecticidal nets (LLIN) was carried out in 2011. The emergence of resistance could threaten the future of these two interventions. As a component of a cluster randomized trial comparing the combination of LLIN and IRS versus LLIN alone the distribution of vectors and prevalence of insecticide resistance is being monitored. From April to December 2011, monthly Anopheles collection using CDC light traps was carried out across 40 villages in the area. Resistance monitoring was carried out on An. gambiae s.l. using WHO cylinder test. CDC bottle bioassays with synergists examined the involvement of metabolic resistance. Species identification and prevalence of knock down resistance (kdr) was confirmed using real time PCR TaqMan assay. A total of 5844 Anopheles mosquitoes were collected over seven months, of these 67% were collected in April, two months after spraying with pyrethroid. 81.8% were An.gambiae s.s. and 17.2% were An. arabiensis. East kdr mutation which is associated with pyrethroid and DDT resistance was present at high frequency in An.gambiae s.s. (97%) but only at 5% in An.arabiensis. Mortality in WHO resistance tests ranged from 0% to 38% for lambda chops, 12% to 40% for DDT, and 84% to 100% for bendiocarb. Result from the CDC bottle assay suggested the presence of elevated level of oxidases and esterases. East kdr mutation seems to have reached fixation in the An.gambiae s.s population. High phenotypic resistance to pyrethroid was observed. In contrast to neighbouring Kenya where An.gambiae s.s. nearly disappeared after vector control despite high kdr frequency, An.gambiae s.s. remains predominant in Kagera even with high coverage of pyrethroid IRS and LLINs.

DISSECTING THE MOLECULAR BASIS OF PYRETHROID RESISTANCE IN FIELD POPULATIONS OF THE MAJOR MALARIAM VECTOR ANOPHELES FUNESTUS IN SOUTHERN AFRICA

Jacob M. Riveron, Helen Irving, Miranda Nduka, Kayla G. Barnes, Charles S. Wondji
Liverpool School of Tropical Medicine, Liverpool, United Kingdom.

Anopheles funestus is a major malaria vector in Southern Africa. It remains unclear whether the many reports of pyrethroid resistance in this region have the same underlying mechanisms spreading between countries through gene flow or if different mechanisms occurred independently. To elucidate these questions we dissected the molecular basis of pyrethroid resistance in three countries, Mozambique, Malawi and Zambia. Microarray analysis using an Agilent chip identified three main P450 genes associated with permethrin resistance (CYP6P9a, CYP6P9b and CYP6M7) but with significant differences in expression patterns between countries. Other genes potentially implicated involved a short chain dehydrogenase and other P450s such as CYP6AA4 and CYP9J14. The most upregulated gene in Mozambique is CYP6P9p with a fold-change (FC) > 88, then CYP6P9p (FC~60) and CYP6M7 (FC~25). Interestingly in Malawi, CYP6P9p is the most upregulated gene (FC~69) then CYP6P9b (FC~30) and last CYP6M7 (FC~12) while in Zambia, CYP6M7 is the top upregulated gene (FC~37) before CYP6P9a (FC~15) and CYP6P9b (FC~11). The overall higher fold-change in Mozambique correlates with the higher level of resistance in this country. The upregulation of these genes was validated by qRT-PCR. Polymorphism analysis of these 3 genes and surrounding microsatellite markers detected selective sweep signatures for CYP6P9p and CYP6P9a but less for CYP6M7. Transgenic in vivo expression of CYP6P9p and CYP6P9b using the GAL4/UAS system indicated that both genes confer resistance in Drosophila to permethrin and deltamethrin. In vitro metabolism assays with recombinant proteins of both genes in E coli cells showed that CYP6P9a and CYP6P9b both metabolise Type I (permethrin) and Type II (deltamethrin and λ-cyhalothrin) pyrethroids but not Etofenprox or DDT. The cloning of the 6kb CDNA of the VGSC gene identified rare clones with potential kdr mutations which remain to be confirmed in field populations. Overall, these results suggest the presence of different resistance fronts in populations of An. funestus in Southern Africa.
EVOLUTION OF INSECTICIDE RESISTANCE AND MALARIA POSITIVITY RATES IN ANOPHELES GAMBIAE AFTER INTRODUCTION LONG LASTING INSECTICIDE TREATED BED NETS IN DIELOM, SENEGAL

Mamadou Ousmane Ndiath, Cheikh Sokha, Jean François Trape
Institut de Recherche pour le Développement, Dakar, Senegal

Despite many efforts in basic and applied research, malaria remains, 120 years after Plasmodium discovery, one of the major health problems, particularly in Africa. Among the different strategies used, vector control is an important component of malaria control. Insecticide-treated nets (ITNs) and indoor residual spraying (IRS) represent the front-line tools for malaria vector control. However, it is a real arms race, anophelines mosquitoes develop more and more resistance. The aim of this study was to identify changes in the principal malaria vectors in Dieulo (Senegal) that occurred before (2006 to July 2008) and after (August 2008 to December 2011) the implementation of LLINs. Adult mosquitoes were collected by HLC monthly from January 2006 to December 2011 and by PSC during the rainy season. Mosquitoes were identified to their species and sub-species by PCR. The presence of circumsporozoite protein (CSP) of *P. falciparum* and the blood meal origin was detected, and kdr mutations were investigated by PCR. From January 2006 to December 2011, 855 (62.0%) *An. gambiae* s.l. and 5,190 (36.3%) *An. funestus* were collected during 744 man night captures. No *An. gambiae* HBR variations was observed during periods before and after the implementation of LLINs (12.0 *An. man/night vs. 11.8 before), whereas *An. funestus* present all year round before LLINs disappeared after. Before the implementation of LLINs, CSP rate was not significantly different between kdr groups (4.55% in RS group and 3.83% in SS group, p=0.6) or in taxa groups (p=0.6). After August 2008, a significant difference was observed among kdr groups with significantly more CSP positive specimens in the RR group (25.45%) than in RS (14.67%) or SS groups (p<0.001). A significant difference in CSP rate was also observed between taxa groups (p=0.004) with more infected mosquitoes in *An. gambiae* S form. No specimen with L1014S kdr mutation was identified after the study. This study demonstrates the exceptional adaptability of *An. gambiae* s.l. to the presence of insecticide inside the houses.

CHANGES IN ANOPHELES FUNESTUS BITING BEHAVIOR FOLLOWING UNIVERSAL COVERAGE OF LONG-LASTING INSECTICIDAL NETS IN BENIN

Nicolas Moiroux1, Cedric Pennetier2, Marinely B. Gomez2, Emmanuel Elanga2, Innocent Djegbe2, Armel Djenontin2, Hélène Guis1, Vincent Corbel1

1Institut de Recherche pour le Développement, Montpellier, France, 2Institut de Recherche pour le Développement, Cotonou, Benin, 3CIRAD, Montpellier, France

Behavioural modification of malaria vectors in response to vector control methods is of great concern. We investigated whether full coverage of Long-Lasting Insecticide-treated mosquito Nets (LLIN) may induce a switch in biting behaviour in *Anopheles funestus*, a major malaria vector in Africa. Human landing collections were conducted indoor and outdoor in two villages (Lokohoué and Tokoli) in Southern Benin prior, 1 year and 3 years after implementation of universal LLIN coverage. Outdoor Biting Rates (OBR) and Median Catching Times (MCT, the hour for which 50% of *An. funestus* mosquitoes were collected) were compared. The resistance status of *An. funestus* to deltamethrin insecticide was monitored using bioassays. MCT of *An. funestus* switched from 02:00 in Lokohoué and 03:00 in Tokoli to 05:00 after 3 years (Mann-Whitney p-value<0.0001). In Tokoli, OBR increased from 45% to 68.1% (OR=2.55;95CI 1.72-3.78;p<0.0001) 1 year after the universal coverage whereas OBR was unchanged in Lokohoué. In this latter place, however, the proportion of *An. funestus* that bites after dawn (06:00) was 26%. Bioassays showed no resistance to deltamethrin. In conclusion, this study provides evidence for a switch in malaria vectors biting behaviour following the implementation of LLIN at universal coverage. We show first evidence for a diurnal activity of a major malaria vector in Africa. These changes may reflect phenotypic plasticity or selection of genetically inherited traits and may have direct consequences on the burden of malaria in Africa. These findings highlighted the need for alternative strategies for better targeting outdoor malaria vectors.

QUANTIFYING THE MOSQUITO’S SWEET TOOTH: MODELING ATTRACTIVE TOXIC SUGAR BAIT FOR VECTOR CONTROL

John M. Marshall1, Michael T. White1, Yosef Schlein2, Gunter C. Muller2, John C. Beier2
1Imperial College London, London, United Kingdom, 2Hebrew University, Jerusalem, Israel, 3University of Miami, Miami, FL, United States

Current vector control strategies focus largely on indoor measures, such as long-lasting insecticide treated nets (LLINs) and indoor residual spraying (IRS); however mosquitoes frequently feed on sugar sources outdoors, inviting the possibility of novel control strategies. Attractive toxic sugar bait (ATSB), either sprayed on vegetation or provided in outdoor bait stations, has been tested in Mali and Israel and has been shown to significantly reduce mosquito densities in these settings. We fitted models of mosquito population dynamics to data from these experiments to gain a better quantitative understanding of mosquito sugar feeding behavior and the potential of ATSB to control mosquito populations. In Mali, we estimate that 42% of female mosquitoes in the experimental setting fed on ATSB solution per day, dying within three hours of ingesting the toxin. A model incorporating the number of gonotrophic cycles completed by female mosquitoes found a higher feeding rate for younger mosquitoes and a slower rate for older mosquitoes. This model was extended to assess the role of ATSB as part of an integrated vector management (IVM) program. Our simulations suggest that an IVM program based on both ATSB and LLINs/IRS is likely to cause substantial reductions in mosquito density as multiple stages of the mosquito’s lifecycle are targeted. In addition, ATSB is expected to be particularly effective against *Anopheles arabiensis*, which is relatively exophilic and therefore less affected by IRS and LLINs. ATSB has a benefit over larvacides in the sense that it skews the age distribution towards younger mosquitoes, which is beneficial for malaria control because only older mosquitoes have time to acquire, incubate and transmit the parasite. These encouraging results suggest that ATSB should be seriously considered as a promising component in future IVM malaria control strategies.
A POTENTIAL ROLE FOR URIC ACID IN ENDOTHELIAL ACTIVATION AND DAMAGE IN PLASMODIUM FALCIPARUM MALARIA

Neida K. Mita-Mendoza1, Diana L. van de Hoeff1, Tatiana M. Lopera-Mesa1, Saibou Doumbia1, Drissa Konaté3, Mory Doumbia3, Jennifer M. Anderson1, Leopoldo Santos-Argumedo4, Ana Rodriguez2, Michael P. Fay5, Mahamadou Diakité1, Carole A. Long1, Rick M. Fairhurst1

1Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States, 2Department of Microbiology, Division of Medical Parasitology, New York University School of Medicine, New York, NY, United States, 3Malaria Research and Training Center, Faculty of Medicine, Pharmacy and Odontostomatologí, University of Bamako, Bamako, Mali, 4Department of Molecular Biomedicine, Centro de Investigación y Estudios Avanzados – Instituto Politécnico Nacional, Mexico City, Mexico, 5Biostatistics Research Branch, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States

Inflammatory cytokinemia and systemic endothelial activation are central to the pathogenesis of Plasmodium falciparum (Pf) malaria. Recently, Pf-derived uric acid (UA) - in both its soluble and precipitated forms - was shown to activate human immune cells in vitro, and elevated plasma UA levels were associated with inflammatory cytokinemia and disease severity in children with malaria. A role for Pf-derived UA in endothelial inflammation has not been investigated. Since UA elevations are associated with endothelial inflammation in a variety of non-malarial diseases, we hypothesized that elevated UA levels contribute to endothelial activation and damage in P. falciparum malaria. To test this, we measured levels of UA and soluble forms of intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), E-Selectin, thrombomodulin (TM), tissue factor (TF) and vascular endothelial growth factor (VEGF) in the plasma of 567 Malian children (aged 6 months - 17 years) with uncomplicated malaria (UM, N=489) and non-cerebral severe malaria (NCNM, N=68). In 69 of these children, we measured these same factors during their malaria episode and twice when they were healthy (before and after the transmission season). We found that levels of UA, sICAM-1, sVCAM-1, sE-Selectin and sTM increased significantly during a malaria episode, returning to ‘healthy’ levels at the end of the transmission season (p<0.0001). In children with UM, UA levels correlated significantly with those of sICAM-1 (r=0.255, p<0.0001) and sTM (r=0.175, p=0.0005). To test the possibility that Pf-derived UA precipitates activate EC, we co-cultured 3D7-infected red blood cells (PRBCs) with primary microvascular endothelial cells (MVECs) with or without uricase, which degrades UA. Our preliminary results show that PRBCs stimulate MVECs to release IL-6 and IL-8 in a dose- and time-dependent manner and that uricase abrogates the production of these cytokines. Our data suggest that parasite-induced elevations in UA levels contribute to malaria pathogenesis by causing endothelial activation and damage.

GLIDING MOTILITY AND ERYTHROCYTE INVASION PROCESSES OF BABESIA MEROZOITES VISUALIZED BY TIME-LAPSE VIDEO MICROSCOPY

Masahito Asada1, Yasuyuki Goto1, Kazuhide Yahata2, Naoki Yokoyama1, Satoru Kawai2, Noboru Inoue1, Osamu Kaneko1, Shin-ichiro Kawazu1

1National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Japan, 2Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan, 3Institute of Tropical Medicine (NEKKEN) and the Center of Excellence Program, Nagasaki University, Nagasaki, Japan, 4Dokkyo University School of Medicine, Mibu, Japan

Babesiosis is a zoonosis caused by tick-transmitted intraerythrocytic protozoa of the Phylum Apicomplexa. Some specific stages of apicomplexan parasites, such as sporozoites of Plasmodium falciparum and tachyzoites of Toxoplasma gondii, invade their target host cells using a unique, active process known as gliding motility. However, it is not thoroughly understood how the merozoites of Babesia parasites target and invade the host red blood cells (RBCs), and the gliding motility has so far not been observed in the parasite. In this study, we revealed the gliding motility of B. bovis merozoites by time-lapse video microscopy. The recorded images delineated that the processes included egress of the merozoites from the infected RBC, gliding motility, and succeeding invasion into new RBCs. Based on these images, the gliding motility of B. bovis merozoites was similar to the helical gliding of Toxoplasma tachyzoites. The trails left by the merozoites were detected by indirect immunofluorescence assay using antiserum against B. bovis merozoite surface antigen 1. This first report of gliding motility in B. bovis is notable and significant for the apicomplexan parasites since merozoites of Plasmodium parasites do not glide on the substrate. Furthermore, inhibition of gliding motility by actin filament polymerizer or depolymerizer indicated that this movement was driven by actomyosin-dependent process. In this study, we also revealed the timing of breakdown of parasitophorous vacuole through time-lapse image analysis. The membrane-stained bovine RBCs showed formation and breakdown of parasitophorous vacuole within ten minutes. Moreover, recent studies in Plasmodium have highlighted the essential role of the thrombospondin related anonymous/adhesive protein (TRAP) family in the gliding and cell invasion of the parasites. Currently, we are in the process of investigating the role of the TRAP-family in the gliding motility of Babesia merozoites.

THE ROLE OF MACROPHAGES IN SCHISTOSOMAL BLADDER PATHOGENESIS

Chi-Ling Fu, Kim Thai, Justin Odegaard, Michael Hsieh

Stanford University School of Medicine, Stanford, CA, United States

Schistosoma haematobium infects 112 million people, rendering it the most prevalent cause of schistosomiasis worldwide. Chronic S. haematobium infection (urogenital schistosomiasis) leads to approximately 150,000 deaths annually from urinary tract fibrosis-induced obstructive renal failure, making it one of the deadliest worm infections globally. Despite the major human impact of urogenital schistosomiasis, the mechanisms of S. haematobium egg-triggered, urinary tract granuloma-associated pathogenesis remain ill-defined. Parallels may be drawn from mouse models of Schistosoma mansoni infection, wherein egg granuloma-associated macrophages play a central role in liver pathogenesis. However, the involvement of macrophages in schistosomal bladder pathogenesis is unknown. To address this deficiency, we have employed our recently developed mouse model of urogenital schistosomiasis, as reported previously. Eight to 12 week old female mice underwent bladder wall microinjection with various single doses of S. haematobium eggs. Macrophages were systemically and locally killed by intraperitoneal, bladder intramural, and transurethral administration of macrophage-
depleting agents. Serial microultrasonography revealed zones of decreased echogenicity in the periphery of egg granulomas in macrophage-depleted versus -replete mice, suggestive of relative hypocellularity. This was confirmed by histology, which revealed hypocellular cavitations in macrophage-depleted granulomas, less fibrosis, and fewer infiltrating leukocytes. None of the control vehicle-treated mice receiving eggs died, whereas 60% of macrophage-depleted mice receiving high doses of eggs died by day 11 post-egg injection, indicating a crucial role for macrophages in prevention of detrimental systemic effects of helmith exposure. Our results confirm a critical role for macrophages in schistosomal bladder pathogenesis, even in the setting of a single exposure to S. haematobium eggs. This suggests that macrophages may be a suitable therapeutic target for advanced schistosomal bladder pathogenesis.

DEVELOPMENTAL AND SPATIAL EXPRESSION OF ANTIGENIC GLYCANS BY LARVAL STAGES OF SCHISTOSOMA MANSONI

Cornelis H. Smit, Angela van Diepen, Dieuwke Kornelis, Manfred Wuhrer, André M. Deelder, Cornelis H. Hokke
Department of Parasitology, Leiden University Medical Center, Leiden, The Netherlands

Many glycans of schistosomes are differentially expressed during the parasite’s lifecycle. In human infections, antibodies are produced against antigenic schistosome glycans, which might form promising novel vaccine targets. Schistosome larvae, up to several days old, appear most vulnerable to immune attack, but their glycosylation is poorly characterized. In this study we determined the structure and localization of the antigenic glycans expressed by early developmental stages of S. mansoni ranging from invading cercariae to transformed schistosomula and mature worms, with the objective of identifying antigens exposed to the immune system of the host. The protein-linked glycans from 14 different lifecycle stages were isolated and profiled using a mass spectrometry-based analysis strategy. Although N-glycans were continuously present during the whole lifecycle, our analysis indicated a gradually changing N-glycome throughout development, whereas others such as the O-glycan expression, often with similar core-xylose and LeX-antennae are abundant in cercariae and shortly after transformation to schistosomula, but expression decreased after 3 days of maturation. On the other hand, glycans with LDN termini become abundant in the adult stages. O-glycan expression, often with similar antennae motifs as N-glycans, strongly diminishes after transformation of the cercariae, but becomes abundant again in eggs. Using a glycan-microarray constructed of schistosome-derived glycans, we determined the fine-specificity of a panel of anti-carbohydrate monoclonal antibodies obtained from schistosome-infected mice. Application of these mAbs in immunofluorescence microscopy assays of the infective cercariae and 1-3 day schistosomula stages of S. mansoni indicated that some glycans epitopes (e.g. LDN, F-LDN, F-LDN-F) identified in the structural studies are expressed at the surface throughout development, whereas others such as the LeX-motif appear at the surface only after transformation. These observations further underline the potential of specific glycans as targets for immune attack.

NEMATODE AUTOPHAGY REGULATES WOLBACHIA POPULATIONS AND IDENTIFIES A NOVEL MODE-OF-ACTION FOR ANTIFILARIAL TREATMENT

Denis Voronin, Darren Cook, Andrew Steven, Mark J. Taylor
Liverpool School of Tropical Medicine, Liverpool, United Kingdom

Filarial nematode parasites are amongst the most important neglected parasitic diseases of humans and animals. Over 150 million individuals are infected with lymphatic filariasis and onchocerciasis and heartworm is an important pathogen of dogs and cats. A new target for antifilarial treatment is the obligate mutualistic endobacteria Wolbachia. Depletion of Wolbachia with antibiotics induces defects in nematode development, fertility and viability. In order to identify novel mechanisms to deplete Wolbachia as part of the A-WOL drug discovery and development programme, we investigated the role of activating host nematode autophagy to control bacterial populations. Our studies revealed that periods of rapid bacterial population growth and expansion were accompanied by activation of the autophagy pathway and that chemical and genetic manipulation of this pathway could directly regulate bacterial populations at an equivalent level to antibiotic treatment. The activation of the autophagy by using drugs or small-molecules resulted in Wolbachia reduction in both in vitro and in vivo treated Brugia malayi. The induction of the host nematode intracellular autophagy defence mechanism can therefore be considered as a novel mode-of-action, which delivers bactericidal activity that can be used to develop improved drugs and regimes for anti-filarial treatment.

FUNCTIONAL REDUNDANCY IN PLASMODIUM HEMOGLOBINASES AND PARASITE DEVELOPMENT INSIDE RETICULOCYTES WITHOUT HEMOGLOBIN DEGRADATION

Jingwen Lin, Elena Aime, Mohammed Sajjidd, Blandine Franke-Fayard, Frans Prins, Philippe E. Van den Steen, Katrien Deroost, Roberta Spaccapelo, Chris J. Janse, Shahid M. Khan
1Department of Parasitology, Leiden University Medical Center, Leiden, The Netherlands, 2Department of Experimental Medicine, University of Perugia, Perugia, Italy, 3Department of Pathology, Leiden University Medical Center, Leiden, The Netherlands, 4Laboratory of Immunobiology, Rega Institute for Medical Research, University of Leuven, Leuven, Belgium

Clinical symptoms of malaria infection manifest when the Plasmodium parasites replicate in host red blood cell. During this intraerythrocytic cycle, the parasite ingests and catabolizes up to 75% of the host cell hemoglobin (Hb). The hemoglobin is broken down by a number of proteases in a semi-order cascade in an acidic, Plasmodium-specific, digestive food vacuole (DFV). Whether this degradation is essential for parasite survival has not been established. To characterize biological roles of various hemoglobinases residing in digestive food vacuole, we attempted targeted gene disruption of all the predicted hemoglobinases in the rodent model P. berghei and found that most of them are functionally redundant. We have also created a double gene-deletion mutant, ApmA4bp2, lacking expression of both plasmspein-4 (equivalent to P. falciparum plasmspein I, II, III and IV) and bergheipain-2 (equivalent to P. falciparum falcipain-2a, 2b and 3), which are thought to be involved in initial cleavage of Hb. Despite severe growth and virulence attenuation, the parasites are able to develop into mature schizonts in reticulocytes. These schizonts produce either no or vastly reduced levels of hemozoin, the crystallized product formed by detoxification of heme that is released early in Hb digestion. This was confirmed by examining hemozoin deposition in both liver and spleen of ApmA4bp2 infected mice, which was greatly reduced compared to wildtype infected mice. The cerebral complication (CM) sensitive C57BL/6 mice were able to clear the infection without visible CM manifestation and survive later wild-type challenge. Our results show that Hb digestion may not be essential for parasite growth in reticulocytes. These findings have implications for the design of drugs against DFV enzymes and for possible mechanisms that underlie Plasmodium resistance to drugs, the majority of which target Hb digestion and heme detoxification.

BIOCHEMICAL CHARACTERIZATION OF UFSP, THE UFM1 ASSOCIATED PEPTIDASE IN LEISHMANIA DONOVANI

Sreenivas Gannavaram, Hira L. Nakhasi
U.S. Food and Drug Administration, Bethesda, MD, United States

Leishmaniasis is a spectrum of diseases caused by protozoan parasites belonging to several different Leishmania species. There are no effective
vaccines against leishmaniases. Currently available therapeutic regimens are often limited in effectiveness due to unwarranted side effects and rapidly emerging drug resistance. Therefore, the quest for a novel vaccine and therapeutic targets acquires urgency towards controlling leishmaniases. Ubiquitin and ubiquitin-like protein modifiers (Ubls) regulate a variety of biological functions ranging from endocytosis, membrane trafficking, protein kinase activation, DNA repair and chromatin dynamics. Studies of Ub functions in human parasitic organisms are limited. Recently, we described the existence of a novel Ub named ubiquitin-fold modifier 1 (Ufm1) that conjugates to parasite proteins in Leishmania donovani. To elucidate the enzymatic activities associated with Ufm1 conjugation, we generated a putative Ufsp in the trypanosomatid genomes. Biochemical analysis of L. donovani Ufsp showed that this protein possesses the 3’hydrolyase activity necessary for processing the precursor Ufm1 into a conjugatable form. To examine the effects of abolition of Ufm1 processing activity, we generated a L. donovani knockout mutant lacking the Ufsp. Analysis of the Ufsp mutant revealed that lack of this protein results in the absence of processing of precursor Ufm1. We also showed that Ufsp null mutant results in reduced survival of L. donovani in infected human macrophages suggesting a role for this protein in Leishmania pathogenesis. This growth defect was reversed by re-expression of wild type but not the mutant of the catalytic cysteine (cys-ser) in the Ufsp indicating the essential nature of this protease for Ufm1 conjugation reactions. Therefore, Leishmania Ufsp has the potential to be a novel drug target. Further, Ufsp/- parasites also provide an opportunity to explore such parasites as live attenuated vaccine candidates.

MOLECULAR AND FUNCTIONAL STUDIES OF THE SCHISTOSOMA MANSONI VENUS KINASE RECEPTORS SMVKR1 AND SMVKR2: POTENTIAL ROLES IN LARVAL DEVELOPMENT AND OOGENESIS

Mathieu Vanderstraete1, Nadege Gouignard1, Edith Browaeys2, Silke Leutner3, Manon Morel1, Svenja Beckmann3, Christoph G. Grevelding1, Katia Cailliau1, Colette Dissous1

1Center of Infection and Immunity of Lille, Lille, France, 2EA 4479, IFR 147, Universite Lille 1 Sciences et Technologies, Villeneuve d’Ascq Cedex, France, 3Institute for Parasitology, Justus-Liebig-University Giessen, Giessen, Germany

Venus kinase receptors (VKRs) form a new family of receptor tyrosine kinases. Atypically, VKRs contain an extracellular Venus Flytrap (VFT) domain, a ligand-binding domain activated by small molecules such as aminoacids. Vkr genes are found in diverse eumetazoa genomes, from cnidarians to echinoderms and are particularly well conserved in protostomian species, as reported previously. In the platyhelminth Schistosoma mansoni, two VKRs have been previously described, SmVKR1 and SmVKR2. Quantitative RT-PCR as well as in situ hybridization indicated a large expression of both genes in larval stages and in female ovariess. RNA interference experiments performed on sporocysts and adult worms further confirmed the implication of SmVKRs in larval development and oogenesis. Using Xenopus laevis oocytes for protein expression, we demonstrated that SmVKR1 could bind and be activated by amino acids, mainly by L-Arginine, whereas SmVKR2 activation was triggered by calcium ions. In order to decipher the downstream signalling pathways of SmVKR1 and SmVKR2, we have started to identify binding partners of these receptors by the screening of an adult worm cDNA library using the yeast two-hybrid system. Our results suggest that both SmVKR1 and SmVKR2 participate in cytoskeleton rearrangement and in developmental mechanisms. Potential substrate/adapters for SmVKR1 have been identified and their function in the activation pathway of the receptor is under investigation.

A PLASMODIUM VIVAX GENETIC CROSS TO INVESTIGATE MOLECULAR DETERMINANTS OF CHLOROQUINE RESPONSE

Juliana M. Sa1, Sarah R. Kaslow1, Jiangbing Mu1, Evan Kessler1, Soundararapandian Velmurugan2, Adam Richman2, Yonas F. Abebe3, Eric R. James2, B. K. Sim2, Stephen L. Hoffman2, Robert W. Gwadz1, Thomas E. Wellens1

1National Institutes of Health, Rockville, MD, United States, 2Sanaria Inc., Rockville, MD, United States

To investigate determinants of Plasmodium vivax chloroquine (CQ) resistance we have generated a genetic cross between two parasite lines with distinct CQ responses. A chimpanzee was infected with a mixture of these parental lines to produce infectious gametocytes for cross-fertilization in Anopheles mosquitoes. Recombinant sporozoites from the infected mosquitoes were purified and cryopreserved and subsequently used to re-inoculate the same chimpanzee after it was completely cured of the parental lines infection. When parasitemia was detected in the
re-inoculated chimpanzee, pools of mixed intraerythrocytic recombinant progeny were collected and inoculated into Aotus monkeys. Progeny in these pools showed responses spanning the range of the parental lines, including some parasites surviving a total CQ dose of 15 mg/kg (5 mg/kg/day x 3 days). Comparison of genetic markers in the mixed progeny before and after CQ treatment identifies regions of chromosomes that may be subject to linkage group selection and contain possible candidate genes. The P. vivax ortholog of the P. falciparum CQ resistance transporter gene (pfCRT ortholog, pvCRT-a) resides in one of these chromosome regions.

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ARTEMISININ RESISTANCE IN PLASMODIUM FALCIPARUM IS ASSOCIATED WITH AN ALTERED PATTERN OF TRANSCRIPTION

Sachal Mok1, Mallika Imwong2, Kek-Yee Lio1, Zhaoting Lin1, Wai-Hoe Ch1,1, Joan Sim1, Poravuth Yi1, Mayfong Mayay1, Kesinee Chotivanich1, Bruce Russell1, Duong Socheat1, Paul N. Newton4, Nicholas P. Day4, Nicholas J. White4, Peter R. Preiser1, François Nosten1, Arjen M. Dondorp5, Zbynek Bozdech1

1School of Biological Sciences, Nanyang Technological University, Singapore, Singapore, 2Department of Molecular Tropical Medicine and Genetics, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand, 3The National Center for Parasitology, Entomology, and Malaria Control, Phnom Penh, Cambodia, 4Wellcome Trust-Mahosot Hospital-Oxford University Tropical Medicine Research Collaboration, Mahosot Hospital, Vientiane, Lao People’s Democratic Republic, 5Singapore Immunology Network, Agency for Science Technology and Research, Singapore, Singapore, 6Mahidol-Oxford Research Unit, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand, 7Shoklo Malaria Research Unit, Mae Sot, Thailand

The emergence of artemisinin resistance in Western Cambodia and spread of resistance evidenced by the recent report of resistance in neighboring Thai-Myanmar border are major obstacles to the global containment and elimination of malaria disease. Although several genome-wide association studies on artemisinin resistance have been carried out proposing candidate genes, yet no definite molecular markers of artemisinin resistance have been commonly identified by the various research groups and none validated so far. Using DNA microarrays, we characterized the transcriptional profile of the ex-vivo intra-erythrocytic stage of total 36 parasite isolates from patients collected from 2008 to 2010 from Laos, Pailin, Western Cambodia and Thai-Myanmar border, of which 15 are resistant to artemisinin as reflected by increased parasite clearance half-lives. Features of the profile associated with artemisinin resistance include reduced expression of metabolic and cellular pathways such as glycolysis, pentose phosphate pathway, protein synthesis, DNA replication and redox metabolism in early stages. In contrast, protein synthesis related functions including cytoplasmic translation, transcription and chaperone-assisted protein folding genes have increased expression in the schizont stage. Hence, artemisinin resistance may be associated with lower metabolic activity of the ring stage that leads to decreased drug activation and simultaneously, increased protein synthesis, folding and turnover, that compensate the loss of proteins damage caused by the drug. In addition, we observed the differential expression of several key regulatory proteins that may underlie the observed transcription profile. The transcriptional profiles of a further 73 samples including 53 Pailin, 18 Lao and 2 Thai isolates from the ongoing TRAC (Tracking Resistance to Artemisinin Collaboration) study has been generated and analyzed and results will be discussed. In order to identify CNVs associated with resistance, we performed comparative genomic hybridizations using genomic DNA sequentially isolated from the same clinical samples and found several genes with copy number variations (CNV) associated with increased clearance half life. The involvement of these CNVs in resistance as well as their relation to the differential transcriptional profile associated with resistance phenotype will be reviewed and discussed.

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ADAPTIVE EVOLUTION OF A RING UBQUITIN LIGASE MEDIATES REDUCED DRUG SENSITIVITY IN PLASMODIUM FALCIPARUM

Ulf Ribacke1, Mackenzie Bartlett3, Saurabh D. Patel1, Niroshini Senaratne1, Daniel L. Park6, Manoj T. Duraisingh1, Pardis C. Sabeti2, Sarah K. Volkman1, Dyann F. Wirth1

1Harvard School of Public Health, Boston, MA, United States, 2Harvard University, Cambridge, MA, United States

The main obstacle to the eradication efforts of Plasmodium falciparum is the parasite’s genome plasticity enabling adaptation to selective pressure exerted by its human host. This has led to the lack of vaccines inducing sterile immunity and a growing dilemma of resistance to existing antimalarials. Recent population genetics approaches have revealed several regions of the genome to be under positive selection, thereby providing candidate loci needed to be scrutinized for their role in parasite biology. A surprisingly large number of the encoded molecules are putatively involved in ubiquitylation arguing that post-translational modification through ubiquitylation is a major pathway for parasite adaptation. We have here characterized a RING ubiquitin ligase (PFF1325c) with one non-synonymous SNP (D113N) under recent positive selection. Recombinant wild type and mutant protein was expressed and were both shown to mediate formation of ubiquitin chains in reactions with human conjugating enzymes UbcH5a-c. This proves PFF1325c to be a true ubiquitin ligase and gives important clues to which conjugating enzymes are likely interactors within the parasite. To directly assess the influence of the D113N mutation on parasite biology, we introduced the two different allelic variants into different parasite genomes. No difference in growth was detected for the allelic variants under normal in vitro growth conditions. However, a clear shift in IC50 to chloroquine (CQ) and amodiaquine (ADQ) was observed, with parasites carrying the mutant allele being less sensitive. To capture the evolutionary benefits of the mutant allele under drug pressure, parasite clones were matched in competition experiments with or without CQ and ADQ. Parasites carrying the mutant allele clearly outcompeted their wild type counterparts at sub-lethal drug concentrations and recrudesced faster after exposure to lethal concentrations of drug. Our data suggest modification of the ubiquitylation cascade to be an important adaptive response and a novel contributor to drug resistance in P. falciparum.

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EX VIVO ANTIMALARIAL DRUG SUSCEPTIBILITY OF PLASMODIUM FALCIPARUM IN WESTERN, NORTHERN AND EASTERN CAMBODIA, 2011

Pharath Lim1, Dalin Dek1, Vorleak Try2, Sokunthea Streng1, Seila Suon1, Sivanna Mao3, Chantha Sopha4, Baramey Sam3, Elizabeth A. Ashley6, Arjen M. Dondorp5, Nicholas J. White6, Jennifer M. Anderson1, Chanaki Amarutunga1, Rick M. Fairhurst1

1Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States, 2National Center for Parasitology, Entomology and Entomology (CNM), Phnom Penh, Cambodia, 3Sampov Meas Referral Hospital, Pursat, Cambodia, 4Makara 16 Referral Hospital, Preah Vihear Cambodia, 5Ratanakiri Referral Hospital, Ratanakiri, Cambodia, 6Mahidol-Oxford Research Unit, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

Artesunate (ART) plus mefloquine (MQ) was introduced as first-line treatment for Plasmodium falciparum malaria in Cambodia in 2001. In 2009, P. falciparum resistance to ART+MQ was reported in Western Cambodia, prompting the National Malaria Control Program to recommend dihydroartemisinin (DHA) + piperaquine (PPQ) for this region. In recent years, however, there have been few reports on the ex vivo susceptibility of P. falciparum to these and other antimalarial drugs in W. Cambodia or elsewhere in the country. To establish profiles of ex...
vivo antimalarial drug susceptibility in W. Cambodia, and compare them with those in Northern and Eastern Cambodia, we obtained *P. falciparum* isolates directly from patients with uncomplicated malaria. Using a SyBR-Green I-based method, we measured the *ex vivo* susceptibility of 252 parasite isolates to 6 antimalarial drugs: chloroquine (CQ), MQ, quinine (QN), PPQ, ART and DHA. Data from 80% (203/252) of these assays were interpretable for ≥ 4 drugs. The proportions of parasite isolates showing reduced *ex vivo* susceptibility to CQ, MQ, QN and PPQ in W. Cambodia were 98%, 22%, 4% and 10%, respectively. The same proportions in N. Cambodia were 97%, 20%, 7% and 7% and in E. Cambodia were 84%, 5%, 0% and 10%. Reduced *ex vivo* susceptibility to ART and DHA was not observed in the 3 regions. The *ex vivo* mean IC$_{50}$ (GM IC$_{50}$) values for CQ, MQ, QN, ART and DHA were significantly higher in W. and N. Cambodia than in E. Cambodia (p<0.001). However, there were no significant differences in the *ex vivo* GM IC$_{50}$ values for PPQ between these regions. We detected significant positive correlations between MQ and ART (r=0.54, p<0.001), MQ and DHA (r=0.32, p<0.001), QN and ART (r=0.62, p<0.001) and QN and DHA (r=0.42, p<0.001). Our data indicate that reduced *P. falciparum* susceptibility to MQ and PPQ is present in all 3 regions of Cambodia. In different regions of Cambodia, where either DHA-PPQ or ART+MQ are the recommended treatments, studies to monitor the clinical efficacy of these drugs is warranted.

**DEVELOPMENT OF ARTESUNATE RESISTANCE IN VIVO USING A *PLASMODIUM FALCIPARUM* HUMANIZED MURINE MODEL OF MALARIA**

Maria B. Jiménez-Díaz1, Sara Viera1, Javier Ibáñez1, Noemí Magán-Martach1, Helena Garuti1, Lorena Cortés-Gil1, Vanessa Gómez1, Teresa Mulet1, Francisco-Javier Gamo1, Laura M. Sanz1, Didier Leroy1, Leonard D. Shultz2, David M. Wilson1, Iñigo Angulo-Barturen1

1GlaxoSmithKline I+D, SL, Tres Cantos (Madrid), Spain, 2Medicines for Malaria Venture, Geneva, Switzerland, 3The Jackson Laboratory, Bar Harbor, ME, United States

Development of resistance against artemisinin-combination therapies (ACTs) is a major threat for the control and eradication of malaria. Humanized murine models of malaria allow the growth of *Plasmodium falciparum* in human erythrocytes engrafted into mice. In this work we show that the *P. falciparum* murine model can be used to analyze the development of resistance against antimalarials. Treatment failure with artesunate was observed after suboptimal therapy in thirteen serial passages whereas atovaquone required two, which is compatible with their corresponding propensity to generate resistance. The artesunate resistant strain showed a marked decrease in the parasite reduction ratio (P RR) whereas the atovaquone resistant strain showed almost complete resistance to treatment *in vivo*. None of the resistant strains showed measurable impairment of proliferative capacity *in vivo*. Interestingly, in contrast with atovaquone, the reduction of susceptibility to treatment with artesunate was not associated with reduced susceptibility *in vitro*. Therefore, these results suggest that the *P. falciparum* humanized model can be a valid model to study the development of resistance caused by sub-therapeutic treatment.
WHAT DETERMINES PARASITE CLEARANCE: A POOLED ANALYSIS OF FREQUENT PARASITE COUNTS AFTER TREATMENT WITH AN ARTEMISININ DERIVATIVE ALONE OR IN COMBINATION WITH OTHER ANTIMALARIALS

Kasia Stepniewska1, Jennifer A. Flegg1, François Nosten2, Rick M. Fairhurst3, Arjen M. Dondorp4, Duong Socheat5, Anders Björkman6, Andreas Märtensson7, Steffen Borrmann7, Mayfong Mayxay8, Paul N. Newton8, Delia Bethel9, Youyi Se9, Harald Noedl10, Abdoulaye A. Djimde11, Nicholas J. White12, Philippe J. Guerin1

1WWARN, Oxford, United Kingdom, 2Shoklo Malaria Research Unit, Maeot, Thailand, 3National Institute of Allergy and Infectious Diseases, Bethesda, MD, United States, 4Mahidol-Oxford Tropical Medicine Research Unit, Bangkok, Thailand, 5Center for Parasitology, Entomology and Malaria Control, Phnom Penh, Cambodia, 6Karolinska Institutet, Stockholm, Sweden, 7Kenya Medical Research Institute/Wellcome Trust Research Programme, Kilifi, Kenya, 8Wellcome Trust-Mahosot Hospital-Oxford Tropical Research Collaboration, Vientiane, Lao People’s Democratic Republic, 9Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand, 10Medical University of Vienna, Vienna, Austria, 11University of Bamako, Bamako, Mali

Parasite clearance (PC) is considered to be the most robust measure of antimalarial effect and is a key component to characterize artemisinin resistance. The dynamics of PC following artemisinin treatment are influenced by several factors other than parasite susceptibility including host immunity, initial parasite biomass, and partner drug efficacy. It is critically important to control for such potential confounding factors to identify changes over time in PC due to reduced parasite drug susceptibility. We have pooled parasitaemia data collected at least every 12 hours from individual patients who participated in completed studies. The WWARN Parasite Clearance Estimator was used to produce standardized estimates of parasite half-life (HL). The effects of covariates such as artesunate dose, partner drug, transmission intensity, year and location of study, age and baseline characteristics on PC were examined in a regression model and the relationship between treatment outcome and HL was evaluated in a subset of patients with efficacy data available using Cox regression. Random effects or frailty were used to account for study effect. Fourteen studies with 4655 patients in Cambodia, Thailand, Laos, Bangladesh, Mali, Tanzania and Kenya were included in the analysis. Clinical outcome was evaluated in 8 studies with 702 patients during 42 (4 studies) or 63 days (4 studies) follow-up period. The median (range) of estimated HLs was 3.2h (0.6 - 21.4). Estimates varied significantly between study location and year (p<0.001), with median HLs ranging 1.9-6.9 h. Among 696 patients with available efficacy outcomes, twenty four had PCR-confirmed recrudescence and slower PC (p<0.001) with a median (range) HL of 6.8h (2.5-11.1) compared to 3.3h (0.9-12.2) in cured patients. HL was not affected by initial parasite count or patient age but was longer in patients with gametocytes, low haematocrit or prolonged fever at enrollment. This analysis provides key reference patient age but was longer in patients with gametocytes, low haematocrit and baseline characteristics on PC were examined in a regression model and the relationship between treatment outcome and HL was evaluated in a subset of patients with efficacy data available using Cox regression. Random effects or frailty were used to account for such potential confounding factors to identify changes over time in PC due to reduced parasite drug susceptibility. We have pooled parasitaemia data collected at least every 12 hours from individual patients who participated in completed studies. The WWARN Parasite Clearance Estimator was used to produce standardized estimates of parasite half-life (HL). The effects of covariates such as artesunate dose, partner drug, transmission intensity, year and location of study, age and baseline characteristics on PC were examined in a regression model and the relationship between treatment outcome and HL was evaluated in a subset of patients with efficacy data available using Cox regression. Random effects or frailty were used to account for study effect. Fourteen studies with 4655 patients in Cambodia, Thailand, Laos, Bangladesh, Mali, Tanzania and Kenya were included in the analysis. Clinical outcome was evaluated in 8 studies with 702 patients during 42 (4 studies) or 63 days (4 studies) follow-up period. The median (range) of estimated HLs was 3.2h (0.6 - 21.4). Estimates varied significantly between study location and year (p<0.001), with median HLs ranging 1.9-6.9 h. Among 696 patients with available efficacy outcomes, twenty four had PCR-confirmed recrudescence and slower PC (p<0.001) with a median (range) HL of 6.8h (2.5-11.1) compared to 3.3h (0.9-12.2) in cured patients. HL was not affected by initial parasite count or patient age but was longer in patients with gametocytes, low haematocrit or prolonged fever at enrollment. This analysis provides key reference baseline data to characterize antimalarial resistance and understand factors affecting measurement of PC.

DEFINING THE ELUSIVE ARTEMISININ RESISTANCE PHENOTYPE IN VITRO

Amanda Hott, Lindsay Morton, Kansas Sparks, Debora Casandra, Matthew Tucker, Dennis E. Kyle

University of South Florida, Tampa, Fl, United States

Artemisinin resistance has emerged in Cambodia and Thailand and is observed clinically as a reduced parasite clearance rate in vivo following treatment with an artemisinin derivative alone or in combination. Recent evidence suggests the in vivo phenotype is linked to heritable genetic trait(s), yet to date a clear artemisinin resistance phenotype in vitro has not been defined. This is in direct contrast to experience with other antimalarial drugs where in vivo drug resistance was clearly evident in cultured parasites either before or simultaneously with the advent of clinical resistance. Through a series of studies we have begun to define the elusive artemisinin resistance phenotype in vitro. First we generated stable artemisinin resistant lines of Plasmodium falciparum, cloned them, and used these clones to assess new in vitro phenotype assays. Secondly we applied the new assays to culture adapted isolates of P. falciparum from Cambodia and Thailand. Isolates with evidence of artemisinin resistance in vitro were immediately cloned and characterized. Results from these studies suggest that both the in vitro generated resistant lines and clones of Cambodian P. falciparum express stable resistance to artemisinin derivatives in vitro. Interestingly, the highest level of resistance in all resistant lines was to artelinic acid (AL), a compound that has never been used clinically. We found 4-8 fold resistance to AL in each of the resistant lines as compared to 3-5 fold resistance to artemisinin. A reduced level of resistance (2-3 fold) was consistently observed for dihydroartemisinin. In addition, each of the artemisinin resistant lines expressed the artemisinin-induced ring stage dormancy phenotype in which the resistant line recovered more rapidly from dormancy than artemisinin susceptible parasites. These new artemisinin resistance phenotypes can be used to monitor emerging resistance in the field and to accelerate the discovery of drug resistance mechanism(s) in stable, culturable, clonal lines.

ASSOCIATION BETWEEN ANTIBODIES TO PLASMODIUM FALCIPARUM AT DELIVERY AND IMPROVED PREGNANCY OUTCOMES AMONG WOMEN EXPOSED TO MALARIA

Alfredo Mayor1, Urwashi Kumar2, Azucena Bardají1, Pankaj Gupta2, Alfons Jiménez1, Amel Hamad3, Betuel Sgágue4, Bijender Singh5, Llorec Quintó6, Sanjeev Kumar7, Puneet Gupta2, Virander S. Chauhan2, Carlota Daboño2, Pedro L. Alonso1, Clara Menéndez1, Chetan E. Chitnis2

1Barcelona Center for International Health Research (CRESIB), Hospital Clinric-Universitat de Barcelona, Barcelona, Spain, 2International Centre for Genetic Engineering and Biotechnology, New Delhi, India, 3Centro de Investigación en Saúde da Manhiça (CISM), Manhiça, Mozambique

Antibodies against VAR2CSA, the variant surface antigen binding to placental chondroitin sulfate A, have been suggested to mediate protection against Plasmodium falciparum in pregnancy, although some studies have indicated that these antibodies at delivery are markers of exposure to P. falciparum. We hypothesized that variations in parasite exposure and HIV infection affect levels of antimalarial antibodies and also their associations with pregnancy outcomes. We measured IgGs against placental and pediatric isolates, VAR2CSA (DBL2X, DBL3X, DBL5e, and other blood stage antigens (DBLε, DBLβ, DBLδ) and other blood stage antigens (DBLγ, DBLβ, MSP1, AMA1, EBA175) in plasmas from 293 Mozambican pregnant women at delivery. The number of antigens recognized by IgG in plasma (breadth of recognition) was higher in women with placental infection (adjusted rate ratio [aRR]=1.59, 95%CI[1.44-1.77]), in women living close to the river (aRR=1.16, 95%CI[1.02-1.32]), in HIV-infected women pregnant during the rainy season (aRR=1.82, 95%CI[1.15-2.86]) and in HIV-infected women not receiving intermittent preventive treatment (IPTp; aRR=1.39; 95%CI[1.1-1.72]). HIV-infection attenuated the parity-dependent increase of IgGs against placental and pediatric isolates, DBLγ and AMA1 (p for interaction between HIV and parity=0.046). Among women who had a malaria episode during pregnancy, high antibody level against VAR2CSA (DBL2X and DBL5e), placental and paediatric isolates and AMA1 were associated with increased weight and gestational age of the newborns (p≤0.036). Anti-parasite IgGs in women at delivery are sensitive to factors influencing malaria exposure and are affected by HIV infection, probably through its impact on the longevity of antibody responses. Reducing the variability of parasite exposure by including in the analysis only women with proven exposure during pregnancy allows the identification of IgGs against merozoite antigens, VAR2CSA and other variant surface antigens that may contribute to reduce the adverse effects of malaria in pregnancy.
CD44 IS A FUNCTIONALLY RELEVANT RECEPTOR FOR ADHERENT PLASMODIUM FALCIPARUM IN THE PLACENTA

Simon O. Owino, Briana Flaherty, Demba Sarr, Samantha Burton, David S. Peterson, Julie M. Moore
University of Georgia, Athens, GA, United States

Plasmodium falciparum infected red blood cells (iRBCs) accumulate in the maternal blood space of the placenta during malaria infection, culminating in pathological consequences deleterious to pregnancy success. The fetal cell in contact with maternal placental blood is a syncytialized epithelium called syncytiotrophoblast (ST). ST has a rich supply of low sulfated chondroitin sulfate A (CSA), a principle ligand for VAR2CSA parasite protein, present on the surface of placenta-adherent iRBCs. It is critical to examine the role CSA-baring proteoglycans on ST play in anchoring iRBCs as well as their potential role as signaling molecules. Because it is known that STs are immunologically active in the presence of CSA-adherent iRBCs, here we examined the role of CD44 proteoglycan, a known CSA-bearing molecule with a transmembrane cytoplasmic domains adept at signaling functions. STs membrane proteins (SMPs) were extracted from cultured primary cells as well as whole placental preparations. SMPs were incubated with CSA-adherent and non-adherent iRBCs; binding of CD44 was specific to CSA-adherent iRBCs as observed by flow cytometry. CD44 from SMPs pre-treated with chondroitinase ABC lost significant iRBC binding activity. In vitro exposure of primary ST to CSA-adherent iRBCs promoted upregulated expression of CD44 as detected by ELISA, and immunohistochemical staining for CD44 antigen in placental tissue from Kenyan women showed a significant increase in expression of this adherence receptor coincident with active placental malaria. Current efforts are exploring the activation state of CD44 following exposure of ST to CSA-adherent iRBCs, as well as the impact of CD44 knockdown by RNA interference on malarial activation of ST. In summary, this work provides evidence that CD44 proteoglycan is an in vivo receptor for VAR2CSA-expressing iRBCs, the expression of which is modulated by malarial infection, and may additionally serve as a signaling molecule, promoting an ST response to placental malaria.

CHRONIC INFECTIOUS EXPOSURE DURING PREGNANCY AFFECTS NEONATAL B CELL SUBPOPULATIONS

Kee Thai Yeo1, Paula Embury2, Tim Anderson2, Peter Mungai2, Penny Holding2, Christopher King2, Arlene Dent2
1Rainbow Babies and Children’s Hospital, Cleveland, OH, United States, 2Center for Global Health and Diseases, Case Western Reserve University, Cleveland, OH, United States

Chronic infections during pregnancy can expose the fetus to antigens that affect fetal B cell development. We hypothesize that resultant changes in B cell subpopulations may affect the infant’s susceptibility to infection and disease. To investigate this, we examined cord blood B cell subpopulations and B cell responses to non-specific polyclonal activation in neonates with and without exposure to chronic prenatal infections. We developed six-color flow cytometry panels to differentiate subpopulations of B cells from cord blood mononuclear cells (CBMC) isolated from North American and Kenyan neonates. North American neonates had no prenatal chronic infectious exposures. Kenyan neonates examined had evidence of prenatal HIV, cytomegalovirus (CMV), P. falciparum malaria or no infectious exposures. Proportions of B cell subpopulations were compared between the exposure groups. Additionally, we examined the ability of B cells in each group to respond to polyclonal activation in culture. We found that neonates exposed to chronic prenatal infections (HIV, CMV and malaria) displayed higher levels of atypical (CD19+CD27−CD21−IgD−) and activated (CD19+CD27+CD21+IgD−) memory B cells compared to Kenyan non-exposed and North American neonates. Little differences were appreciated in naive B cell (CD19+CD21+CD27−CD10−) or classic isotype switched memory B cell (CD19+CD27+CD21+IgD+) populations. Neonates exposed to HIV had a lower proportion of CD5+ B cell compared to all other groups. Polyclonal activation of B cells resulted in subtle shifts in CD5 and TLR2 expression, which were similar among the exposure groups. The results of our study suggest that the presence of chronic infections during pregnancy affects B cell development, leading to increased levels of atypical and activated memory B cells. The functional effects of these differences will need to be further investigated.

THE SUPPRESSION OF MALARIA ANTGEN-SPECIFIC RESPONSES BY REGULATORY T CELLS ACQUIRED IN UTERO PERSISTS INTO EARLY CHILDHOOD

Christopher L. King1, Ruth Nyakundi2, Elton Mzungu3, Peter Mungai1, Indu Malhotra1
1Case Western Reserve University, Cleveland, OH, United States, 2Institute for Primate Research, Nairobi, Kenya, 3Division of Vector Borne Diseases, Mambweti, Kenya

Prenatal exposure to malaria blood stage antigens has been associated with impaired malaria-Ag-specific Th1-type immune responses in early childhood as well as increased risk of malaria infection. Here we examined the hypotheses that thologous fetal natural (CD25hi, FoxP3+CD4+) and adaptive (high IL-10) regulatory T cells develop in utero, and that these cells persist into early childhood, impairing T cell-assisted production of protective antibody to malaria blood stage antigens. We show that depletion of CD25hiCD4+ T cells or neutralization of IL-10 in cord blood is associated with 2.3 to >10 fold increased IFNγ production by and/or proliferation of malaria blood stage-specific lymphocytes in samples from newborns of a subgroup of malaria infected women; otherwise these newborns (classified as putatively tolerant, N=10) show weak or absent malaria Ag-driven proliferation or IFNγ production. By contrast, offspring of women uninfected with malaria (not exposed, N=24) or offspring who develop a malaria Ag-driven predominantly Th1-type response in the face of maternal prenatal malaria infection (exposed-sensitized, N=13) fail to show consistent augmentation of lymphocyte proliferation and/or IFNγ production with CD25hiCD4+ depletion and/or IL-10 neutralization. Repeat examination of these same children at 12 to 24 months of age shows persistence of these phenotypes, with putatively tolerant offspring showing an overall increased lymphocyte proliferation with CD25hiCD4+ depletion and enhanced IFNγ production with IL-10 neutralization compared to children identified as exposed-sensitized or not exposed (p=0.006, 0.01 and p=0.02, 0.009 respectively). Thus, in utero exposure to malaria blood-stage antigens can induce a form of immune tolerance that is probably regulatory T cell-mediated and likely modulates malaria antigen-specific immune responses throughout early childhood.

INTERACTIONS BETWEEN THE GENETIC DIVERSITY OF PLASMODIUM FALCIPARUM INFECTIONS AND BREADTH OF ANTIBODY RESPONSES IN RELATION TO IMMUNITY TO MALARIA

Josea K. Rono1, Faith H. Osier1, Leah Faraja2, Ingegard Rooth3, Anna Färnert1
1Kenya Medical Research Institute-Wellcome Trust Research Programme, Kilifi, Kenya, 2Nyamisati Malaria Research, Dar es Salaam, United Republic of Tanzania, 3Infectious Diseases Unit, Department of Medicine, Solna, Karolinska Institutet, Stockholm, Sweden

In areas of high malaria transmission, the presence of asymptomatic infections with genetically diverse Plasmodium falciparum clones is associated with reduced risk of malaria by a yet unknown mechanism. Asymptomatic P. falciparum parasitemia can modify the association between antibodies to both merozoite and variant red blood cell surface antigens and the risk of malaria. Antibody responses to merozoite antigens are short-lived in the absence of continued infection. Considering these
observations and the step-wise reduction in risk of malaria with increasing breadth of antibody responses to merozoite antigens, we hypothesize that the presence of genetically diverse P. falciparum infections interacts with antibody responses to enhance the acquisition of immunity to malaria. To test this hypothesis, we have studied a longitudinally followed population in an area of high malaria transmission in Tanzania. A cross-sectional survey was conducted in March and April of 1999, just before the rainy season in which whole blood was collected. All the participants were monitored in the subsequent 40 weeks and episodes of malaria were recorded by a passive case detection system. We assessed the genetic diversity of P. falciparum infections at baseline by genotyping the P. falciparum merozoite surface protein 2 (msp2) gene by fluorescent PCR and capillary electrophoresis. We measured antibody levels to four of the leading malaria vaccine candidate antigens; 2 alleles of MSP-2, two alleles of MSP-3, two alleles of apical merozoite antigen 1, and the 19-kilodalton fragment of MSP-1 using a multiplex platform. Increasing breadth of antibody responses and presence of increasing number of genetically distinct clones at baseline were associated with reduced risk of malaria both individually and when analyzed in combination. These findings suggest that in an area of high malaria transmission, genetic diversity and antibody responses are additive or synergistic in conferring protection from malaria.

USE OF TETRAMER STAINING TO ENUMERATE AND CHARACTERIZE MALARIA ANTIGEN-SPECIFIC CD8+ T-CELLS INDUCED IN VOLUNTEERS IMMUNIZED WITH ADENOVIRUS SEROTYPE 5 PLASMODIUM FALCIPARUM MALARIA VACCINES

Glenna Banania1, Soren Buus2, Robert J. Schwenk1, Yohan Kim1, Bjorn Peters1, Maria Belmonte1, Hanni Ganeshan1, Jun Huang1, Cindy Tamminga1, Alessandro Sette3, Carter Diggs3, Lorraine Soisson3, Michael R. Hollingdale1, Thomas L. Richie1, Martha Sedegah1

1U.S. Military Malaria Vaccine Program, Naval Medical Research Center, Silver Spring, MD, United States, 2Laboratory of Experimental Immunology, University of Copenhagen, Copenhagen, Denmark, 3U.S. Military Malaria Vaccine Program, Walter Reed Army Institute of Research, Silver Spring, MD, United States, 4Division of Vaccine Discovery, The La Jolla Institute for Allergy and Immunology, La Jolla, CA, United States, 5United States Agency for International Development, Washington, DC, United States

Protection against pre-erythrocytic stages of malaria is believed to be mediated in part by cytotoxic T-lymphocyte (CTL) responses against epitopes of protective antigens presented by HLA on the surface of infected liver cells. Because IFN-γ secreting CD8+ T cells have been implicated in protection against pre-erythrocytic stage malaria through IFN-γ-mediated induction of nitric oxide to kill intra-hepatic stage malaria parasites, it has become common practice to attempt to associate the magnitude of malaria vaccine-induced and CTL-mediated IFN-γ production with protective immunity, since antibody responses are usually poor. However, malaria antigen-specific CD8+ T-cells might kill infected hepatocytes by non-IFN-γ mediated mechanisms, such as the direct killing of hepatocytes through release of perforin and granzyme. In order to test this hypothesis, we have studied a longitudinally followed population in an area of high malaria transmission in Tanzania. A cross-sectional survey was conducted in March and April of 1999, just before the rainy season in which whole blood was collected. All the participants were monitored in the subsequent 40 weeks and episodes of malaria were recorded by a passive case detection system. We assessed the genetic diversity of P. falciparum infections at baseline by genotyping the P. falciparum merozoite surface protein 2 (msp2) gene by fluorescent PCR and capillary electrophoresis. We measured antibody levels to four of the leading malaria vaccine candidate antigens; 2 alleles of MSP-2, two alleles of MSP-3, two alleles of apical merozoite antigen 1, and the 19-kilodalton fragment of MSP-1 using a multiplex platform. Increasing breadth of antibody responses and presence of increasing number of genetically distinct clones at baseline were associated with reduced risk of malaria both individually and when analyzed in combination. These findings suggest that in an area of high malaria transmission, genetic diversity and antibody responses are additive or synergistic in conferring protection from malaria.

GENERATION OF NOVEL “HUMAN-IMMUNE-SYSTEM” HUMANIZED MOUSE STRAINS CO-EXPRESSING HLA CLASS I AND CLASS II MOLECULES IN NOD.RAGKO.II2RGCKO BACKGROUND

Sai Majji1, Rebecca Danner1, Eileen Villasante1, Thomas L. Richie1, Teodor D. Brummeau2, Sofia A. Casares3

1U.S. Military Malaria Vaccine Program/Naval Medical Research Center, Silver Spring, MD, United States, 2Uniformed Services University of the Health Sciences, Bethesda, MD, United States

The current animal models that are used to test approaches that target the immune system (i.e., vaccines) are imperfect and accounts for many failures when human vaccines are tested in clinical trials. Development of humanized mouse models able to generate a surrogate human immune system is a highly pursued goal for investigating human immunology and for testing human vaccines. We previously showed that humanized mice expressing HLA class II (DR4) molecules in NOD.RagKO.II2rgcko background and infused with HLA-DR-matched human hematopoietic stem cells, develop a functional human immune system and respond to vaccination (PLoS One 6: e19826, 2011). While the frequency of human CD4 T cells, B cells, and dendritic cells in blood and lymphoid organs of humanized DRAG mice was similar to that in humans, the frequency of human CD8 T cells was however lower. This was attributed to the lack of HLA class I expression in humanized DRAG mice, since HLA class I molecules are required for thymic selection and survival of human CD8 T cells. Herein we have generated a new humanized mouse strain co-expressing HLA class I (A2) and HLA class II (DR4) molecules in NOD. RagKO.II2rgcko background, and provide evidence for human immune cell reconstitution as well as function of human CD8 T cells upon infusion of HLA-matched human hematopoietic stem cells.

QTL MAPPING OF PLASMODIUM FALCIPARUM GENES THAT ALLOW EVASION OF THE MOSQUITO IMMUNE SYSTEM

Alvaro Molina-Cruz, Amy Alabaster, Lois Bangiolo, Ashley Haile, Jared Winikor, Lindsay Garver, Corrie Ortega, Carolina Barillas-Mury

National Institutes of Health, Bethesda, MD, United States

The mosquito Anopheles gambiae L3-5 strain is capable of eliminating some lines of Plasmodium falciparum but not others. This elimination involves the mosquito immune complement-like system. A Quantitative Trait Loci (QTL) mapping was carried out to identify the P. falciparum gene(s) that allow some parasite strains to evade the A. gambiae immune system. The gene mapping was done in a P. falciparum cross between 2 lines that differ in their survival in A. gambiae L3-5; GB4 successfully infects the mosquito while 7GB is mostly eliminated by melanoctic encapsulation. Phenotyping of parental lines and progeny lines for survival/encapsulation in A. gambiae L3-5; GB4 successfully infects the mosquito while 7GB is mostly eliminated by melanoctic encapsulation. Phenotyping of parental lines and progeny lines for survival/encapsulation in A. gambiae L3-5; GB4 successfully infects the mosquito while 7GB is mostly eliminated by melanoctic encapsulation. Phenotyping of parental lines and progeny lines for survival/encapsulation in A. gambiae L3-5; GB4 successfully infects the mosquito while 7GB is mostly eliminated by melanoctic encapsulation. Phenotyping of parental lines and progeny lines for survival/encapsulation in A. gambiae L3-5; GB4 successfully infects the mosquito while 7GB is mostly eliminated by melanoctic encapsulation. Phenotyping of parental lines and progeny lines for survival/encapsulation in A. gambiae L3-5; GB4 successfully infects the mosquito while 7GB is mostly eliminated by melanoctic encapsulation. Phenotyping of parental lines and progeny lines for survival/encapsulation in A. gambiae L3-5; GB4 successfully infects the mosquito while 7GB is mostly eliminated by melanoctic encapsulation. Phenotyping of parental lines and progeny lines for survival/encapsulation in A. gambiae L3-5; GB4 successfully infects the mosquito while 7GB is mostly eliminated by melanoctic encapsulation.

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41 genes identified 15 genes with non-synonymous polymorphisms between GB4 and 7G8 lines. Based on the expression differences and sequence polymorphisms, 5 candidate genes were selected for detailed genetic analysis by testing phenotype changes between survival and encapsulation, after allele replacement in P. falciparum. Identification of P. falciparum gene(s) that allow evasion of the mosquito immune system may be important to understand malaria transmission and could be a target for transmission blocking strategies.

EVIDENCE OF RECOMBINATION IN THE X-CHROMOSOME CENTROMERIC REGION IN ANOPHELES GAMBIAE MOLECULAR FORMS FROM AN AREA OF PUTATIVE SECONDARY CONTACT

Beniamino Caputo1, David Weetman2, Emiliano Mancini1, Marco Pombi1, José L. Vicente1, Amabelia Rodrigues1, Martin J. Donnelly1, Joao Pinto1, Alessandra della Torre1

1University of Rome Sapienza, Rome, Italy, 2Liverpool School of Tropical Medicine, Liverpool, United Kingdom, 3Universidade Nova de Lisboa, Lisbon, Portugal, 4Instituto Nacional de Saúde Pública, Bissau, Guinea-Bissau

Anopheles gambiae M and S molecular forms are typically strongly reproductively isolated and clearly identifiable based on a SNP in the multi-copy IGS rDNA region, co-segregating with the single-copy insertion of a SINE element located approximately 1 Mb apart from the X-centromeric IGS region. However, an area of putative secondary contact has been recently detected at the westemmost extreme of M and S range. Preliminary indications of discordant M and S genotypes at the two X-linked markers near the centromere in female samples suggests that introgression and inter-locus recombination may be occurring in this area. This hypothesis is intriguing because recombination is known to be highly reduced in centromeric regions, and this is believed to have played a significant role in the incipient speciation process ongoing within A. gambiae. Here we present data from M and S female (N=275) and male (N=392) samples collected in Safim village in Guinea Bissau. Notably, males provide the opportunity to separate recombination, as distinct from heterozygosity, along the hemizygous X-chromosome. Results from IGS and SINE PCR-genotyping show: i) a 22% frequency of SINE MS-heterozygotes in females (consistent with previous data) and an absence of heterozygotes in males (as expected for a single-copy X-linked marker); ii) a 34% and 9% frequency of IGS MS-heterozygous pattern in females and males, respectively, strongly supporting the occurrence of recombination within even the most centromere-proximal region of the X chromosome; iii) the occurrence of discordant SINE/IGS genotypes in 12% and 18% of SINE-M and SINE-S females, respectively, and in 10% of SINE-M and SINE-S males, showing that recombination is occurring in both molecular forms. Moreover, multilocus SNP analysis carried out on a subsample of males provides estimates of recombination along the whole X-chromosome and novel original insights on M and S form status in their putative secondary contact zone.

THE GENETIC BASIS OF HUMAN HOST CHOICE IN THE MALARIA VECTOR ANOPHELES GAMBIAE

Giri Athrey1, Theresa K. Hodges1, Luciano Cosme1, Willem Takken2, Michel A. Slotman1

1Texas A&M University, College Station, TX, United States, 2Wageningen University and Research Center, Wageningen, The Netherlands

The predominant malaria vector Anopheles gambiae s.s preferentially takes it blood meals from human hosts, often at rates as high as 90% in natural populations. The adaptation of these mosquitoes to human hosts has a genetic basis in the olfactory system, which includes several key gene families - the odorant receptors (ORs), odorant binding proteins (OBPs) and ionotropic receptors (iRs). To identify An. gambiae genes responsible for human host preference, we conducted a quantitative trait loci (QTL) mapping experiment based on introgressive backcrosses between the anthropophilic An. gambiae and the zoophilic An. quadriannulatus, in which F1 females were backcrossed to An. quadriannulatus males. These backcross females were subjected to a host-choice experiment in an olfactometer in which they were presented with a human and cow odor. Only individuals that selected the same odor on three consecutive days were included in the experiment. A total of ~15,000 individual backcross females were run through host-choice experiments, resulting in two pools totaling 432 mosquitoes with divergent host preferences. We are using 24 microsatellite markers to genotype individuals from the two pools and performed QTL analysis using RQTL. Preliminary results based on 13 markers identified one highly significant QTL that explains 16% of the phenotypic variance. This QTL region is estimated to span a 10 Mb region.
and contains several ORs, OBPs and IRs. These genes are candidates for being involved in the adaptation of *An. gambiae* to its human host and were sequenced in six anopheline species to identify those that show evidence of positive selection.

### 1001

**DENGUE 2 INFECTION ALTERS MICRORNA EXPRESSION IN *Aedes aegypti***

Corey Campbell, Ann Hess, Gregory D. Ebel  
**Colorado State University, Fort Collins, CO, United States**

Emerging studies show that important avenues in the post-transcriptional regulation of gene expression occur via small RNA regulatory pathways. Non-coding RNAs (ncRNAs) are key features of these pathways, and critical molecules involved in their biogenesis and function are conserved in plants, insects and mammals. To identify products of anti-viral RNA interference in vector mosquitoes, deep sequencing small RNA (sRNA) libraries were prepared from DENV2-fed *Ae. aegypti* females (RemD strain) and matched un-infected controls at 2, 4, and 9 days post-infection (dpi). An earlier publication described DENV2-derived viral sRNAs (viRNAs) across three size classes: unusually small RNAs (usRNAs) (14-19nts), canonical siRNAs (20-24nts), and piRNAs (25-30nts) (Hess et al, BMC Microbiology, 2011). In the present analysis, these libraries were mined to determine whether substantive changes occur in mosquito microRNA (miRNA) levels during DENV2 infection. Reads were aligned to miRBase release 17 hairpin database (mirbase.org). miRNAs with over 50 reads across treatment groups and showing ≥2 fold-changes were chosen for further study. Our analysis reveals that significant changes to specific miRNA levels occur in DENV2-infected mosquitoes compared to un-infected controls. Moreover, some miRNAs showed coordinated enrichment or depletion at both 2 and 4 dpi, substantiating the hypothesis that they are important to the establishment of virus infection, whether by being exploited by the virus or as part of an anti-viral mechanism. Coordinately co-regulated miRNAs or *miRNAs (complementary to miRNA), non-canonical miRNAs were classified by type: conserved (homologous to previously reported miRNAs), *miRNA (complementary to miRNA), non-canonical or unclassified. Although the precise part played by each differentially expressed miRNA remains to be elucidated, orthologous miRNAs in other animals are important effectors of cellular differentiation, neurogenesis, transcriptional regulation, nutritional metabolism and the regulation of apoptosis. Our results show an intriguing new way in which major cellular processes of mosquitoes respond to arbovirus infection.

### 1002

**GENETIC REGULATION OF VECTOR MOSQUITO SALIVARY GLAND DEVELOPMENT**

Molly Duman Scheel1, Chilinh Nguyen2, Zeinab Annan1, Emily Andrews1, Christy Le2, Longhua Sun2, Anthony Clemons2, David W. Severson3  
1*Indiana University School of Medicine South Bend at Notre Dame, South Bend, IN, United States*, 2*University of Notre Dame, Notre Dame, IN, United States*

Understanding mosquito salivary gland development is critical given the importance of this tissue in blood feeding and pathogen transmission. Our recent survey of the mosquito genomes indicated that mosquitoes have orthologs of many genes that regulate embryonic salivary gland development in *Drosophila melanogaster*, a well-characterized insect genetic model organism. The expression patterns of a large subset of these genes were assessed during development of *Aedes aegypti*, an emerging model for vector mosquito development. These studies revealed that the early stages of *Ae. aegypti* salivary gland development significantly differ from that of *D. melanogaster*. We are now using an RNAi knockdown strategy to investigate the roles of genes expressed in the developing *Ae. aegypti* salivary gland. Functional characterization of cyclic-AMP response element binding protein A (crebA) indicates that this gene encodes a key regulator of secretory function in the *Ae. aegypti* salivary gland. These studies highlight the need for further analysis of mosquito developmental genetics and may foster comparative studies of salivary gland development in additional vector insect species.

### 1003

**MEDUSA: A NOVEL GENE DRIVE SYSTEM FOR CONFINED SUPPRESSION OF MOSQUITO POPULATIONS**

John M. Marshall1, Bruce A. Hay2  
1*Imperial College London, London, United Kingdom*, 2*California Institute of Technology, Pasadena, CA, United States*

Following successful field trials of sterile GM mosquitoes designed to control dengue fever, interest is now growing in the use of gene drive systems, such as X-shredders, capable of inducing a population crash as they spread. These systems hold much promise for wide-scale disease control; however issues arise from their potential to spread across international borders. We propose a novel gene drive system, Medusa, capable of inducing a local population crash without spreading into neighboring populations. Medusa consists of four components - two at a locus on the X chromosome and two at a locus on the Y chromosome. A maternally-expressed, X-linked toxin and a zygotically-expressed, Y-linked antidote suppress the female population because only males can protect themselves against the effects of the toxin. A zygotically-expressed, Y-linked toxin and a zygotically-expressed, X-linked antidote ensure that the two constructs are always inherited together. We use simple population genetic models to explore the dynamics of the Medusa system. An all-male release is preferred since males don’t bite and, if released over two generations, Medusa is expected to induce a population crash within seven generations for modest release sizes. Re-invasion of wild mosquitoes can lead to the population eventually rebounding; however this can be prevented by small, regular releases of Medusa males. The Medusa system could serve as a proof of principle for invasive population suppression systems such as X-shredders. We describe molecular solutions to chromosomal anomalies that could interfere with Medusa dynamics.

### 1004

**VACCINATION WITH EXCRETORY/SECRETORY PRODUCTS CONFER PARTIAL PROTECTION IN A MURINE MODEL OF FILARIASIS**

C. Paul Morris, Marina Torrero, Kristin Killoran, Edward Mitre  
**Uniformed Services University of the Health Sciences, Bethesda, MD, United States**

Infection with filarial worms can cause severe and debilitating diseases in both humans and animals. While many vaccine candidates have been studied in filariasis, our understanding of protective immune responses in a permissive model to filariasis is incomplete. In this study, we evaluated preparations of worm antigens for protection against challenge infection in the BALB/c *Litomosoides sigmodontis* model. The fractions included LS, soluble antigens of a homogenate of adult worms, and ES, excretory/secretory products of adult female worms. 6-8 week old female BALB/c mice were given 3 intraperitoneal injections of 10 micrograms LS or ES with CpGAlum and subsequently challenged with 40 infectious larvae subcutaneously. 8 weeks after infection mice were euthanized and parasite burdens were determined. Mice that were vaccinated with LS antigen showed no significant protection against challenge infection compared to control mice. Mice vaccinated with ES product, however, harbored 60% fewer adult worms than control mice. While mice vaccinated with LS and ES produced similar levels of IgG antibodies to both antigen preparations, analysis by western blot demonstrated that ES vaccinated mice recognized different ES proteins than LS-vaccinated mice. Currently, we are in the process of conducting mass spectroscopy to identify a ~160kda protein that was strongly preferentially recognized by ES-vaccinated mice. No substantially large differences were observed between the two vaccinated groups with regards to lymphocyte