Efficacy, safety and tolerability of dihydroartemisinin-piperaquine for treatment of uncomplicated Falciparum malaria in pregnancy in Ghana

a randomized, non-inferiority trail

Osarfo, Joseph; Tagbor, Harry; Magnussen, Pascal

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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 quantify 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 Grey 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 brief 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, Coquillettidida, Haemagogus, Mansonia, Psorophora, Trichopsophon); South American Phlebotomine Sand flies (include male and female keys of genera, subgenera, and vector species of Dampomyia, Evandromyia, Helcocyrtomyia, Lutzomyia, Nyssomyia, Pintomyia, 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

Karalo 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 CLIP98, 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 Culverwell3, Mary Cooke1, Samuel Kahindi4, Chrispin Owaga4, Elizabeth Ayoma4, Robin Oriango4, Ralph Harbach1, 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, 3Centre 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 Kenyan 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, mosquitos 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 mosquitos 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, mosquitos 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 GAMBIAE 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, 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 mosquitos prefer glucose and sucrose. Initial data suggests that mosquitos exhibit strong aversion to berberine, a canonical noxious compound used in insect gustatory preference assays. We will report further progress in the analysis of sugar preference and noxious compound sensing in An. gambiae, and initial RNA interference results characterizing requirements for different GPCRs in sugar and noxious compound gustation. The overall goal of this project is to understand the functional roles of the mosquito gustatory GPCRs and exploit this system to enhance development of sugar-meal based, vector-targeted interventions that will decrease vectorial capacity of An. gambiae and other malaria vectors.

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OUTBREAK CLUSTERS OF FASCIOLA HEPATICA IN ARGENTINA

Jose Debes1, Liliana Nieto Sosa2, Andres Barnes2, William Stauffer1, Leonardo Marianelli2
1University of Minnesota, Minneapolis, MN, United States, 2Hospital Rawson, Cordoba, Argentina

Fasciola hepatica is a trematode responsible for the human disease fascioliasis. It differs from other human liver flukes in that it is present worldwide. Fascioliasis is transmitted through the ingestion of the metacercaria stage parasite found in infected vegetables such as watercress. Although it generally presents as chronic indolent individual infections, outbreaks of acute fascioliasis 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 fascioliasis 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 fascioliasis in patients living and traveling to endemic areas.

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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.Prelvalence 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.
**DEVELOPMENT OF A RECOMBINANT PROTEIN VACCINE AGAINST SCHISTOSOMA MANSONI INFECTIOIN 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 Huttinger2, Shawn Coyle3, James Tidwell1, Kyle Schneider1, Oumar Talla Diaw2, Mouhamadou Mbacke Seye4, Djibril S. Faye5, Kevin Lafferty4, 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, United States Geological Survey, 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 1984, 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 Twegonyere1, Shona Wilson2, Peter Naniima3, Stephen Case4, Edridah M. Tukahebwa5, Alison M. Elliott5, 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 Schistoma mansoni infected individuals, in which we investigated the effects of co-culturing human eosinophils with peripheral blood mononuclear cells (PBMCs)
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 HAEMATOBIFORM RECOMBINANT PROTEINS AS A VACCINE CANDIDATE FOR HUMAN SCHISTOSOMIASIS**

Kwaku B. Ahmed1, Iain W. Chalmers2, Martha Turscott2, Maria Yazdanabakhsh2, Cornelis H. Hokke3, 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 endemcity. 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 hits (S Tetraspanin proteins, 5 CD59-like proteins, 2 MEG-8 proteins, 2 Saponin proteins, 1 FOG precursor, 1 Stomatin-related protein) from *S. mansoni* sequences. This bio-informatic lead analysis was performed using tBLASTn search of Wellcome Sanger *S. haematobium* ESTs libraries against 52 *S. mansoni* vaccine candidates (with e-value 3.90e-30-3.00e-110 and identity value 56%-93%). These 17 orthologs were characterised by non-allergen, had no human orthologs and was expressed during vertebrate infections (definitive host). 3 of such orthologs (1-Tetraspanin, 1-MEG and 1-CD59 like proteins) were produced as recombinant proteins and being used as lead proteins towards the discovery of *S. haematobium* vaccine 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. Prasapanich1, Anthony Luyai2, Megan L. Mickum3, Ziad S. Kawar2, Jamie Heimburg-Molinaro2, Yi Lasanajak2, Xuezheng Song2, David F. Smith2, Richard D. Cummings2

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 LactoNac (GalNAcβ1,4-GlcNAc, LDN) and fucosylated LactoNac (GalNAcβ1,4-GlcNAcβ1-(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 (LecBGFT) abundantly on its glycoproteins. Immunizing mice with these cells induced glycan-specific antibodies and a sustained booster response. The LecBGFT 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 inactivate 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 fulfill 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 ex 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.
ASSESSING THE HEALTH IMPACT OF IMPROVED RURAL SANITATION: DESIGNING AND CARRYING OUT A CLUSTER-RANDOMIZED, CONTROLLED TRIAL IN ORISSA, INDIA

Thomas Clasen1, Sophie Boisson1, Paramita Routray1, Jeroen Ensink1, Miriam Jenkins2, Matthew Freeman2, Wolf-Peter Schmidt1
1London School of Hygiene & Tropical Medicine, London, United Kingdom, 2University of California, Davis, Davis, CA, United States, 3Emory University, Atlanta, GA, United States

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 the 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 Joma 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, 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 outbursts 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 contact 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 DRC. 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.

AN UNUSUAL PARTNERSHIP TO ENSURE SAFE DRINKING WATER TO THE RURAL POPULATION IN INDIA

Camille A. Saade
FHI 360, Washington, DC, United States

Water treatment at the point-of-use (POU) can reduce diarrhea caused by waterborne pathogens by 30 to 50 % (WHO 2007). The goal of the project was to demonstrate a comprehensive strategy aiming at increasing use of POU water treatment methods among poor urban and rural populations and thereby reduce childhood diarrhea in the state of UP in India. The at-scale goal was to achieve 30 % rural and 40 % urban use of an effective POU method. By January of 2009, the partnership between POU manufacturers and NGO partners was formalized through MOUs. The project reached 674,064 households residing in 1120 urban slum areas and 1350 rural villages in UP. A quantitative study of 1400 households at baseline, showed only 2.5 % of households (4.1 % urban and 1.1 % rural) reported ever using a POU method promoted by the project (boiling, disinfection products, or filtration). In contrast, the outcome evaluation found very high rates of POU use in both the intervention and comparison areas, with 96.8 % of intervention households reporting they had ever used a recommended POU method, along with 71.0 % of households in the comparison areas. The biggest difference between intervention and comparison districts was in the use of chlorine liquid for
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 childrens’ feces and low levels of handwashing, especially after defecation and the handling of childrens’ 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 hygiene and sanitation 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, HSPP, 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 Kundu1, Abu Mohd. Naser1, Mahmudur Rahman1, Stephen P. Luby4

1International Centre for Diarrhoeal Disease Research, Bangladesh and Institute of Epidemiology, Disease Control and Research (ICDDR), Dhaka, Bangladesh, 2International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh, 3Institute of Epidemiology, Disease Control and Research (ICDDR), 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 affected 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 provision is difficult in municipalities where water supply is intermittent, 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 provision is difficult in municipalities where water supply is intermittent, 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 provision is difficult in municipalities where water supply is intermittent, 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 provision is difficult in municipalities where water supply is intermittent, 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 provision is difficult in municipalities where water supply is intermittent, 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 provision is difficult in municipalities where water supply is intermittent, 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

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 Naushin1, Mahfuzur Rahman1, Samir Ghosh1, Shahnook Akter1, 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 from the 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 community where BRAC, a non-governmental organization, implemented a project in 2007 providing dual pit latrines for households who 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 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, Pavani K. Ram3, Fazlul K. Chowdhury1, Md. A Mamun1, Smriti Roy1, Subas C. Biswas1, Rouha Anamika Sarker1, Sania Ashraf1, Thomas Clasen4, Peter J. Winch1, 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 study households at the 2nd month (n=129) and 14th month (n=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. Faldetta¹, 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 Vujcic¹, Pavani K. Ram¹, Leanne Unicombe², Faruque Hussain³, Partha Sarathi Gope³, Zahid Hayat Mahmud³, Jaynal Abedin³, Md. Sirajul Islam³, 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 ARTEСUNATE-AMODIAQUINE VS. COMPARATOR TREATMENTS FOR UNCOMPLICATED ФАLСIPARUM MALARIA IN SUB-SАHАRAN AFRICA, AN INDIVIDUAL PATIENT DATA ANALYSIS

Julien Zwang1, Grant Dorsey2, Djimédé Aboulaye3, Corine Karena4, Andreas Mårtensson5, Jean-Louis Ndiaye6, Sodiomon Sirima7, Piero L. Olliaro8

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-piperazine 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 and dihydroartemisinin-piperazine compared to other AEs (not different from other treatments). The overall incidence of serious AEs (SAEs) with ASAQ was nine per thousand (29/3,113) and a mortality of one per thousand (three 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

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

Ymkje Stienstra1, Yves Barogui2, Sander Klis3, Honoré Sourou Bankolé, Ghislain Sopoh4, S. Mamo5, Lamine Baba-Moussa6, Willem L. Manson7, Christian Johnson8, Tijp S. van der Werf9

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 Générale 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, 8University Medical Center Groningen, Department of Microbiology, Groningen, The Netherlands, 9Fondation Raoul Follereau, 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 for 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 Ndiaye3, Michael Alifrangis4, Babacar Faye5, Cheikh T. Ndour5, Jean L. Ndiaye1, Pascal Magnussen6, Ib C. Bygbjerg3, Oumar Gaye7

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 ethiology 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 Boncotto 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 $\geq 11$ 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 176 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-thalassaemia (OR=1.82, 95%CI [1.23-2.35]), stunting (OR=3.37, 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 parasitism, 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. Coulibaly\(^1\), Siaka Y. Coulibaly\(^2\), Boubacar Fofana\(^3\), Modibo Keita\(^4\), Mahamadou M. Keita\(^4\), Ilo Dicko\(^4\), Moussa B. Sangare\(^4\), Seydou Doumbia\(^4\), Adama Dao\(^4\), Oumar Maiga\(^4\), Samba O. Sow\(^4\), Thomas B. Nuttman\(^4\), Amy D. Klon\(^4\), Adama D. Keita\(^4\)

\(^1\)Centre National d’Appui a la lutte contre la Maladie (CNAM), Bamako, Mali, \(^2\)Malaria Research and Training Center, Bamako, Mali, \(^3\)Hospital du Point G, Bamako, Mali, \(^4\)National 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 testis 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.

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

Elena Grigorenko\(^1\), Carolyn Fisher\(^1\), Sunali Patel\(^1\), Clark Tibbets\(^1\), Moussa Kourou\(^1\), Anjan Purkayastha\(^2\), Hira L. Nakhasi\(^3\), Robert C. Duncan\(^2\)

\(^1\)Life Technologies, Corp., Beverly, MA, United States, \(^2\)Food and Drug Administration/CBER, Rockville, MD, United States, \(^3\)TessArae, 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\(^4\) 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\(^4\) system can perform approximately 3,000 individual real-time polymerase chain reaction tests simultaneously on a microscope slide-size wafer. The RPM utilizes an Affymetrix\(^5\) GeneChip\(^5\) based and a particular arrangement of oligonucleotides. Hybridization to these oligonucleotides leads to sequence identification. We assembled and tested a blood pathogen OpenArray\(^4\) with primer and probe sets for viruses (HIV-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.

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**CENTRE-BASED CLINICAL MANAGEMENT OF CYSTIC ECHINOCOCCOSIS**

Thomas Junghans

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.
TUBERCULOUS MENINGITIS AND RABIES ARE THE MOST COMMON CENTRAL NERVOUS SYSTEM INFECTIONS IN THE NATIONAL REFERRAL HOSPITAL FOR INFECTIOUS DISEASE, THE PHILIPPINES

Emi Kitashoji†, Nimfa M. Putong‡, Efren M. Dimanono‡, Maiko Kojiro†, Motoi Suzuki†, Benito J. Villarama‡, Koya Ariyoshi†

†Department of Clinical Medicine, Institute of Tropical Medicine, Nagasaki University, Nagasaki, Japan, ‡San Lazaro Hospital, Manila, Philippines

Central nervous system (CNS) infections are significant causes of mortality and morbidity in low-middle income countries. To improve clinical diagnosis, management and public health intervention, it is essential to clarify the comprehensive picture of CNS infections. However most published studies focused limited pathogens. The objective of this study is to describe the present picture of whole CNS infections in the Philippines. We conducted a hospital-based retrospective descriptive study in San Lazaro Hospital (SLH), the national referral hospital for infectious and tropical diseases in the Philippines. We collected demographic and clinical information of all patients who were admitted with any suspected CNS infection from 1st January 2008 to 30th September 2011. It included all patients who were diagnosed CNS infections as initial and/or final diagnosis and all hospitalized patients who required a lumbar puncture (LP) examination, except for patients in HIV ward. A total of 1,264 patients were analyzed, 937 of them showed CNS infections as final diagnosis. There were more males (62%) and nearly half of the cases (43%) was under 12 years old. Tuberculous meningitis (TBM) and Rabies were the most common CNS infections with 312(27%) cases of TBM and 217(19%) of rabies. This was followed by other bacterial meningitis 169(15%) and viral encephalitis 135(12%). Case fatality rate (CFR) for rabies was 100%; likewise, the CFR for non-rabies CNS infection was also high at 238/703(33.9%). 131(11%) of the patients who were initially diagnosed as CNS infections were confirmed not CNS infection in the final diagnosis. Febrile convulsions and seizures were the most common non-CNS infections. 131(11%) of the patients who were initially not diagnosed as CNS infections were later diagnosed as CNS infection: typhoid fever was the most common misdiagnosis upon admission. LP was performed in 277(22%) cases but its performance was often substantially delayed since many of the patients were critically ill upon admission and none of CSF was positive for bacterial culture. There is ample room for improvement of clinical diagnosis and management of CNS infections.

DENGUE AND DIARRHEAL DISEASE RISK FACTORS IN RURAL AND SUBURBAN VILLAGES IN THAILAND AND LAOS

Hans J. Overgaard1, Nsa Dada2, Nanthasane Vannavong1, Razak Seidu1, Ram Rangsit2, Theraphap Chareonviriyaphap2, Audrey Lenhart3, 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.

SAFETY AND EFFICACY OF ARTEMETHER-LUMEFANTRINE AGAINST UNCOMPROMICATED PLASMODIUM FALCIPARUM MALARIA DURING PREGNANCY: A SYSTEMATIC REVIEW

Christine Manyando1, Kassoum Kayentao2, Umberto D’Alessandro3, Henrietta U. Okafor4, Elizabeth Juma5, Kamal Hamed6

1Tropical Diseases Research Centre, Ndola, Zambia, 2Malaria Research and Training Centre, Bamako, Mali, 3Medical Research Council Unit, Fajara, Gambia, 4Department of Pediatrics, College of Medicine, University of Nigeria, Enugu, Nigeria, 5Kenya Medical Research Institute, Kisumu, Kenya, 6Novartis Pharmaceuticals Corporation, East Hanover, NJ, United States

PLASMODIUM falciparum malaria during pregnancy, linked to increased morbidity and mortality, must be reduced by preventive measures and effective case management. Although, the World Health Organization (WHO) recommends artemisinin-based combination therapy (ACT) to treat uncomplicated P. falciparum malaria during the second and third trimesters of pregnancy, and quinine plus clindamycin during the first trimester, the national policies of many African countries currently recommend quinine throughout pregnancy. Our objective is to analyze available data on the safety and efficacy of artemether-lumefantrine (AL) in pregnancy. English-language search identified 16 publications from 1989 to October 2011 with reports of artemether or AL exposure in pregnancy, including randomized clinical trials, observational studies, and systematic reviews. There were 1,103 reports of AL use in pregnant women: 890 second/third trimester exposures; 212 first trimester exposures; and 1 case where the trimester of exposure was not reported. In the second and third trimesters, AL was not associated with increased adverse pregnancy outcomes compared with quinine or sulphadoxine-pyrimethamine, showed improved tolerability relative to quinine, and its efficacy was non-inferior to quinine. Few reports suggest that the pharmacokinetics of anti-malarial drugs may change in pregnancy, however, the majority of studies reported high cure rates and adequate tolerability. Additional data are required to assess the potential to use AL in the first trimester. These findings reinforce the WHO recommendation to treat uncomplicated P. falciparum malaria with quinine plus clindamycin in early pregnancy and ACT in later pregnancy.

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MICROBIAL ETIOLOGY OF TRAVELERS’ DIARRHEA:
EXPERIENCE OF A TRAVEL CLINIC IN TOKYO

Yasuyuki Kato1, Hidemasa Izumiya2, Taichiro Kobayashi1, Mugen Ujije1, Nozomi Takeshita1, Shuzo Kanagawa1
1National Center for Global Health and Medicine, Tokyo, Japan, 2National Institute of Infectious Diseases, Tokyo, Japan

Travelers’ diarrhea (TD) is the most common illness in international travelers visiting developing regions of the world. Published studies provide relatively limited data on the microbial etiology of TD from South and Southeast Asia, which is popular destinations for tourism and business from Japan, compared with that from Africa and Latin America. Travelers who visited at the Travel Clinic of the National Center for Global Health and Medicine, Tokyo, with acute diarrhea (<14 day) that started during or shortly after a stay abroad during December 2009 and March 2012 were eligible for this study. After the participants provided informed consent, clinical data and stool samples were collected. The stool samples were screened by PCR for conventional diarrheagenic bacterial pathogens and cultured by standard methods. Commercially available antigen detection kits for Giardia, Cryptosporidium, rotavirus, norovirus, and adenovirus were also used. A total of 121 cases were analyzed. The major destinations included Southeast Asia (44%), South Asia (30%), and Africa (13%). Diarrheagenic pathogens were detected in 66% of the TD cases. In 23% of them, multiple pathogens were detected in the stool samples. Enteropathogenic Escherichia coli was the most common pathogen in all the destinations (36%). Enterogroupaggregative E. coli was the second most common pathogen overall (12%) and more frequently detected in the cases who had returned from Southeast Asia. Campylobacter, Shigella, and rotavirus followed in this order. Rotavirus was more frequently detected in the cases who had returned from South Asia (P <0.05). Ciprofloxacin resistance in diarrheagenic E. coli was rare in all the destinations, but broad-spectrum β-lactam resistance was found in the strains from South Asia. Further investigation focusing on antimicrobial resistance of pathogens of TD is needed.

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 polyarthritis 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%).

ESTABLISHING A TROPICAL MEDICINE TRAINING PROGRAM FOR THE US DEPARTMENT OF DEFENSE (DOD) IN KINTAMPO, GHANA: OVERCOMING CHALLENGES

Eyako K. Wurapa1, David Brett-Major2, Bradley Lloyd3, Damien Punguyire4, Karl Kronmann5, Chris Duplisis6, Naakai Tagoe6
1GEIS Kenya, Nairobi, Kenya, 2Uniformed Services University of the Health Sciences, Bethesda, MD, United States, 3Landstuhl Regional Medical Center, Landstuhl, Germany, 4Kintampo District Hospital, Ghana, Kintampo, Ghana, 5Naval Medical Center Portsmouth, Portsmouth, VA, United States, 6Naval Medical Research Unit 3, Cairo, Egypt

Endemic diseases remain key concerns when deploying US forces. A critical challenge for us is to maintain a solid fund of knowledge in tropical medicine. DoD has courses that provides such training. We discuss challenges of developing a Ghana field site as part of an advanced course, Military Tropical Medicine-Field. Logistics: The annual 2 week event launched in August, 2008, at War Memorial Hospital in Navrongo. This provided a remote setting. However, an 18 hour drive from Accra was problematic. The site was moved to Kintampo, 7 hours from Accra. The right partner: We initially partnered with the Ghana Army at the 37 military hospital. This lacked the disease burden for the course objectives. The Kintampo site had a District Hospital and a Clinical Research Center (CRC). Our partnership began at CRC. It had a strong lab program. However, the students already complete a lab curriculum during pre-requisite courses. Direct patient contact embedded in Ghanaian conduct of clinical care and public health was critical. A stronger relationship with the hospital resulted. An exchange program: The Ghana team is limited to ten physicians. While team backgrounds vary, the course faculty focuses on Preventive Medicine and Infectious Diseases. The hospital provides additional focus in Surgery, Pediatrics, and through educational collaborations, Emergency Medicine. Neither the US students nor the faculty practice independently. Through active shadowing, Ghanaian led care delivery teaches the team. Differences in care and differential diagnoses are discussed. Nurturing the relationship: Continuity is important and we maintain contact with our hosts during the 11 month hiatus. Each year we execute a planning visit. We also support our Ghanaian partners. For instance, we nominated our host physicians to activities such as the University of Florida epidemiology course. Humanitarian Assistance: In 2008, we provided US led direct care in a village. We have discontinued this activity because of the risk of undermining host medical infrastructure and difficulties following patients. Financial resources restricted by law instead are applied to resource our hosts with locally procured consumables based upon their and our needs assessments as well as the course’s curricular objectives. This durable and multi-faceted relationship has allowed us in the last four years to optimize this episodic learning environment on the ground.
DECREASING OCCURRENCE OF TROPIC NEUROINFECTIONS: CEREBRAL MALARIA, MENINGOCOCCAL MENINGITIS AND SLEEPING SICKIES IN SOUTH SUDANESE RURAL HOSPITALS

Emilia Ceploova1, Alexandra Mamova2, George Benca3, Lubica Alumbasi Timon3, Pavlena Bukovinova4, Jana Kralova1, Eva Smrekova2, Jaroslava Sokolova2, Nada Kulkova4, Gertruda Mikolosova5, Inoent Nkonwa1, Vladimir Krcmery4

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 Bukwe, St. Elizabeth University of Health and Social Sciences, Bukwe, 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. cryptoccocal 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 Yirol 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 (SSI) 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 were 10 years, the area of South Sudan was under control of Sudan’s People Liberation Army (SPLA) and medical serviced were served by Médecins Sans Fronterìes (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, geohelmits 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/fomefatin. 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 travelers. 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, Miriam Balazova1, Petra Stullerova1, Nada Kulkova4, Jaroslava Sokolova2, Vladimir Krcmery4

1Our Lady Fatima Hospital, St. Elizabeth University College of Health and Social Sciences Tropical Program, Gordim, South Sudan, 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

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 Fronterìes (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/fomefatin. 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 travelers. Also, occurrence of tropical neuroinfections, such as sleeping sickness, cerebral malaria and meningococcal meningitis, was sporadic.

SLEEPING SICKIES IN SOUTH SUDANESE RURAL HOSPITALS

CEREBRAL MALARIA, MENINGOCOCCAL MENINGITIS AND SLEEPING SICKIES IN SOUTH SUDANESE RURAL HOSPITALS

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

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 (200ug/kg). Leucocytes and eosinophils were counted and total serum IgE was determined. The concentration of IL-4, IL-5, IL-10, INF-γ, TNF-α and TGF-β 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 INF-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 (0-11months). 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 Golenbock5

1University of Massachusetts, Worcester, MA, United States, 2Hospital Clinico 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 Clinico Viedma is a public hospital in the heart of South America that serves the most underprivileged population in the city. It is also 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 other 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 PEPFAR 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.

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 even low levels of impoverishing and often stigmatizing diseases is evidence of persistent inequities.

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 dissuade 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.

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

Erasmo Malekela1, Albina Chuwa2, Mohamed Rajab3, Anne Cross4, Joanna Lowell4, Aldegunda Komba5, Geoffrey Somi5, Renata Mandike6, Abdul-wahid Al-mafazy7, Mary Kibona8, Jessica Kafuko9, Ritha Njau10, Gilly Arthur8, Raz Stevenson1, Peter McElroy1

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 THMIS 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 anemia by April 2012. The 2007/08 HIV and malaria prevalence (5.7% and 17.7%, respectively) will be compared to 2011/12 estimates. The THMIS required four months to complete data collection compared to three to four months per each stand-alone survey. In-country costs for 2011/12 THMIS ($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 Kakuma², Sharon Fonn³, Chimaraoke Izugbara⁴, Margaret Thorogood⁵, Imelda Bates⁶

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 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.
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.

INSSCALE 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

Karín Källander1, James Tibenderana1, Betty Kirkwood2, Zelee Hill3, Daniel Strachan3, Seyi Soremekun2, Raghu Lingam3, Anna Vassal4, 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 INSSCALE 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 LONGITUDINAL PEDIATRIC DENGUE AND INFLUENZA COHORT STUDY IN NICARAGUA: IMPROVING QUANTITY, QUALITY, TRACEABILITY AND TIMELINESS OF DATA COLLECTION AND MANAGEMENT

William Avilés1, Heather Zornetzter1, Brenda Lopez1, Guillermina Kuan1, Lionel Gresh1, Angel Balmaseda1, Aubree Gordon1, Eva Harris1
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 asymptomatic 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 Ruiz3
1Instituto de Investigación Nutricional, Lima, Peru, 2Universidad Peruana Cayetano Heredia, Lima, Peru, 3CRESB, 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.

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BUILDING CAPACITY FOR CONDUCTING CLINICAL TRIALS IN VIETNAM

Peggy L. Coyle1, Giang D. Dao2, Quang N. Nguyen3, Thang C. Tran4, Dzung V. Truong2, Tien K. Nguyen5, 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 ICRN), 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.

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REDUCING 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. PHTPs 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.

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

Davis R. Mumbengegwi1, 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 HCW 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 such as UNICEF 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 pools organized by income vs. geo-spatial proximity; and adherence to RDT results.

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A PROTOCOL TO OPTIMISE MICROSATELITE DNA AMPLIFICATION OF TRYPANOSOMA BRUCELLI GAMBIENSIS FROM BODY FLUIDS

Jacques Kabore1, Vincent Jamonneau2, Thierry De Meeus2, Hamidou Ilboudo1, Paul Capewell3, Mamady Camara4, Adrien Marie Belem5, Bruno Bucheton2, Annette Macleod1
1CIRDES URBIO, Bobo-Dioulasso, Burkina Faso, 2IRD/CIRDES, Bobo-Dioulasso, Burkina Faso, 3University of Glasgow, Glasgow, United Kingdom, 4PNETHA, Conakry, Guinea, 5Université Polytechnique de Bobo-Dioulasso, Bobo-Dioulasso, Burkina Faso

Microsatellite genotyping of Trypanosoma brucei gambiense, the causative agent of human 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 Guinean 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 nymph 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. Martins1, John B. Malone2, Marta M. Silva1, Luciana L. Cardim1, Deborah D. Carneiro1, Jennifer McCarroll3, 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 0.890); 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 then 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. Riley2, Mary E. Wilson1

1Hospital Universitário Professor Edgard Santos, Universidade Federal da Bahia, Salvador, Brazil; 2Division of Infectious Diseases, School of Public Health, University of California - Berkeley, Berkeley, CA, United States; 3Departments 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 amplicon 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 Hailu1, Workagegnehu Hailu1, Welelta Shiferaw1, Shewaye Belay1, Aysheshem Kassahun1, Asrat Bezuneh1, Hirut Wondimu1, Ibrahim Abassi1, Samar Aramin1, Alon Warburg1

1Addis Ababa University, Addis Ababa, Ethiopia; 2Mekelle University, Mekelle, Ethiopia; 3The Hebrew University of Jerusalem, Jerusalem, Israel

Globally, visceral leishmaniasis (VL), a systemic protozoan infection caused by Leishmania donovani spp. is estimated to afflict some 500,000 persons annually. In Africa, the worst affected regions are southern Sudan (15,000-20,000 cases/yr) and Ethiopia (4,000-5,000 cases/yr). VL 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 (PTC) 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 k-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 k-DNA 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 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 Gorgias 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, Panamá, 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. trapiida 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-EPIEPIDEMIOLOGY 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 Kitron1, Ricardo E. Gürtler2

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 Toba; 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, Aimin 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 recalled seeing triatomin insect in Latin America, and 27% of participants reported being bitten by triatomin 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 participants' country of birth. Of the 13% of participants who had heard of CD, 62% thought CD was a problem in their 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 CD. 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 Toba; 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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MODELING THE DISTRIBUTION OF CUTANEOUS LEISHMANIASIS IN BRAZIL BASED ON ENVIRONMENTAL AND SOCIOECONOMIC RISK FACTORS

Maria E. Bavia, Moara S. Martins, John B. Malone, Marta M. Silva, Luciana L. Cardim, Deborah D. Carneiro, Jennifer McCarron, Michael T. Kearney, Joara S. Santos

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 UB Nh education increased (p<0.0001) but notification of decrease decreased as UNB Nh 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, UB Nh model); subsistence (33.7%) and unemployment (26%; AUC=0.77, UB Nh model). A final model was executed combining environmental and socioeconomic data and it was found that the variables contributing in the model were UB Nh sanitation (39.6%), UB Nh 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 TRYPANOSOMA CRUZI IN ARGENTINA, HONDURAS, AND MEXICO: AN ONGOING STUDY

Pierre Buekens, Maria-Luisa Cafferata, Jackeline Alger, Fernando Althabe, Jose Belizan, Yves Carrier, Alvaro Ciganda, Eric Dumonteil, Rubi Gamboa-Leon, Elizabeth Howard, Maria Luisa Matute, Sergio Sosa-Estani, Craine Truyens, Dawn Wesson, Concepcion Zuniga

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, 6Universidade Autónoma de Yucatán, Merida, Mexico, 7National Chagas Program, Ministry of Health, Tegucigalpa, Honduras, 8Instituto Nacional de Parastitologia “Dr. Mario Fatale Chaben”-ANLIS, Buenos Aires, Argentina, 9National Chagas Program, Ministry of Health, Tegucigalpa, Honduras

Trypanosoma cruzi has been divided into different lineages: T. cruzi I (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, and TcI in 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, 7,500 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. Beadell3, Loyce M Okedi3, Serap Aksoy4, Chineke Enyioha2, John C. K Enyaru4, Elizabeth Opio1, Wendy Gibson4, 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 endemics areas and continued spread to new unaffected areas. Uganda harbors the two forms of Trypanosoma brucei subspecies; Trypanosoma bbaruaci 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. gambienne 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. gambienne foci. All the 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) Bразилиensis

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. Miranda3, Jeffrey J. Shaw4, Sinval P. Brandão Filho2

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. 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 SYLVATIC TRANSMISSION CYCLE OF Trypanosoma cruzi IN THE HUMID CHACO OF ARGENTINA

Marcela Orozco1 Orozco1, Gustavo Enriquez1, Julián Alvarado-Otegui1, Victoria Cardinal1, Alejandro Schijman1, Uriel Kitron1, Ricardo Gürtler1

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 variation 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 xenodiagnosis-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

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vellerus. A total of 15 xenodiagnosis-negative animals were kDNA-PCR-positive, including 4 D. albiventris 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. vellerus and T. m. atrox from Argentina. A PCR-based strategy showed that all opossums were infected with DTU TcI 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). Symbiotic transmission cycles of T. cruzi in the dry and humid Chaco differ in the composition of the main reservoir hosts.

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**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 Suce, Sencelco, 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 Suce 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, Sencelco 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 *Le. (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.

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

Claudia P. Nieto-Sanchez, Darwin F. Guerrero-Jimenez, Esteban G. Baus, Mario J. Grijalva

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-infestation of treated dwellings. To address this issue, we designed in 2010 the Healthy Living Initiative (HLI), a holistic program 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.

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

Aaron M. Samuels, Eva Clark, Gerson Galdos-Cardenas, Ryan Wiegand, Lisbeth Ferrufino, Silvio Menacho, Jose Gil, Jennifer Spicer, Julia Budde, S. Pamela Cajal, Ruben Cimino, Michael Z. Levy, Ricardo W. Bozo Gutierrez, Robert H. Gilman, Caryn Bern, Working Group on Chagas Disease in Bolivia and Peru

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 Universitarrio 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 Municipio 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 (χ²(1) = 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.
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SPATIOTEMPORAL CLUSTERING OF VISCERAL LEISHMANIASIS AND LEISHMANIA DONOVANI INFECTIONS IN BIHAR, INDIA

Albert Picado1, Paritosh Malaviya2, Epcio Hasker3, Rudra Pratap Singh2, Marleen Boelaert4, Shyam Sundar2
1Barcelona Centre for International Health Research (CRESIB), Barcelona, Spain, 2Banaras Hindu University, Varanasi, India, 3Institute of Tropical Medicine, Antwerp, Belgium

In the Indian subcontinent, visceral leishmaniasis (VL), also known as kala-azar, is caused by Leishmania donovani, which is transmitted from man to man by the sand fly Phlebotomus argentipes. VL tends to cluster in certain hamlets in remote rural villages but the spatiotemporal dynamics of the disease and leishmania infection are not fully understood. We analysed the clustering of VL cases and L. donovani infections in a VL endemic area covering over 80,000 people in Muzzafarpur district, Bihar. The people living in the study area are regularly monitored and demographic information is been gathered as part of NIH funded project on VL in India. VL cases occurring from 2007 to 2011 were identified by yearly house to house surveys in the whole study area. Incident L. donovani infections were identified as seroconverters (using DAT and rK39 ELISA) in high transmission areas by means of two serosurveys in 2008 and 2009 (n=1,000 people). Yearly edge-corrected kernel density maps, the K-function and the scan-statistic were used to evaluate the spatiotemporal dynamics of VL and L. donovani infection over the study period. The implications of VL clustering and spatial variation for the VL control program in the Indian subcontinent will be discussed.

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EXAMINING LEVEL OF USE OF CHEMOTHERAPY, CHEMOPROPHYLAXIS AND INTERMITTENT-PREVENTIVE TREATMENT OF MALARIA IN PREGNANCY BY PREGNANT WOMEN IN NIGERIA

Obinna Onwujekwe1, Oguchukwu Onwujekwe2, 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.

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DESCRIPTIVE SURVEILLANCE ON USE OF ARTEMETHER-LUMEFANTRINE IN PEDIATRIC AND ADULT RETURNING TRAVELERS WITH MALARIA

Alyson M. Gray1, Marc Cousin2, Paul M. Arguin1, Kamal Hamed3
1Centers for Disease Control and Prevention, Malaria Branch, Atlanta, GA, United States, 2Novartis Pharma AG, Basel, Switzerland, 3Novartis Pharmaceuticals Corporation, East Hanover, NJ, United States

Data from clinical studies show that artemether-lumefantrine (AL) is effective and well tolerated in children and adults with uncomplicated Plasmodium falciparum malaria. However, data on effectiveness and safety of AL in patients in non-endemic settings are limited. Our 5-year prospective surveillance plan includes AL-treated pediatric and adult patients with suspected or confirmed P. falciparum malaria in the US, as reported to the National Malaria Surveillance System at the Centers for Disease Control and Prevention. Descriptive analyses include demographics and baseline characteristics, including malaria immune status; treatment effectiveness; prior and concomitant medication use; and occurrence of adverse events. In the first 17 months (1 May 2010 to 30 September 2011), demographics, treatment adherence, and safety data were collected on 24 patients. Treatment effectiveness data were collected on 21 (91.3%; 2 patients were lost to follow-up) of 23 patients with confirmed (smear 95.7%, PCR 41.3%) or suspected malaria. The mean age of patients was 40.3 years (SD=19.3; range 12-83) and the median BMI was 27 kg/m² (range 16.8-33.8). The majority were male (58.3%) and malaria non-immune (91.7%). Half were non-Hispanic Black. The most common malaria species was P. falciparum (65%; others were P. vivax, P. ovale, and P. malariae, 22%; undetermined, 13%). Of 22 patients taking AL, 18 (81.8%) adhered to treatment. The overall cure rate of patients treated with AL was 83.3% (95% CI= 56.6-96.4%) on Day 7 and 82.4% (95% CI=56.6-96.2%) on Day 28 (patients with missing effectiveness data excluded from analysis). The most common prior and concomitant medications included analgesics, other antimalarials, vitamins, and supplements. There were no deaths, but 3 serious adverse events (severe malaria, incorrectly diagnosed as uncomplicated malaria) were reported. Treatment of P. falciparum malaria in non-immune patients with AL is effective and well tolerated without any unexpected or new safety findings with approved 3-day treatment regimen.

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EFFICACY OF SHORT PROPHYLACTIC COURSE OF ATOMAQUONE- PROGUANIL

Eyal Leshem, Eyal Meltzer, Shmuel Stienlauf, Eran Kopel, Eli Schwartz
Sheba Medical CTR, Ramat Gan, Israel

Current guidelines recommend continuation of Atovaquone proguanil hydrochloride (AP) prophylaxis for seven days after leaving Plasmodium falciparum endemic areas. Evidence from previous studies suggest that continuation for one day after malaria exposure ends may be sufficient. We conducted a retrospective survey of travelers who terminated AP prophylaxis one day after leaving malaria endemic areas to identify falciparum malaria cases. A retrospective telephone survey of travelers to sub-Saharan Africa. Travelers who visited our pre-travel clinic and used AP prophylaxis were included. After returning from their trip, travelers were contacted and questioned regarding prophylaxis adherence, duration, and malaria diagnosis during or after travel. In Israel, malaria is a reportable disease. A retrospective analysis was performed looking at all falciparum malaria cases reported to the Israeli ministry of health (MOH) between 2003-2008. Information about prophylaxis use among these patients was retrieved. The survey included 454 travelers between the years 2010-2011 (total 4771 days in endemic areas). AP was discontinued one day after leaving the malaria endemic areas by 341/454 (75%) travelers. No cases of malaria were noted. The MOH registry survey included 118 falciparum patients between the years 2003-2008. The majority (100; 85%) did not
impact of intermittent preventive treatment in pregnancy with sulfadoxine-pyrimethamine on placental infection and infant birth outcomes in malawi

julie gutman, dyson mwandama, ryan wiegand, doreen ali, don p. mathanga, jacek skarbinski

centers for disease control and prevention, atlanta, ga, united states, malaria alert center, university of malawi college of medicine, blantyre, malawi, national 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, 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), apr=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 dihydroartemisinin-piperaquine for treatment of uncomplicated falciparum malaria in pregnancy in ghana: a randomized, non-inferiority trial

joseph osarfo, harry tagbor, pascal magnussen

kwanne nkrohmah university of science and technology, kumasi, ghana, university 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 dihydroartemisinin-piperaquine for which there is limited efficacy and safety data in pregnancy. with its introduction on the ghanaiian 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 dihydroartemisinin-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 (lccs) when they are sick. this study sought to assess the practices of pharmacies and lccs with respect to malaria in ghana. a random sample of 216 pharmacies and 306 lccs were drawn from the registry of pharmacies and lccs 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 lccs 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 lccs and 57% of pharmacies visited asked about the symptoms of the patient. also, 26% of lccs and 35% of pharmacies asked about the medication history of the patient. about 88% of lccs and 90% of pharmacies visited recommended some drugs to the

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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.

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**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.

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**SONTOCINH AS A GUIDE TO DEVELOPMENT OF DRUGS AGAINST CHLOROQUINE RESISTANT MALARIA**

Michael Riscoe¹, Sovitj Pou¹, Rolf Winter¹, 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, “pharmachins”, 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 pharmachin derivatives have the potential for use in treating drug resistant malaria. A detailed structure-activity profile will be presented.

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**WEIGHT BASED DOSING CAUSES SIGNIFICANTLY LOWER CHLOROQUINE CONCENTRATIONS IN YOUNGER CHILDREN**

Johan Ursing¹, Poul-Erik Kofoed², Amabelia Rodrigues³, Staffan Eksborg¹, Lars Rombo²

¹Karolinska Institutet, Stockholm, Sweden, ²University of Southern Denmark, Kolding, Denmark, ³Bandim Health Project, Bissau, Guinea-Bissau, ⁴Centre for Clinical Research, Sørmland 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 50 mg/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.

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**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. Abdulla³, 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 for those who did not receive treatment, but the difference was not statistically significant (p=0.07). Data are preliminary. Study results will be used to improve the management of non-malarial febrile illness.

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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. Lanteri, Susan Charman, Montip Gettayacamin, Rawiwon Imerbsin, Brandon Pybus, Jason Sousa, Pattaraporn Vanachayanakul, Larry Walker, Colin Ohts

1 Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand, 2 Monash Institute of Pharmaceutical Sciences, Parkville, Victoria, Australia, 3 Walter Reed Army Institute of Research, Silver Spring, MD, United States, 4 University 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. Kangwana, Sarah Kedenge, Abdisalan Noor, Victor Alegana, Andrew Nyandigisi, Jayesh Pandit, Gregory Fegan, Jim Todd, Simon Brooker, Robert Snow, Catherine Goodman

1 Kenya Medical Research Institute - Wellcome Trust Research Programme, Nairobi, Kenya, 2 Division of Malaria Control, Nairobi, Kenya, 3 Pharmacy and Poisons Board, Nairobi, Kenya, 4 London 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 endline 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.
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%, 100%, 97.8%, 96.8%) and DP (100%, 100%, 99.1%, 98.7%).

Permeability experiments with increasing levels of CQ showed a marked resistance to artemisinins were noted.

The effect of CQ on the plasma permeability of the multidrug-resistant human liver cell line, C3H mouse cells, and its primary plasma metabolite, carboxyprimaquine, has been reported. The overall effect observed was a decrease in Cmax with a corresponding decrease in Cl/F and increase in AUC. To better understand these effects, transporter inhibition studies were carried out using both MDRI-MDCK and Caco2 cell lines. Permeability experiments with increasing levels of CQ showed a marked dose dependence in B-A permeability, indicative of MDRI 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 forming 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/6J 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 C57Bl/6J 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 Atuguba2, Timothy Awine1, Patrick Ansah1, Victor Asoala1, 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), Artemether Lumefantrine(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 artemisinin 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-
The impact of intensive malaria microscopy training on district hospitals laboratory staff in Tanzania

Sarah Chiduo1, Awalludin Sutamihardja1, Edward Sekonde1, Tiffany Hamm2, Colin Ohrt2, Charles Massambu, Fidelis Mgohamwende, Robert A. Gramzinski

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; U.S. Military HIV Research Program, Rockville, MD, United States; Ministry of Health and Social Welfare, Dar es Salaam, United Republic of Tanzania; National Malaria Control Program, Dar es Salaam, United Republic of Tanzania

Malaria microscopy training of laboratory staff responsible for definitive malaria identification, quantification, and speculation has a direct impact on achieving proper malaria diagnosis. We investigated the impact of a malaria diagnostics training program developed at the Malaria Diagnostics Center in Kisumu, Kenya on the overall understanding and performance of malaria microscopists in Tanzania. The objective of this analysis was to determine the optimal course syllabus and training duration necessary for strengthening clinical laboratory diagnosis of malaria in Tanzania. Pre- and post-course test results from 2009 to 2011 were compared against trainees' stratified laboratory training background. The time dedicated to performing microscopy practical sessions was recorded to determine if the optimal training time was enough to maximize accurate malaria diagnosis. For the 78 course participants tested it was observed that there is an improvement on the performance of laboratory in reaching a proper malaria diagnosis. Individual average scores for sensitivity ranged from 44.5% to 81.5% with an average improvement of 37% from pretest to post test. Specificity test scores from 34.5% to 86% with an average improvement of 51.5%, written test scores from 2.8% to 82.5% with an average improvement of 29.5% species identification from 40% to 71.5% with an average improvement of 31.5%, parasite detection from 64% to 79.5% with an average improvement of 15.5%, and parasite counting from 42.5% to 74.0% with an average improvement of 31.5%. There is a clear need for conducting regular and standardized malaria microscopy training for all laboratory staff dealing with malaria microscopy on a day-to-day basis. A two week training course can increase the level of malaria microscopy proficiency in Tanzania.
to strengthen malaria diagnostic services in Tanzania. Implementing these changes will be the focus of ongoing efforts increasing accurate diagnosis to improve the effectiveness of treatment in test interpretation; and the implementation of QA/QC procedures. Improvement in the support provided to testing staff, including job aids, performance and only one of 16 health facilities achieved 90%. Our 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 Hann, Colin Ohrt, Robert A. Gramzinski, Fidelis Mgohamwende, Sigsbert Mkude

A walludin Sutamihardja, Sarah Chiduo,
Edward Sekonde, Tiffany Hann, Colin Ohrt, Robert A. Gramzinski, Fidelis Mgohamwende, Sigsbert Mkude

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 ≤60% for testing performance and only one of 16 health facilities achieved 90%. Our overall analysis of the baseline assessments indicated 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.

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 Hann, Colin Ohrt, Robert A. Gramzinski, Fidelis Mgohamwende, Sigsbert Mkude

A Walludin Sutamihardja, Sarah Chiduo, Edward Sekonde, Tiffany Hann, Colin Ohrt, Robert A. Gramzinski, Fidelis Mgohamwende, Sigsbert Mkude

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MISCLASSIFICATION OF PLASMODIUM SPECIES BY CONVENTIONAL MICROSCOPY

Peter Obare1, Bernhard Ogutu2, Christine Adhiambo1, Seth Owusu1, Fred Binka3, Jacob Johnson1

1United States Medical Research Unit, Kisumu, 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 was 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 MICROSCOPY 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
aggregate number of slides would be 24, out of a target number of 40 slides (ten visits), 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, Gonzalez 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-aminoquinolone 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. Our findings indicate that most of the tests examined in a blinded manner for parasite identification, determination of several parasite profiles in addition to being simple to execute and providing results within 2 hours, including preparation time. Also, the gelified format of the assay was stable for 7 days at room temperature and for 2 months at 4 C. We believe that the gelified assay format can streamline the use of real-time PCR for confirmatory diagnosis of malaria.

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A METHOD FOR IMPROVING THICK BLOOD FILM SLIDE ADHERENCE FOR MALARIA DIAGNOSIS

Andrew P. Norgan, Heather E. Arguello, Lynne M. Sloan, Emily C. Fernholz, Bobbi S. Pritt

Mayo Clinic, Rochester, MN, United States

The gold standard for malaria diagnosis remains the examination of thick and thin blood films. The thick film contains a greater amount of blood than the thin film and thus provides the greatest sensitivity for screening. Unfortunately, the larger quantity of blood may not adequately adhere to the thick film and some or all of the droplet may separate from the slide during staining. The possibility of sample loss has led to recommendations that thick films be allowed to dry from 3 hours to overnight to improve the blood droplet adherence to the slide. This delay in preparation of the thick film can delay diagnosis and treatment decisions if parasitemia is not evident on the thin film. Here we describe a simple and inexpensive ‘scratch method’ for improving thick blood film adherence, ameliorating the need for extended drying times. Standardized blood droplets (35 microliters) from twenty-six previously examined EDTA whole blood specimens (22 positive and 4 negative) were used to prepare Giemsa-stained thick films either by a traditional or scratch method. By the traditional method, the droplet was gently spread to an approximate nickel-sized area (22 mm diameter) on the slide using the edge of a traditional method, the droplet was gently spread to an approximate nickel-sized area (22 mm diameter) on the slide using the edge of a second glass slide. Using the scratch method, the droplet was smeared while forcefully grinding or ‘scratching’ it into the slide with the point of a second glass slide. All slides were dried for 1 hour in a laminar flow hood and Giemsa-stained using established protocols. Slides were then examined in a blinded manner for parasite identification, determination of percent parasitemia, and degree of blood droplet adherence by 4 independent trained examiners. There was no difference in detection of parasites or parasite morphology between the two methods, but blood droplet adherence was significantly improved by the scratch method. The scratch method is a simple and effective way to improve thick film adherence and thus facilitate rapid screening. This method does not require additional equipment or significant changes in sample preparation methods.

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, Maniphet Xayavong2, de Almeida E. Marcos2, Naomi Lucchi2, Alexandre J. da Silva2

1IOCruz, 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 laboratorian 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 cycler 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 gelified format of the assay was stable for 7 days at room temperature and for 2 months at 4 C. We believe that the gelified 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 18S. 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.

PLASMODIUM FALCIPARUM HISTIDINE-RICH PROTEIN 2 IN HUMAN MALARIA: DYNAMICS AND DETECTION BY RAPID DIAGNOSTIC TESTS

Louise Marquart, Alice Butterworth, James McCarthy, Michelle Gatton
Queensland Institute of Medical Research, Herston, Australia
Rapid diagnostic tests (RDTs) based on Plasmodium falciparum histidine-rich protein 2 (PfHRP2) are popular for diagnosis of this most virulent malaria infection. However, concerns have been raised about the longevity of the PfHRP2 antigenemia following curative treatment in endemic regions. It is also unclear how early in an infection the PfHRP2-detecting RDTs become positive. These are important questions for the widespread use of malaria RDTs in diagnosing malaria in clinical settings. We developed a mathematical model of PfHRP2 production and decay to mimic the kinetics of antigenemia during human infections. Fitting this PfHRP2 dynamics model to data from human infection studies in malaria naïve hosts allowed the amount of PfHRP2 produced per parasite to be estimated at g per replication cycle. The limit of detection for four commercial malaria RDTs was assessed in the laboratory; the minimum detection thresholds were between 6.9 and 27.8 ng/mL. The low detection threshold and long-life of PfHRP2 is predicted to cause the RDTs to remain positive for at least 7 days after treatment. However the actual duration of positive test results following curative treatment is heavily dependent on the duration and density of parasitemia prior to treatment. Combining the model output and RDT sensitivity data, it is predicted that good quality PfHRP2-based RDTs should be able to detect parasites on the first day of symptoms in malaria naïve individuals, and that as few as 8 parasites/µl may be required to maintain a positive RDT in a chronic P. falciparum infection. The impact that host anti-PfHRP2 antibodies may have on RDT positivity in endemic regions is currently being investigated.

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

Naomi W. Lucchi¹, Jothikumar Narayanan¹, Manijphet Xayavong¹, Simon Kariuki², Alexandre J. Dasilva³, Vincent Hill³, Venkatchalam Udhayakumar¹
¹Centers for Disease Control and Prevention, Atlanta, GA, United States, ²Kenya 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 parasitemia, 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 Giemsa-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 immunooassay was employed to detect and semi-quantitative Plasmodium falciparum (Pf) and Plasmodium vivax (Pv) 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-
quantitative test was 95%, the new fluorescence immunoassay may be used as a POCT diagnostic tool and has potentials as a fast, accurate, reliable, easy, and suitable tool for the semi-quantitative analysis for \( P.f \) and \( P.v \) malaria diagnosis.

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MODELING HEALTH SYSTEMS BARRIERS TO SUCCESSFUL MALARIA MANAGEMENT

V. Bhargavi Rao\(^1\), David Schellenberg\(^2\), Azra Ghani\(^3\)

\(^1\)imperial College London, London, United Kingdom, \(^2\)London 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 (upto 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.

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RAPID DIAGNOSTIC TEST PERFORMANCE IN THE SETTING OF DIFFERING TRANSMISSION INTENSITIES: THE MALAWI ICEMR EXPERIENCE

Atupele Kapito-Tembo\(^1\), Don Mathanga\(^1\), Jacqueline Fiore\(^2\), Karl Seydel\(^1\), Mike Liomba\(^1\), Andrew Balukeni\(^1\), Paul Pensulo\(^1\), Rabia Mukadam\(^1\), Osward Nyrenda\(^1\), Terrie Taylor\(^2\), Miriam Lafer\(^1\)

\(^1\)Malaria Alert Centre, University of Malawi College of Medicine, Blantyre, Malawi, \(^2\)College of Osteopathic Medicine, Michigan State University, East Lansing, MI, United States, \(^1\)Blantyre Malaria Project University of Malawi College of Medicine, Blantyre, Malawi, \(^1\)University of Malawi College of Medicine, Blantyre, Malawi, \(^1\)Center 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 used to confirm the diagnosis of malaria because access to microscopy is limited. Much attention has been given to the sensitivity of RDTs, but their positive predictive value has not been explored, especially in areas such as Malawi, where malaria continues to be endemic. RDTs may be positive for weeks following successful treatment. In cases of false positive RDTs evaluation and treatment of alternative diagnoses might be neglected because of the presumed malaria diagnosis. Through the Malawi International Center of Excellence in Malaria Research, we 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.

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IMPROVING ANTIMALARIAL DRUG RESISTANCE SURVEILLANCE THROUGH EXTERNAL QUALITY ASSESSMENT AND PROFICIENCY TESTING PROGRAMS

Chris Lourens, WWARN QA/QC Group

Worldwide Antimalarial Resistance Network, QA/QC Programme, Bangkok, Thailand

Comprehensive antimalarial drug resistance surveillance includes measures of recurrent parasitemia, in vitro drug susceptibility, drug concentrations to differentiate true resistance from inadequate drug exposure, and genotyping to distinguish recrudescence from re-infection. External quality assessment and proficiency testing (PT) are key components of quality control for laboratory procedures. The goals of the WWARN QA/ QC program are to improve and maintain high-quality antimalarial drug analysis, in vitro drug susceptibility testing, and genotyping, thereby improving the quality of data. The program includes PT for pharmacology laboratories, a reference material program that provides pure antimalarial drug standards, metabolites and internal standards for pharmacology and in-vitro laboratories, and a molecular PT program. WWARN is developing international networks of laboratories doing antimalarial drug testing and genotyping. The reference material program distributed accurately weighed quantities of antimalarial drug standards, metabolites and internal standards to 25 laboratories. The pharmacology PT program sent samples to 8 laboratories in 4 rounds of PT. The molecular PT program includes biannual PT to differentiate parasite recrudescence from re-infection for 8 laboratories in 6 countries. Data will be presented showing how participating laboratories have improved significantly over subsequent rounds of PT. The benefits of the reference material program include cost savings for the laboratories and provision of a uniform standard of material, reducing inter-laboratory variability. Benefits of participating in the PT program include identification of technical difficulties encountered in the analysis of drug compounds and genotyping. Technical experts provide advice for correcting problems to improve performance in subsequent analysis, and ultimately improve the quality of drug resistance surveillance data and facilitate pooled analyses.

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PFMDR1 IS ASSOCIATED WITH RECRUDESCENCE AFTER TREATMENT WITH ARTMETHER-LUMEFAENTRINE IN WESTERN KENYA

Fredrick L. Eyase, Ogutu Bernhards, Hoseah Akala, Angela Omondi, Luicer Ingasia, Dennis Wekesa, Agnes Cheruiyot, Charles Okudo, Redempta Yeda, Ben Andagaluu, Elizabeth Wanjia, Jacob D. Johnson
United States Army Medical Research Unit, Walter Reed Project, Kisumu, Kenya

Single Nucleotide Polymorphisms (SNPs) in PFMDR1 and PFCRT have been associated with Plasmodium falciparum resistance to drugs including chloroquine (CQ), amodiaquine (AQ), lumefantrine (LU) and mefloquine (MQ). Artmether-Lumeфантрин (AL) is currently the first line antimalarial used in Kenya with arsunesate-amodiaquine (ASAQ) and dihydroartesimin piperquine (DHA-PPQ) being readily available from private retailers. During an open-label randomized clinical study evaluating the efficacy of AL in Ombeyi, a malarial endemic district in Western-Kenya, we investigated the role of PFMDR1 and PFCRT in modulating tolerance to artemisinin partner drugs. All recurrent samples were genotyped for SNP1, SNP2 and GLURP at day 0 and day of recurrence. Additionally all samples were assayed for SNPs in PFMDR1 codon 86 and PFCRT codon 72-76 as well as copy numbers in PFMDR1. All day 0 samples were assayed for drug susceptibility using the SYBR Green method. Among the 454 subjects enrolled in the study, there were 162 recurrent cases of which 134 were reinfections while 17 were recrudescences and 11 underdetermined. PFMDR1 N86 was significantly associated with recrudescence compared to both day 0 and reinfection. There was no significant association between PFCRT and recurrent infections and amplification of PFMDR1 gene was not observed. Significant positive correlation was observed between LU and MQ (r=0.5, r²=0.27, p<0.05). This data demonstrates an association between PFMDR1 N86 and recrudescence after treatment with AL in Western Kenya. Co-resistance between LU and MQ indicates that LU pressure may lead to MQ resistance, an important prophylaxis for malaria naive visitors to Kenya.

ANTI PLASMODIUM FALCIPARUM MO15-RELATED PROTEIN KINASE (PFMRK) AND P. FALCIPARUM PROTEIN KINASE 5 (PPFKS) ACTIVITIES OF NATURAL PRODUCTS FROM PLANTS

Hosemah A. Akala1, Veronica M. Zhang2, Cassandra L. Woodard3, Fredrick L. Eyase1, Maud K. Kamatens-Mugisha4, Abiy Yenesew5, Bernard T. Kiremire6, Ben Andagaluu1, Douglass S. Walsh1, Elizabeth Wanjia1, Jacob D. Johnson1, Norman C. Waters6
1Kenya Medical Research Institute (KEMRI)/United States Army Medical Research Unit-Kenya (USAMRU-K), Kisumu, Kenya, 2School of Chemistry and Molecular Biosciences, University of Queensland, St. Lucia, Queensland, Australia, 3Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Silver Spring, MD, United States, 4School of Biological Sciences, College of Natural Sciences, Makerere University, Kampala, Uganda, 5Department of Chemistry, University of Nairobi, Nairobi, Kenya, 6Department of Chemistry, Makerere University, Kampala, Uganda, 7Department of Immunology and Medicine, U.S. Army Medical Component, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand, 8Department of Chemistry and Life Science, United States Military Academy, West Point, NY, United States

There is urgent need to develop new chemothapeutic anti-Plasmodium falciparum to replenish loses to resistance. Natural products, including flavonoids and quinones, currently being explored as anticancer agents inhibiting cyclin dependent kinases (CDKs) are also antimalosomal, but information on their mechanism of action is scanty. This study assessed the in-vitro flavonoids and quinones inhibition of Plasmodium falciparum MO15-related protein kinase (PFmrk) and Plasmodium falciparum protein kinase 5 (PPFKS) using luciferase-coupled kinase assay. These compounds were obtained from six East African plants. Compounds coded as BA-4E, 0.26μM and BA-6S 0.22μM were the most active against Pfmrk and PFPKS respectively while BA-6U (0.4μM) and BA-4C (1.03μM) showed specificity against Pfmrk. Flavonoids of the subclass flavanones were the most active compounds. Flavanones having two prenyl substituents (diprenylated compounds) on ring B with a hydroxyl or methoxy group at position 4’ had highest activity regardless of these groups’ position. These findings suggest that inhibition of Pfmrk and/or PFPKS may be among ways that flavonoids inhibit Pf replication.
CHLOROQUINE-RESISTANT PLASMODIUM FALCIPARUM MALARIA IN TRAVELERS FROM HAITI AFTER THE 2010 EARTHQUAKE

Myriam Gharbi1, Jennifer Flegg2, Magatte Ndiaye3, Bruno Pradines4, Cally Roper4, Véronique Hubert5, Eric Kendjo5, Philippe Brasseur6, Gunmar Gaye7, Abdoulaye Dijmé8, Ako Berenger9, André T. Offianan10, Louis Penali11, Jacques Le Bras1

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 pfcrf76T 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 travellers 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 information, treatment, history of travel and malaria infection were collected systematically. Prior to the earthquake, all isolates (n=29) had the wild-type pfcrf76T 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[CI], 23 to 31). After the earthquake, two of ten showed CQ resistance in vitro after culture adaptation. Both isolates had high CQ IC50 (506nM and 708nM) and high CQ IC50 isolates showed Pf3D7 (CQ susceptible clone) ratio (20 and 27). Resistance was confirmed by the molecular analysis demonstrating the presence of the CQ-resistant associated pfcrf76T allele (mixed K76) only in these two isolates. Our data confirm the presence of CQ-resistant strains in Haiti. They highlight the importance of implementing 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 Roper4, Véronique Hubert5, Eric Kendjo5, Philippe Brasseur6, Gunmar Gaye7, Abdoulaye Dijmé8, Ako Berenger9, André T. Offianan10, Louis Penali11, 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 Cote 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 (slope1=0.03, slope2=0.03, respectively, p=NS), SN (β1=0.17, β2=0.21, respectively, p=NS) and ML (β1=0.19, β2=0.17, respectively, p=NS). These results are supported by in vitro analysis in SN (β1=0.03, β2=0.05, 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=-0.22, 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
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 Chulaibo, Ahero, chulaibo, Emakakha and Kisian shows susceptibility to both deltamethrin and ICON with the mortality rates ranging between 80% - 85% but mosquitoes from Bungoma and Emitete shows resistance to both ICON and deltamethrin with mortality rates ranging from 69%- 74%. Sprayed artificial walls shows lower mortality rates compared to sprayed filter papers. ICON had high mortality rates on the mosquitoes compared to Deltamethrin. Mosquitoes from Bungoma and Emitete 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 Chen1, Dennis E. Kyle2, Michelle L. Gatton1, Qin Cheng1

1Australian Army Malaria Institute, Brisbane, Australia, 2University of South Florida, Tampa, FL, United States

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 underlying 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 artesinin resistance may involve two steps. The molecular events important in each step are currently being investigated to determine whether full artesinin resistance develops as a stepwise process or whether the two stages arise independently of each other.
The data also reveal differences in efficacy between artemisinins 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.

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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-PYRIMETAMINE (IPTP-SP) IN GABON

Marielle K. Bouyou-Akotet, Denise P. Mawili-Mboumba, Marie-Lou Tchibola, Gladys Tsoumbou-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 P. 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.

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

Gisela C. Henriques1, Khalid Beshir1, Teun Bousem1, Halidou Tinto2, Umberto D’Alessandro1, Paul Hunt4, Pedro Cravo5, Colin Sutherland1, Rachel Hallett1

1London School of Hygiene and Tropical Medicine, London, United Kingdom, 2Centre Muraz, Instituto de Investigacion 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 µ chain of the AP2 adaptor protein complex (pcap2-µ) 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.

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

Elena M. Artimovich1, Ananias A. Escalante2, Kristan Schneider3, Terrie E. Taylor4, James G. Kublin5, Miriam K. Lauf3, Christopher V. Plowe1, Shannon Takala-Harrison1

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 most 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, pfcr. 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-mutations 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 Haplotypic Homozygosity was estimated, and showed increased linkage disequilibrium (LD) in regions flanking both dhfr and dhps. Selective 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.

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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...
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 reemergence 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, PfRh3 and PfRho1 (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.

Persistently Plasmodium Falciparum Infections Drive Expansion of Atypical Memory B Cells as Well as Exhausted T Cells

Joseph Illingworth, Noah S. Butler, Peter D. Crompton, Sophie Roetynck, Susan K. Pierce, John Harty, Kevin Marsh, Philip Bejon, Francis M. Ndungu

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 Teff CD8 T cells, respectively, but only in the presence of asymptomatic parasitaemia. Together, these results suggest that Pf drives expansion of atypical lymphocytes. The implication is that these cells may dampen inflammatory responses to malaria, thus reducing pathogenesis, but may also impede the generation of protective responses.

The Effect of Maternal Malaria and Helminth Infections on Childhood Malaria: A Birth Cohort in Entebbe, Uganda

Juliet Ndiabaza, Emily L. Webb, Swaib A. Lule, Harriet Mpaine, Miriam Akello, Gloria Odurui, Moses Kizza, Helen Akurat, Lawrence Muhangi, Birgitte Vennervald, Alison M. Elliott

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

Helminths 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 malaria-free (18%), and 0.6% had malaria (0.4%). Of these, 0.5% had malaria parasitaemia, and 0.1% had P. falciparum parasitaemia. 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 PREVENTIVE TREATMENT DURING PREGNANCY (IPTp): A CROSS-SECTIONAL STUDY

Fatuma Namusoke1, Muhammad Ntale1, Mats Wallgren2, Fred Kironde1, Florence Mirembe1
1Makerere 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 Sulfadoxine-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 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 of 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, Peerayuth Boonpan2, Rujira Lerdprom2, Ming-Chieh Lee3, Guiyun Yan4, Jetsumon Prachumsri4, Liwang Cui5, Jeeraphat Sirichaisinthop2
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. Kedenge1, Beth B. Kangwana1, Evelyn W. Waweru1, Andrew J. Nyandisu2, 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 Nasr3, Denis Rubahika4, Steven S. Yoon5, Kevin Sullivan1, Achuuyt 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=0.03] 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<.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 interaction<0.01). 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 Gutman1, Raz Stevenson1, Peter McElroy2, 1Ihpiego, 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, 4Ihpiego, 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, 7United States Agency for International Development, Dar es Salaam, United Republic of Tanzania, 8Centers for Disease Control and Prevention and President’s Malaria Initiative, Dar es Salaam, United Republic of Tanzania

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.

FINE-SCALE SPATIAL VARIATION IN TRANSMISSION INTENSITY, IN SECULAR TRENDS OF TRANSMISSION INTENSITY, AND IN THE AGE PROFILE OF FEBRILE MALARIA IN KILIFI, KENYA

Philip Bejon1, Abdisalan M. Noor1, Janet T. Midega1, Thomas N. Williams1, Mark Otieno2, Judith Pesu1, Mafudh Bashraihe1, Dave L. Smith2, Kevin Marsh1, 1Kenyan Medical Research Institute, Kilifi, Kenya, 2Johns Hopkins Bloomberg School of Public Health, Bethesda, MD, United States

Malaria transmission is spatially heterogeneous. Maps of malaria episodes at fine spatial scales often show clusters of transmission comprising groups of homesteads or “hotspots”. These hotspots make malaria control measures less effective than might have been expected, but targeting intensive control interventions at the hotspots could be highly effective. At present, there are few epidemiological descriptions of the properties of hotspots. We have previously shown that hotspots of asymptomatic parasitaemia are stable over several years, but hotspots of febrile malaria are unstable. The risks of asymptomatic parasitaemia and febrile malaria were closely related to proximity of Anopheles larval sites, interacting with wind direction. We hypothesise that immunity offsets the high rate of febrile malaria that might otherwise occur in stable hotspots, whereas unstable hotspots necessarily affect a population with less prior exposure to malaria. We present data from 4,200 episodes of malaria among 4,800 homesteads monitored from a local dispensary in Kilifi, Kenya, from 2003 to 2011. There was marked spatial clustering of febrile malaria episodes. Spatial clustering of febrile malaria among younger children was more stable over time compared with among older children. Reasoning that febrile malaria risk in younger children was less confounded by immunity, we used data from children below one year of age to classify homesteads into high or low mean transmission intensity, and into rising or falling secular trends of transmission intensity. This classification predicted the age-profiles of febrile malaria by homestead. At high mean transmission, the peak febrile malaria risk was at 3 years of age, and at low mean transmission intensity the peak febrile malaria risk was at 8 years of age. A rising secular trend of transmission predicted a sustained risk of malaria in children above 10 years of age, whereas a falling secular trend predicted a falling risk of malaria. We conclude that aggregated febrile malaria incidence is inadequate to represent the complexity of
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.

ESTIMATING TRANSMISSION INTENSITY FROM PLASMODIUM FALCIPARUM SEROLOGICAL DATA USING ANTIBODY DENSITY MODELS

Emilie Pothin, Neil Ferguson, Chris Drakeley, Azra Ghani

Imperial College London, London, United Kingdom, London 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.

PREVALENCE OF MALARIA AND ANEMIA AMONG PREGNANT WOMEN ATTENDING ANTE NATAL CARE CLINICS IN THE EJISU-JUABEN AND SEKYERE-EAST DISTRICTS OF GHANA

Gifty D. Antwi, Harry Tagbor, Imelda Bates

Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, Liverpool 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 IPTp 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.

REVITALIZING ROUTINE HEALTH FACILITY DATA FOR MALARIA CONTROL

Jessica Butts, Melody Miles, Carrie Nielsen, Achuyt Bhattarai, 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.

MOLECULAR EPIDEMIOLOGY OF PLASMODIUM VIVAX RELAPSES IN THE PERUVIAN AMAZON

Raul Chuquiyauri, Pablo Peñataro, Kimberly C. Brouwer, Manuel Fasabi, Maritza Calderon, Sonia M. Torres, Shiria Abeles, Robert Gilman, Alejandro Llanos-Cuentas, Margaret Kosek, Joseph M. Vinetz

University of California at San Diego, San Diego, CA, United States, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, Universidad Peruana Cayetano Heredia, Lima, Peru

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 northeast 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 Pvmsp-3a 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 (p<0.001) (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, 5.5; 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 Hossain2, Abdullah Abu Sayeed2, Sanjib K. Paul2, Walijur Rahman2, Rapeekhan R. Maude1, Nidhi Vaid1, Aniruddha Ghose1, Robin Amin2, Rasheda Samad2, Erman Bin Yunus2, M. Ridwanur Rahman2, A. M. Bengali2, M. Goftanul Hoque1, Nicholas P. Day1, Nicholas J. White1, Arjen M. Dondorp1, M. Abul Faiz2

1Wellcome 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, 5Sheheed Shwarwardhy 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 Plasmodium 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 Chuquiyauri1, Maribel Paredes2, Pablo Peñataro2, Sonia M. Torres4, Silvia Marín2, Alexander Tenorio2, Kimberly C. Brouwer3, Shara Abeles1, Alejandro Llanos-Cuentas4, Robert Gilman1, Margaret Kosek1, Joseph M. Venetz1

1University of California at San Diego, San Diego, CA, United States, 2Asociacion Benefica Prisma, Lima, Peru, 3Voehns 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 hypoendemic. 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 polychotomous 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.
relapse, suggesting a genetic basis to relapse. Pvmsp1 individual appearance of novel variants supports the notion that latent hypnozoites reactivate in concert to cause relapse. At the same time, the common all or a subset of the multiple clones found in an initial infection can relapse. By accounting for the polyclonality of infections in Cambodia, we compared, two specific variants were associated with subsequent from initial infections of 45 “relapsers” and 62 “nonrelapsers” (those sharing at least half their variants. At the same time, novel variants 3National Malaria Center, Phnom Penh, Cambodia, 4University of North Carolina School of Medicine, Chapel Hill, NC, United States, 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 co-infecting 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 “relapsers” and 62 “nonrelapsers” (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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GENOTYPIC PATTERNS OF RELAPSING PLASMODIUM VIVAX INFECTIONS IN CAMBODIA

Jessica T. Lin1, Jonathan J. Juliano1, Oksana Kharabora1, William O. Rogers2, Sinuon Muth3, Chansuda 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 co-infecting 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 “relapsers” and 62 “nonrelapsers” (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. Ali3, Issa Garimo1, Fabrizio Molteni4, Mwinyi I. Mselenium1, 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 reporting, 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, Johansan Mawole1, John Mutuku Mul1, Victor Namulanda1, Jirina Kafkova1, Mario Jancovic1, Daria Pechacova1, Jaroslava Sokolova1, Vladimir Krcmery1

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 anemia (<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 school year age and also for all mothers coming to maternity check since 2009. Seasonal variation has been observed as well with maximum in June - October (rainy season) and minimum in November - December. In conclusion, decrease of annual incidence of microscopically positive cases of malaria in 2010-2011 has been observed in slum area of Mukuru in Nairobi, Kenya. 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 Kigozi3, Stella Kakeeto3, Humphrey Wanzira3, Fred Kizito3, Denis Rubahika4, Sussann Nasr5, Melody Miles5, Steven S. Yoon3, Michelle Chang5, Sarah G. Staedke6, Moses Kamya7, Grant Dorsey8

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%), Mubende (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 overestimated the burden of malaria when compared to surveillance program data: 27 percent higher in Kambuga (25% vs. 52%), 24 percent higher in Tororo, (61% vs. 85%), 18 percent 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 Siharova1, Andrej Bebjak1, Petra Mikulasova1, Emilia Ceploova1, 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 (84,7 %) 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 units and 5 years of 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 PEACETIME AND DEPLOYMENT IN EAST AFRICA

Refaat Hanna1, Christopher Arrumm2, Priya Baliga3, Adam Mwabulanga4, Juma Mwinula2, Zuberi Muvunyi5, Godfrey Bwire2, Marc Nimburana6, Robert Miller1, Robert Holmes1, Annette Von Thun1

1United States Africa Command, Holzgerlingen, Germany; 2Kenya Defense Forces, Nairobi, Kenya; 3Amed 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.

Burundi remains an important parasitic disease of public health concern, especially in Africa. Malaria is a problem for military forces because of its ability to cause sudden epidemics which can hinder or halt operations. During the Malaria Symposium hosted by the United States Africa Command in April 2011, representatives from several African militaries proposed formation of a multi-national Malaria Task Force to address 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.

EXPLORING THE IMPACT OF DRUG STORE ACCREDITATION AND PRACTICES OF TANZANIA’S DRUG RETAILERS: EXPLORING THE IMPACT OF DRUG STORE ACCREDITATION

Boniface Johannes1, Rebecca Thomson-2, Charles Festo1, Admirablis Kalolel1, Mark Taylor1, Katia Bruxvoort1, Sarah Tougher1, Yazoume Ye3, Andrea Mann1, Ruilin Ren1, Barbara Willey2, Fred Arnold3, Kara Hanson1, 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 I 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.

OCCURRENCE OF MALARIA IS DECREASING WITH HIGHER ATTITUDE IN BURUNDI HIGHLANDS

Eva Misikova1, Renata Machalkova2, Jozef Marada2, Lucia Paskova1, Jan Dubeª3, Eva Uderzo3, Nada Kulkova2, Vladimir Krcmery1

1Tropical Program in Murago, 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. Buraniro 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 Muragro 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 Muragro (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 Muragro where only few people are travelling to large cities or crowded places (such as Great Lake Tanganyika).

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EVALUATING THE COST-EFFECTIVENESS OF INTERMITTENT SCREENING AND TREATMENT (IST) COMPARED TO INTERMITTENT PREVENTATIVE THERAPY (IPTP) DURING PREGNANCY IN PREVENTING LOW BIRTH WEIGHT: A MODEL-BASED ANALYSIS

Patrick Walker1, Azra C. Ghan1, Feiko Ter Kuile1, Matt Cairns3
1MRC Centre for Outbreak Analysis and Modelling, London, United Kingdom, 2Liverpool School of Tropical Medicine, Liverpool, United Kingdom, 3London School of Hygiene and Tropical Medicine, London, United Kingdom

Malaria during pregnancy is the leading preventable cause of low birth weight in many areas of sub-Saharan Africa. The current recommended intervention is to administer up to 3 doses of Sulphadoxine Pyrimethamine (SP) to pregnant women during antenatal clinic visits (IPT-SP) to clear any existing infection and protect against re-infection. However, with the emergence of SP resistance in many parts of Africa, alternative strategies to IPT-SP are currently being evaluated. One such alternative is intermittent screening and treatment (IST), whereby long-acting artemisinin combination therapy is administered to women with a positive rapid diagnostic test (RDT). By linking a model of the progression of Plasmodium falciparum malaria during pregnancy to the risk of low birth weight, we explored the impact and cost-effectiveness of IST and IPT-SP in areas with different transmission intensity and levels of SP resistance. Our results suggest that in areas where the parasite is still sensitive to SP, IPT will be more cost-effective than IST. This is due to the limited sensitivity of RDTs to detect low-grade infections and the additional cost of the RDT relative to SP. However, in areas of East Africa with high levels of SP resistance, our results suggest that a switch to IST would lead to a reduction in the burden of malaria-attributable low birth weight. Whether IST is also more cost-effective depends mainly on the difference in cost between SP and the chosen RDT and less on the level of transmission, the level of immunity acquired or the relative cost of the antimalarial provided to those with a positive test. For example we found that in areas where SP fails to clear infections in 35% of parasitaemic women, IST would be cost-effective provided costs associated with an RDT are below $1 per test. In summary, our results suggest, conditional on our model assumptions, that a switch of policy to IST would only be effective in reducing the burden of low birth weight in areas where there are moderate to high levels of SP resistance, with the degree of resistance necessary to make such a decision cost-effective depending primarily upon the cost of the RDT used.

DEFINING THE MALARIA BURDEN IN NCHELENGE DISTRICT USING THE WHO MALARIA INDICATORS SURVEY

Michael Nambozi1, Phidelis Malunga1, Jean-Pierre van Geertruyden2, Modest Mulenga1, Umberto D’Alessandro1
1Tropical Diseases Research Center, Ndola, Zambia, 2University of Antwerp, Antwerp, Belgium, 3Malaria Research Center, Gambia, Gambia

Malaria is considered as one of the major public health problems and among the diseases of poverty. In areas of stable and relatively high transmission, besides children under 5 years of age, pregnant women and their new born babies are among the higher risk groups. A multicentre trial on the safety and efficacy of several ACTs during pregnancy is currently on-going in 4 African countries, including Zambia, whose study site is in Nchelenge district. As the study outcomes may be influenced by the local malaria endemicity, this needs to be characterised. Therefore, in March-April 2012 we carried out a cross-sectional survey to determine the prevalence and intensity of malaria infection among <10 years old children in Nchelenge district, on the shores of Lake Mweru. The sampling unit was the household where all children < 10 were included in the survey. We used a simple random selection of households using the GPS coded list. Individual consent to participate was collected from parents/guardians. A blood sample for Hb measurement and the detection of malaria infection was collected as well as information on the use of preventive measures such as Long-Lasting Insecticidal Nets (LLIN). Three hundred twelve households were sampled and 358 children included in the survey. Malaria parasite prevalence was 31.3% (95% CI: 26.6-36.4%); anaemia prevalence (Hb <11g/dl) was 49.1% (95% CI: 43.8-54.6%), a higher value than those previously found in the province. Though malaria has declined substantially in Zambia, there are still pockets of high endemicity such as Nchelenge district. These areas should be targeted for achieving high coverage of preventive interventions such as LLIN and indoor residual spraying.

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 - 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.
CHANGES IN MALARIA PREVALENCE AND HEALTH PROVIDER’S BEHAVIOR TOWARDS FEVER WITH THE INTRODUCTION OF ACT AND RDT AT PERIPHERAL HEALTH CENTRE LEVEL IN SOUTHWESTERN SENEGAL (2000-2011)

Philipppe Brasseur1, Malik Badiane2, Moustafa Cisse3, Michel Vaillant4, Piero L. Olliaro1
1Institut de Recherche pour le Développement (IRD), UMR 198 Dakar, Senegal, 2District Médical d’Oussouye, Oussouye, Senegal, 3Programme National de Lutte contre le Paludisme (PNLP), Ministère de la Santé et de la Prévention, Dakar, Senegal, 4Unité d’Épidémiologie Clinique et de Santé P Centre d’Etudes en Santé, CRP-Santé, Luxembourg, Luxembourg, 5World 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 -74%, consultations -51%.

No change was seen in any of the above. ASAQ accounted for 12% of antimalarial treatments overall (41% of treatments for P+, 7% P+, 9% P0). Comparing 2007-11 (ASQA + RDT deployed) to 2000-06, the yearly number of fevers halved, non-malaria fevers doubled, malaria treatments dropped -86%. ASAQ increased from 17% to 30% of antimalarial treatments and from 57% to 94% of P+ cases. There was no difference in the proportion of fevers tested parasitologically (75% with microscopy during 2000-06, 70% with RDT during 2007-11), nor in the P.falciparum positive rate (29% vs. 31%). Case management of fever improved (better detection of non-malaria fevers, few malaria treatments). Practice compliance with malaria policy increased (almost all treatments are ASAQ), ca. three-quarters of fevers are tested parasitologically. However, introduction of RDTs did not boost testing significantly (presumably because of prior successful training in microscopy), and confidence in RDT is still limited (presumably because the proportion turning out positive is low compared to clinical suspicion - the established prior practice).

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

Ulrika Morris1, Berit Aydin-Schmidt1, Pedro Ferreira2, Louise Jörnågen1, Abdullah S. Ali3, Delére Shaleký1, Mwinyi I. Msellem1, Andreas Mårtensson1, J. Pedro Gil4, Anders Björkman1
1Malaria Research Laboratory, Department of Medicine Solna, Karolinska Institutet, Stockholm, Sweden, 2Department of Protozoology, Institute of Tropical Medicine, Nagasaki University, Nagasaki, Japan, 3Zanzibar Malaria Control Program, Zanzibar, United Republic of Tanzania, 4Drug 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 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 P. falciparum parasites diluted in malaria negative whole blood. The level of P. 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 P. 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 Lundblom1, Linda Murungi2, Victoria Nyaga2, Daniel Olsson1, Josea Rono3, Faith Osier2, Edna Ogada2, Scott Montgomery1, Anthony Scott1, Kevin Marsh2, Anna Färnert1
1Karolinska Institute, Stockholm, Sweden, 2Kenya 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 P. 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 P. falciparum by microscopy or PCR in at least one sample from birth conferred an increased risk of severe malaria and particularly if a multicolonial infection, as defined by genotyping of the polymorphic merozoite surface protein 2 gene, was ever detected. Antibodies to P. falciparum schizont extract were similar 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.
MEASURING *PLASMODIUM FALCIPARUM* TRANSMISSION IN LOW-ENDEMIC SETTINGS USING A COMBINATION OF COMMUNITY PREVALENCE AND HEALTH FACILITY DATA

Joshua O. Yukič1, Olivier Briët2, Michael Bretscher1, Adam Bennett1, Seblewengel Lemma3, Yemane Berhan4, 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 1000 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.

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 (66,000/yr), 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).

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, Rwanda, Atlanta, GA, United States

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 netbed coverage (1 net per 2 people) in February 2011after distributing 6.1 million LLINS since 2009. In December
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 insecticide 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% (PHI>768 threshold; polyethylene: 3-10%; polyester: 7-30%) of LLINs would require replacement after 6 months and 32% after 12 months (PHI>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, Sylla 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-DPHO).
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 malarious 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. Quantitative 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 sustainability 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 ASYMPOTOMATIC CARRIERS WITH ARTEMETHER-LUMEFANTRINE ON MALARIA TRANSMISSION

Wamdaogo M. Guelbeogo¹, Alphonse Traore¹, Antoine Sanou¹, John Lucas², 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 gambiensi 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 leads to a sustained reduction in malaria burden beyond that. However, in some settings, even with sustained coverage, clinical incidence of malaria increases as the population loses its naturally acquired immunity. In some low to medium transmission settings, our simulations 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 Cohen1, Sarah Alphs2, Jean Arkedisi3, Julius Massaga4
1University of Michigan, Ann Arbor, MI, United States, 2Harvard University, Cambridge, MA, United States, 3Results for Development, Washington, DC, United States, 4National Institute for Medical Research, Dar es Salaam, United Republic of Tanzania

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 Feb 2011 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 ANOPELESES 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 18 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. Suleiman2, Abdul- wahid H. Al-mafazy3, Rosemary Lusinde1, Peter McElroy2, 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 Thior3, Mame Birame Diouf4, Cheikh Thiam4, Ouleye Tall Beye1, Ibrahima Diallo2, Fatou Ba Fall2, Medoune Ndop3, Moustapha Cisse4, Cheikh Tacko Dio4
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. It 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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IMPACT OF COMMUNITY SCREENING AND TREATMENT OF ASYMPTOMATIC CARRIERS OF PLASMODIUM FALCIPARUM WITH ARTEMETHER-LUMEFANTRINE ON ASYMPTOMATIC AND GAMETOCYTE CARRIAGE: A 12-MONTH, CLUSTER-RANDOMIZED STUDY IN SUB-SAHARAN AFRICA

Alfred B. Tiono1, Alphonse Ouédraogo1, Bernhards Ogutu2, Amidou Diarra1, Sam Coulibaly1, Marc Cousin2, Christine Remy3, Amitava Mukhopadhyay4, Issiaka Soulama1, Sodiomon B. Sirima1, Kamal Hamed1
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 1-4 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 higher 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).

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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 mechanism 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.

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SEROPREVALENCE OF TOSCANIA VIRUS INFECTIONS AMONG RURAL POPULATIONS IN AZERBAIJAN

Jason M. Blaylock1, Emilya Nasirova2, Afrail Imsayilov2, Eric Garges1, Luis J. Martinez2
1Walter Reed Army Institute of Research, Silver Spring, MD, United States, 2Republican Anti-Plague Station, Azerbaijan Republic Ministry of Health, Baku, Azerbaijan

The sandfly fever viruses are endemic to the Mediterranean region and well known causes of human illness there. The Toscana virus (TOSV) serotype displays a unique neurotropism and can cause a meningitis or meningoencephalitis in some cases. Little is known about the geographic distribution of Toscana virus outside of the Mediterranean basin. While the Phlebotomus spp. sandfly vector has been associated with visceral leishmaniasis in Azerbaijan, the presence of TOSV infection in this region has never been assessed. In Azerbaijan, the etiology of febrile illnesses and cases of meningitis are underreported due to low healthcare utilization and lack of laboratory diagnostics. However, public health officials suspect high rates of arboviral infections along the Azerbaijan-Russia border. Previous studies have attempted to document the seroprevalence of several zoonotic infections in this region, however no studies to-date have evaluated the prevalence of TOSV infection there. We obtained 755 serum samples that were previously accessed from adults from the villages of Guba, Gusar, and Xachmaz as part of a cross-sectional study [USAMRID HUC FY07-31 (APS TNK 004)WRAIR 1435]] and analyzed the prevalence of TOSV antibodies using an IgG TOSV detection kit [DIESSE, Siena, Italy]. The subject population included 796 adults over the ages of 18 years, 72% of who were less than 50 years of age, 56.4% of whom were female, and 75.3% of Azerbaijaniani ethnicity. We found a total of 18 positive samples, resulting in a seroprevalence of 2.38%. This initial study is the first to document the presence of TOSV infection in the Republic of Azerbaijan. Although the majority of TOSV infections are asymptomatic, further testing may be warranted for suspected cases in which subjects present with fevers, headaches, and/or symptoms of meningitis or meningoencephalitis of unknown etiology. This study also helps to establish a need for further epidemiologic and surveillance studies in this region.

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DETECTION OF EASTERN EQUINE ENCEPHALOMYELITIS VIRUS RNA IN NORTH AMERICAN SNAKES

Andrea M. Bingham1, Sean P. Graham2, Nathan D. Burkett-Cadena3, 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 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. Drebot1, Kristina Dimitrova1, Maya Andonova1, Stephen Turner2, Bouchra Serhir3, Michel Couillard3, Cecile L. Tremblay1

1National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, MB, Canada, 2Hospital Fleury, Montreal, QC, Canada, 3Laboratoire de santé publique du Québec – Institut national de santé publique du Québec, 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 titres 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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**IDENTIFICATION OF NEUTRALIZING ANTIBODY EPITOPES ON CHIKUNGUNYA VIRUS ENVELOPE PROTEIN**

Kristen M. Kahle1, Rachel Fong1, Suganya Selvarajah2, Kimberly-Anne Mattia1, Trevor Barnes1, Joseph Rucker1, Cheryl Faes1, Graham Simmons1, Benjamin J. Doranz1

1Integral Molecular, Inc., Philadelphia, PA, United States, 2Blood Systems Research Institute, San Francisco, CA, United States

To obtain anti-Chikungunya (CHIKV) Envelope monoclonal antibody (MAb) epitope maps at the resolution of individual amino acids, we individually mutated 920 residues of CHIKV (S27 strain) Envelope protein (E2/E1) to alanine, expressed each mutant in human cells, and analyzed them for effects on antibody reactivity and viral infectivity. This ‘Shotgun Mutagenesis’ approach offers the capability of mapping both linear and conformational epitopes, even for structurally complex proteins including oligomeric and glycosylated Envelope proteins such as CHIKV E2/E1. The neutralizing human anti-CHIKV MAbs used in our studies were derived from infected patient B-cells using phage display library panning against purified CHIKV virus like particles (VLPs) and from B-cell cloning. Critical amino acids required for the binding of each MAb were identified and visualized on the E2/E1 protein structure. We also determined the binding affinity and kinetics of these MAbs to intact CHIKV VLPs on a biosensor. Our goal is to map epitopes on CHIKV Env protein, determine how they
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

University of Texas Medical Branch, Galveston, TX, United States

Western equine encephalitis virus (WEEV), is a member of the family Togaviridae, genus Alphavirus, has a single-stranded, positive-sense RNA genome, and is an important mosquito-transmitted human and veterinary pathogen in North and South America. Infection with WEEV can result in severe neurological sequelae in human survivors, with an economic impact ranging from 21,000 to 3 million dollars per case. WEEV is also considered a bioterrorism agent since aerosolized virus causes high primate mortality. Regrettably, there is no vaccine or antiviral therapies to aid in mitigating a natural outbreak, bioterrorist attack, or accidental lab exposure. The objective of this study was to develop a safe and efficacious WEEV vaccine. 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 passaging 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, bioterrorism 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 KIR2DS3, KIR2DS4 and KIR2DL3 were statistically different between Andeans and Mestizos. When using ethnic admixture proportion, significant differences were observed for KIR3DL1 and KIR2DS4s in addition to those genes, among the three groups defined. No significant differences were detected in haplotypes and inhibitory-activating KIR genes using either the questionnaire or AIM-based classification. AIM helps minimizing both the bias in ethnic group 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. Edgel, 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 incidence 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),...
to explore the prevalence of human infection to these viruses among
SFV and herpes viruses in captive NWPs in Peru. We plan follow-up studies
they are human or NWP species. Our results show a high prevalence of
herpes virus. Molecular characterization of the viruses is ongoing to
Saimiri. Twenty-one (15%) of 144 tested NWPs were PCR positive for
and Ateles, Callicebus, Callithrix, Cebus, Lagothrix, Pithecia, Saguinus
positive for SFV, including animals from the genera
(antibody in retroviruses correlates with active virus infection), and for
SFVs by enzyme immunoassay, with confirmatory Western blot analysis
of infection of these zoonotic viruses to humans with exposure to these
fomites, often resulting in a fatal outcome. We assessed the prevalence
from their reservoirs to accidental hosts through direct contact and
herpes virus and its reservoir species. Herpes viruses are easily transmitted
that infect a variety of animals, with co-evolution between each unique
Callithrix, and Cebus, Saimiri, Ateles, Callicebus, and Callithrix.
These data are limited to small numbers of captive monkeys from genera
Previous reports show genetically distinct SFV variants among NWPs but
pathogens. Simian foamy viruses (SFV) are retroviruses found in high
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frequently illegally captured for pet trade, traditional medicine, or
Many species of New World primates (NWPs) exist in Peru and are
often illegally captured for pet trade, traditional medicine, or
consumption. Government confiscation and placement of these animals
has led to extreme crowding in zoos and rescue and rehabilitation centers,
providing ideal conditions for animal-human transmission of zoonotic
pathogens. Simian foamy viruses (SFV) are retroviruses found in high
prevalence in various simian species. Infection has occurred in humans
exposed to Old World monkeys and apes in captivity and in nature.
Previous reports show genetically distinct SFV variants among NWPs but
these data are limited to small numbers of captive monkeys from genera
Cebus, Saimiri, Ateles, and Callithrix. Herpes viruses are ubiquitous agents
that infect a variety of animals, with co-evolution between each unique
herpes virus and its reservoir species. Herpes viruses are easily transmitted
from their reservoirs to accidental hosts through direct contact and
fomites, often resulting in a fatal outcome. We assessed the prevalence of
SFV and herpes virus infection in captive NWPs to help assess the risk
of infection of these zoonotic viruses to humans with exposure to these
animals. Serum samples were collected and tested for antibodies to
SFVs by enzyme immunoassay, with confirmatory Western blot analysis
(antibody in retroviruses correlates with active virus infection), and for
herpes virus by PCR. Sixty (38%) of 157 tested NWPs were antibody
positive for SFV, including animals from the genera Alouatta, Aotus,
Ateles, Callicebus, Callithrix, Cebus, Lagothrix, Pithecia, Saguinus and
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herpes virus. Molecular characterization of the viruses is ongoing to
identify the specific viruses and, in the case of the herpes viruses, whether
they are human or NWP species. Our results show a high prevalence of
SFV and herpes viruses in captive NWPs in Peru. We plan follow-up studies
to explore the prevalence of human infection to these viruses among
Peruvians in contact with captive NWPs.

**SIMIAN FOAMY VIRUS AND HERPES VIRUS IN CAPTIVE NEW WORLD PRIMATES IN PERU**

Bruno M. Ghersi¹, Ana Patricia Mendoza², Hugo Raizuri¹, Ada Romero¹, Andrew Bennett³, William M. Switzer⁴, Hongwei Jia⁴, Joe Zunt⁵, Daniel G. Bausch⁴, Joel M. Montgomery⁴

¹Naval Medical Research Unit No. Six, Lima, Peru, ²Wildlife Conservation Society, Lima, Peru, ³Tulane School of Public Health and Tropical Medicine, New Orleans, LA, United States, ⁴Centers for Disease Control and Prevention, Atlanta, GA, United States, ⁵University of Washington, Seattle, WA, United States

Many species of New World primates (NWPs) exist in Peru and are
frequently illegally captured for pet trade, traditional medicine, or
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has led to extreme crowding in zoos and rescue and rehabilitation centers,
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Cebus, Saimiri, Ateles, and Callithrix. Herpes viruses are ubiquitous agents
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to explore the prevalence of human infection to these viruses among
Peruvians in contact with captive NWPs.

**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 Cuong¹, Jeremy Farrar¹, Eddie Holmes³, Tran Thinh Hien³, Alex Cook⁴, Cameron Simmons⁴, Ottar Bjornstad⁴, C. Jessica E. Metcalf⁴, Nguyen van Vinh Chau⁴, Bryan T. Grenfell⁴, 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
HANTAVIRUS RODENT RESERVOIRS IN BULGARIA

Mustafa A. Aboualy1, Iva Christova2, Hristo Dimitrov3, Emad W. Mohareb1

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 due to respiratory 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.

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 Mukunzi2, Finniey Osuna2, James Njiri2, Janet Nyambura1, Julia Wangui1, Benjamin Opot1, Steven Ocholla1, Rose Nyawira1, Eyako Wurapa2

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 NA's natural substrate and bind 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 (Y225) in influenza A/H3N2. 64% (7) of the 2008 seasonal influenza A/
H1N1 isolates had resistant marker H275Y. 4 (36%) of the seasonal A/ H1N1 isolates, lacked the drug resistant marker depicting sensitivity to the class of drugs. Genetic analysis of the 48 pandemic influenza A/ H1N1 strains in 2009 showed that all were sensitive to oseltamivir through possession of histidine at position 274 of the neuraminidase protein sequence. The same pattern was duplicated in 2 of the pandemic influenza A/ H1N1 isolates analyzed in the year 2010. All the 14 isolates belonging to influenza A/H3N2 subtype lacked the H275Y substitution in the neuraminidase protein. Genotypic data obtained in this study demonstrate antiviral resistance in seasonal influenza A/H1N1 viruses isolated in Kenya in 2008-2009 through possession of H275Y (N1 numbering) marker in the neuraminidase protein.

APPLICATION OF IN VIVO IMAGING IN THE CHARACTERIZATION OF OLFACTORY INFECTION OF MICE WITH WEEV

Aaron T. Phillips1, Charles B. Stautf1, Tawfik A. Aboellail1, Ann M. Powers2, Ken E. Olson1

1Colorado State University, Fort Collins, CO, United States, 2Division of Vector-Borne Infectious Diseases, Centers for Disease Control and Prevention, Fort Collins, CO, United States

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 led to the infection of the anterior olfactory nucleus, basal ganglia, hypothalamus, amygdala, thalamus, hippocampus, and cerebrum. IHC staining showed intense neurotropism with very few supportive cells infected. Neuronal processes were highly stained for FLUC expression and presented patterns consistent with dissemination of virus through neuronal connectivity. Immunopositive axons were often seen in areas connecting immunopositive foci, even when foci were separated by substantial distances. An additional route of neuroinvasion through the trigeminal nerve pathway was observed and resulted in significant reporter expression within the brainstem (pons). Although recombinant virus was observed to be attenuated when compared to wild-type virus in both replication kinetics and induction of immunological markers of disease, manifestation of disease was comparable. Therefore, we feel that this system provides a quantifiable determination of WEEV infection. This may prove beneficial to future assessments of antiviral strategies aimed at treating disease arising from olfactory infection with New World alphaviruses.

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

John P. McCracken1, Wences Arvelo2, Maria Renée López2, María Luisa Müller1, Chris Bernart1, Antonio Paredes3, Fabiola Moscoso1, Jennifer Gray1, Alejandra Estévez1, Juan Carlos Moir4, Jose Ortiz5, Kim Lindblade2

1University of the Valley, Guatemala City, Guatemala, 2International Emerging Infections Program, Centers for Disease Control and Prevention Regional Office for Central America and Panama, Guatemala, 3Ministry of Public Health and Social Assistance, Guatemala City, Guatemala, 4Ministry of Public Health and Social Assistance, Quetzaltenango, Guatemala, 5Instituto Guatemalteco de Seguridad Social, Guatemala City, Guatemala

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 persons ≥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=0.7, 95% CI: 0.6, 0.8), mechanical ventilation use (OR=0.7, 95% CI: 0.5, 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.

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

Amruta Dixit1, Curtis Condon2, Ryan Ramos2

1University of California Irvine, Irvine, CA, United States, 2County of Orange Health Care Agency, Santa Ana, CA, United States

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 Ram,1 Manoshi Islam,2 Kaniz Jannat3, Margaret DiVita1, Emily Cercone1, Kimberly Rook1, Eduardo Azizz-Baumgartner1, Badrul M. Sohel2, W. Abdulllah Brooks2, Jihneee Yu1, Alicia M. Fry2, Stephen P. Luby2

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, 4International 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/or 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 index case-patient households 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 Hattendorf1, Ani Gil2, Hector Verastegui2, Daniel Mausezahl1

1Swiss Tropical and Public Health Institute, University of Basel, Basel, Switzerland, 2Instituto de Investigacion 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-blinding 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 takers 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-Baumgartner1, Timothy M. Uyeki2, Robert H. Gilman3, 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...
INCIDENCE OF HUMAN METAPNEUMOVIRUS IN RURAL AND URBAN POPULATIONS IN KENYA, 2006-2011

Victor Omballa1, Leonard Cosmas1, Allan Audi1, Gilbert Kikukwai1, Juliette Ongus1, Dean Erdman1, Joe Oundo1, Godfrey Bigogo1, Daniel Feikin2, Barry Fields3, Robert Breiman2, 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 by 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 - 24 years [14.6/1000 pyo (95%CI 14.5 - 14.7)]. In Kibera, 33% (14/43) 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.

ROLE OF TEMPERATURE, HUMIDITY AND RAINFALL ON INFLUENZA TRANSMISSION IN GUATEMALA, EL SALVADOR AND PANAMA

Radina P. Soebiyanto1, Nivaldo Linares-Perez2, Luis Bonilla3, Jorge Jara4, John McCracken5, Eduardo Aziz-Baumgartner6, Marc-Alain Widdowson7, Richard Kiang1

1NASA Goddard Space Flight Center, Greenbelt, MD, United States, 2Influenza Program, Centers for Disease Control and Prevention Regional Office for Central America Region, Guatemala, Guatemala, 3Centro de Estudios en Salud, Universidad del Valle de Guatemala, Guatemala, Guatemala, 4Centers for Disease Control and Prevention Influenza Division, Atlanta, GA, United States

Worldwide, seasonal influenza causes about 500,000 deaths and 5 million severe illnesses per year. The environmental drivers of influenza transmission are poorly understood especially in the tropics. We aimed to identify meteorological factors for influenza transmission in tropical Central America. We gathered laboratory-confirmed influenza case-counts by week from Guatemala City, San Salvador Department (El Salvador) and Panama Province from 2006 to 2010. The average total cases per year were: 390 (Guatemala), 99 (San Salvador) and 129 (Panama). Meteorological factors including daily air temperature, rainfall, relative and absolute humidity (RH, AH) were obtained from ground stations, NASA satellites and land models. For these factors, we computed weekly averages and their deviation from the 5-yr means. We assessed the relationship between the number of influenza case-counts and the meteorological factors, including effects lagged by 1 to 4 weeks, using Poisson regression for each site. Our results showed influenza in San Salvador would increase by 1 case within a week of every 1 day with RH>75% (Relative Risk (RR)= 1.32, p=.001) and every 1°C increase in minimum temperature (RR=1.29, p=.007); it would decrease by 1/20 cases for every 1mm-above mean weekly rainfall (RR=0.93, p<.001) (model pseudo-R²=0.58). Within 2 weeks, influenza in Panama was increased by 1 case for every 1% increase in RH (RR=1.04, p=.003), and it was increased by 2 cases for every 1°C increase of minimum temperature (RR=2.01, p<.001) (model pseudo-R²=0.4). Influenza counts in Guatemala had 1 case increase for every 1°C increase in minimum temperature in the previous week (RR=1.21, p<.001), and for every 1mm/day-above normal increase of rainfall rate (RR=1.03, p=.03) (model pseudo-R²=0.54). Our findings that cases increase with temperature and humidity differ from some temperate-zone studies. But they indicate that climate parameters such as humidity and temperature could be predictive of influenza activity and should be incorporated into country-specific influenza transmission models.
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, Capitán Zeuz Capitán1, Javier Nieto1, Melissa Nieto1, José Calzada1, Hector Paz1, Mara Kreishman-Deitrick2, Karen Kopydlowski2, Diane Ullman2, William McCarthy2, Janet Ransom3, Chuck Scott3, William Ravis4, Max Grogl5

1Gorgias 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% reapethelization of the lesion by Day 63 and 100% reapethelization 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 MALARIASIA IN SABAH, MALAYSIA: HIGH INCIDENCE BUT NO MORTALITY WITH EARLY REFERRAL AND ARTESUNATE THERAPY

Bridget E. Barber1, Timothy William2, Matthew J. Grigg1, Jayaram Menon3, Nicholas M. Anstey4, Tsun W. Yeo4

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 P. knowlesi, P. falciparum 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 monoinfection. 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 hyperparasitemia (>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), anemia (N=2) and abnormal bleeding (N=2). Severe knowlesi malaria occurred in 27/57 (47%) patients ≥50 years old compared to 1/173 (15%) <50 years. However using logistic regression, only parasite density independently predicted severe malaria, excluding hyperparasitemia as a sole severity-criterion (OR [log-increase in density count] 2.01, p<0.0001). Nearly all (92%) patients with knowlesi malaria received artesiminin 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. P. knowlesi 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 Plasmodium 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 antimarial treatment during pregnancy. At delivery, we measured the prevalence of maternal anemia (Hb< 8g/dL), peripheral parasitemia, placental parasitemia (impression smear) and low birth weight (LBW) by number of IPTp doses administered during pregnancy. Among multigravidae, infants born to women who received no IPTp, or 1, 2, or 3 or more doses of IPTp had LBW prevalence of 28%, 21%, 18% and 15%, respectively. Spontaneous premature delivery occurred in 8%, 7%, 6% and 5% of infants born to women who received no IPTp, and 1, 2, or 3 or more doses of IPTp. Among multigravidae, significant trends by number of SP doses received were slightly higher in the Paromomycin Alone 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.
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. Tong²

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 bone and joint (57%) and pleuropulmonary (17%); endocarditis was uncommon (6%). The median length of stay was 23 days (mean 27, SD 16.6, range 2-68). 14% were readmitted within 1 year for related reasons. There were no 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 Goodhew¹, Shawn Hunter², Jan Zajdowicz³, 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 reduviid 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 Mayaudo, Cono Arito, 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=8,346), 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, 57.2; 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 Leishmaniosis, 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.

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

Grace Kaguthi1, Videlis 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 symptoms 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 investigated for TB through gastric aspirates, sputum induction, chest radiographs, HIV tests and TST. Definite cases were those culture confirmed and probable cases had Keith Edward(KES) 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 seroexposed 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, Dhillon Nyachieo1, John M. Williamson3, Leonard Cosmos4, Beatrice Olack2, George Okoth1, Henry Njuguina5, Lucy Nganga-Wanjuik6, Rebecca Bunnell7, Warren Dalal8, Daniel R. Feikin9, Heather Burke4, Katherine A. Robinson6, Nancy Knight9, Joel M. Montgomery5, Robert F. Breiman4

1Centers for Disease Control and Prevention, Nairobi, Kenya, ‘Kenyan Medical Research Institute, Centers for Disease Control and Prevention, International Emerging Infections Program, Nairobi, Kenya, 2Kenyan Medical Research Institute, Centers for Disease Control and Prevention, Influenza Program, Nairobi, Kenya, 3Centers for Disease Control and Prevention, Division of Global HIV/AIDS, 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 household contacts of HIV-infected residents 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 household 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-negative 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.
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 Ugarte3, Jorge Paz3, Juan Echevarria3, Eduardo Gotuzzo3

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 Baguïya1, Bertrand Méda1, Adjia Mariam Ouédraogo1, Aristide Romaric Bado1, Damien De Walque2, Seni Koundà2

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 or increase by their more risky sexual behaviors. This study aimed to compare PLHIVs’ 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 84.6% 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 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 condom 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 PLHIVs in Burkina Faso.

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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-1(e), 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+ (>0.048). However, proportions of CD14+CXCR4+ cells were reduced in the mal(-)/HIV-1- group relative to mal(+)/HIV-1- group (P<0.039) and mal(+)/HIV-1- group (P<0.077). 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+ group (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.
SPATIOTEMPORAL DYNAMICS OF CHOLERA EPIDEMICS IN THE FAR NORTH REGION, CAMEROON

Mohuman Arabi1, Ningchuan Xiao2, Song Liang3, Rebecca Garabed4, Mark Moritz5
1Department of Environmental Sciences, The Higher Institute of the Sahel University of Maroua, Maroua, Cameroon, 2Department of Geography, Ohio State University, Columbus, OH, United States, 3College of Public Health, The Ohio State University, Columbus, OH, United States, 4Centers for Disease Control and Prevention, Kisumu, Kenya, 5University of Maroua, Maroua, Cameroon

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 Rajasingham1, Richard Omore1, Maurice Ombo2, Alex O. Avuoor2, Fenny Moke3, John Vulu1, Kayla Laserson3, Tamer H. Farag4, Dilruba Nasrin1, Sandra Panchalingam2, James P. Nataro2, Karen L. Kutiolf1, Myron M. Levine2, Jeffrey Switchenko1, Tracy Ayers1, Gordana Derado1, Eric D. Mintz1, Robert F. Breiman6
1Centers 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, Kisumu, 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.

RELATIONSHIP BETWEEN USE OF WATER FROM COMMUNITY-SCALE WATER TREATMENT REFILL KIOSKS AND CHILDHOOD DIARRHEA IN JAKARTA

Laura C. Sima, Desai M. Mayur, Kathleen M. McCarty, Menachem Elimelech
Yale University, New Haven, CT, United States

In developing countries, safe piped drinking water is generally unavailable, and bottled water is unaffordable for most people. Purchasing drinking water from community-scale decentralized water treatment and refill kiosks (referred to as isi ulang depots in Indonesia) is becoming a common alternative. This study investigates the association between diarrhea risk and water kiosks. We monitored daily diarrhea status and water source for 1,000 children aged 1–4 years in Jakarta, Indonesia, for up to 5 months. Among children in an urban slum, rate of diarrhea per 1,000 children-day varied significantly by primary water source: 8.13 for tap water, 3.60 for bottled water, and 3.97 for water kiosks. In multivariable Poisson regression analysis, diarrhea risk remained significantly lower among water kiosk users (adjusted rate ratio [RR] = 0.49, 95% confidence interval [CI] = 0.29–0.85) and bottled water users (adjusted RR = 0.44, 95% CI = 0.21–0.94), compared with tap water users. Purchasing water from low-cost water kiosks is associated with a reduction in diarrhea risk similar to that found for bottled water.

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

Md. Fosial A. Nizame1, Md. Nuruzzaman1, Notan C. Dutta1, Elli Leontsini2, Pavani K. Ram3, Peter J. Winch2, Shahana Parveen1, Stephen. P. Lub1, 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 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.
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.

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INACTIVATION OF HELMINTH IN A SOLAR CONCENTRATOR

Andrew M. Foote1, Emily Woods1, Fernando Fredes2

1Georgia Institute of Technology, Atlanta, GA, United States, 2University of Chile, Santiago, Chile

More than 1 billion people worldwide are infected with helminths. Typical pit latrines and composting conditions do not inactivate helminths in faecal matter effectively. By concentrating solar energy and reaching pathogen inactivation temperatures (50°C and higher), a solar concentrator, with projected capital costs of $0.30 per person per year, has the potential to inactivate helminths in faecal matter. The goal of this work was to evaluate the efficacy of a solar concentrator in inactivating helminth in faecal matter and meet World Health Organization (WHO) guidelines for safe disposal and reuse of faecal matter. Inactivation was assessed by evaluating the viability of *Toxocara canis* eggs. *T. canis* is a helminth in the same taxonomic order as *Ascaris lumbricoides*, which is a WHO indicator for safe faecal disposal and reuse. Three trials were conducted from December 2011 through February 2012 in Santiago, Chile. The first two trials evaluated *T. canis* viability daily. To calculate the inactivation rate for the solar concentrator unit, the third trial evaluated *T. canis* viability hourly. In each trial, *T. canis* eggs were isolated from canine faecal matter, concentrated, placed in semi-permeable tea bags (1,500 eggs each) and inoculated into 40 liters of fresh canine faecal matter. *T. canis* eggs were incubated into two control conditions: indoors in the dark and in a mimic pit latrine. At the end of each trial, eggs were incubated and classified as viable if they contained a motile larva. In all 3 trials, temperatures reached 60°C at the center and 70°C at the edges of the solar concentrator for at least 4 hours daily. During all three trials, after one day in the solar concentrator, the die-off of eggs was greater than 99%. In the third trial, the inactivation rate was 0.67 Log10 eggs/hr and there was 99% inactivation after 4 hours. These results suggest that a solar concentrating unit can be used to rapidly inactivate helminths in faecal matter, and therefore, faecal matter treated by a solar concentrator can be safely disposed and reused on edible crops.

CONTINUOUS ENVIRONMENTAL FECAL CONTAMINATION FOLLOWING IMPLEMENTATION OF SANITATION HARDWARE

Sania Ashraf1, Faruque Hussain1, Eli Leontis2, 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 hoe like 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 households 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 defeation 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 produced 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 OFANOPELLES GAMBIAE AND ITS ROLE IN INSECTICIDE RESISTANCE

Christopher M. Jones1, Fiacre R. Agossa2, Toë 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.

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THE EVOLUTION OF RESISTANCE TO CARBAMATES AND OXONOPHOSPHATE INSECTICIDES IN *ANOPOHELES GAMBIAE*

Luc S. Djogbéno1, David Weetman2, Roch Dabiré3, Guillaume Ketto4, Fabrice Chandre5, Mylène Weill6, Martin Donnelly7

1 Institut Régional de Santé Publique/Université d’Abomey-Calavi, Ouidah, Benin, 2Liverpool School of Tropical Medicine and Hygiene, Liverpool, United Kingdom, 3RSS-Centre Muras, Bobo-Dioulasso, Burkina Faso, 4URETIUniversité de Lomé, Lomé, Togo, 5Laboratoire de Lutte contre les Insectes Nuisibles/IRD, MIVEGEC, Montpellier, France, 6Institut des Sciences de l’Évolution de Montpellier/Université Montpellier, Montpellier, France

Determining the number of origins of insecticide resistance-associated mutations is important not only from an evolutionary perspective but also for modelling its spread, which is increasingly important for the implementation and monitoring of malaria vector control programmes. In the mosquito *Anopheles gambiae* s.s., the main African vector of malaria, organophosphate (OP) and carbamate resistance is strongly associated with a single amino-acid substitution (G119S) in the insecticide target site, acetylcholinesterase 1 (Ace-1). This mutation apparently incurs a high fitness cost in the absence of insecticidal pressure. Recently, a duplication of the Ace-1 locus was observed in *An. gambiae*, which results in a wildtype and resistant-associated allele occurring on the same haplotype (Ace-1′). It is proposed that the Ace-1′ haplotype is in effect a “fixed” heterozygote that may confer similar levels of resistance but with reduced fitness effects. We have screened for diversity in and around the Ace-1 locus in mosquitoes from a large number of sites in West Africa.

**Results**

- **Early Stage**
  - The duplication of the Ace-1 locus is confirmed in field populations. Overall, these results suggest 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.

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DISSECTING THE MOLECULAR BASIS OF PYRETHROID RESISTANCE IN FIELD POPULATIONS OF THE MAJOR MALARIA VECTOR *ANOPOHELES FUNESTUS* IN SOUTHERN AFRICA

Jacob M. Riveron, Helen Irving, Miranda Ndula, Kayla G. Barnes, Charles S. Wondji

Liverpool School of Tropical Medicine, Liverpool, United Kingdom

*An. 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 CYP6P9b with a fold-change (FC) $>88$, then CYP6P9a (FC~60) and CYP6M7 (FC~25). Interestingly in Malawi, CYP6P9a 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 CYP6P9b and CYP6P9a but less for CYP6M7. Transgenic In vivo expression of CYP6P9a and CYP6P9b using the GAL4/UA 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.
Institut de Recherche pour le Développement, Montpellier, France,
Nicolas Moiroux

INSECTICIDAL NETS IN BENIN
FOLLOWING UNIVERSAL COVERAGE OF LONG-LASTING
CHANGES IN BITING BEHAVIOR
demonstrates the exceptional adaptability of An. gambiae s.l with L1014S kdr mutation was identified along the study. This study with more infected mosquitoes in S form. No specimen difference in CSP rate was also observed between taxa groups (p=0.004) (25.45%) than in RS (14.67%) or SS groups (p<0.001). A significant groups with significantly more CSP positive specimens in the RR group After August 2008, a significant difference was observed among kdr in RS group and 3.83% in SS group, p=0.6) or in taxa groups (p=0.6). LLINs, CSP rate was not significantly different between kdr groups (4.55% year round before LLINs disappeared after. Before the implementation of LLIN's. Adult mosquitoes were collected by HLC monthly from January 2006 to December 2011 and by PSC during the rainy season. Mosquitoes were identified down to their species and sub-species by PCR. The presence of circumsporozoite protein (CSP) of P. falciparum and the blood meal origin was detected by ELISA, 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 along 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. Gomez3, Emmanuel Elanga3, Innocent Djegbé2, Armel Djenontin2, Hélène Guis3, Vincent Corbel2

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. Müller2, John C. Beier3
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 LLIN/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 larvicides 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 Hoeve1, Tatiana M. Lopera-Mesa1, Saibou Doumbia1, Drissa Konaté3, Mory Doumbia3, Jennifer M. Anderson1, Leopoldo Santos-Argumedo4, Ana Rodriguez2, Michael P. Fay5, Mahamadou Diakité6, 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 Infectious Diseases, Instituto Nacional de Salud, Lima, Peru, 4Department of Molecular Biomedicine, Centro de Investigación y Estudios Avanzados – Instituto Politécnico Nacional, Mexico City, Mexico, 5Institute of Biostatistics 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 (NCSM, N=68). In 69 of these children, we measured these same factors during their malaria episode and twice with or without uricase, which degrades UA. Our preliminary results show that Pf-derived UA precipitates activate EC, we co-cultured 3D7-infected red blood cells with or without uricase, which degrades UA. 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 (PfRBCs) with primary microvascular endothelial cells (MVECs) with or without uricase, which degrades UA. Our preliminary results show that PfRBCs 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 Goto2, Kazuhide Yahata3, Naoaki Yokoyama1, Satoru Kawai4, 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 antisera 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-
developed. Serial micro-ultrasound revealed zones of decreased echogenicity in the periphery of egg granulomas in macropbage-depleted versus -replete mice, suggestive of relative hypopcellularity. This was confirmed by histology, which revealed hypopcellular cavitations in macropbage-depleted granulomas, less fibrosis, and fewer infiltrating leukocytes. None of the control vehicle-treated mice receiving eggs died, whereas 60% of macropbage-depleted mice receiving high doses of eggs died by day 11 post-egg injection, indicating a crucial role for macropbages in prevention of detrimental systemic effects of helminth exposure. Our results confirm a critical role for macropbages in schistosomal bladder pathogenesis, even in the setting of a single exposure to *S. haematobium* eggs. This suggests that macropbages may be a suitable therapeutic target for advanced schistosomal bladder pathogenesis.

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**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 during development. Expression of immunogenic glycan elements such as 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 were abundant in the adult stages.

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

Jingwen Lin¹, Elena Aime², Mohammed Sajjd³, Blandine Franke-Fayard¹, Frans Prins¹, Philippe E. Van den Steen³, Katrien Deroost³, Roberta Spaccapelo³, Chris J. Janse³, Shahid M. Khan³

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 intraerythocytic 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 semic-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, ⁴pm4apbp₂, lacking expression of both plasmepsin-4 (equivalent to *P. falciparum* plasmepsin 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 crystalline product formed by detoxification of heme that is released earlier in Hb digestion. This was confirmed by examining hemozoin deposition in both liver and spleen of ⁴pm4apbp₂ 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.
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 Ubl functions in human parasitic organisms are limited. Recently, we described the existence of a novel Ubl named ubiquitin-fold modifier 1 (Ufm1) that conjugates to parasite proteins in Leishmania donovani. To elucidate the enzymatic activities associated with Ufm1 conjugation, we identified a putative Ufsp in the trypanosomatid genomes. Biochemical analysis of L. donovani Ufsp showed that this protein possesses the 3′hydrolase 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 knock out 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 Browaes2, Silke Leutner3, Manon Morel1, Christophe G. Grevelding1, Katia Cailliau1, Colette Duss1

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 euetazonae 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 ovaries. 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.

**DETECTING CRYPTIC GENETIC EXCHANGE IN TRYPANOSOMA CRUZI TCI**

Louisa A. Messenger1, Martin S. Llewellyn1, Tapan Bhattacharya1, Oscar Franzen2, Michael D. Lewis1, Juan-David Ramirez3, Hernan J. Carrasco4, Bjorn Andersson2, Michael A. Miles1

1London School of Hygiene and Tropical Medicine, London, United Kingdom, 2Karolinska Institutet, Stockholm, Sweden, 3Universidad de los Andes, Bogota, Colombia, 4Universidad Central de Venezuela, Caracas, Bolivarian Republic of Venezuela

Clonal propagation is considered to be the predominant mode of reproduction among many parasitic protozoa. However, this assumption may overlook unorthodox, infrequent or cryptic sexuality. Trypanosoma cruzi, the causative agent of Chagas disease, is known to undergo non-Mendelian recombination in vitro, while evidence of natural genetic exchange is more limited. T. cruzi displays remarkable genetic heterogeneity and is recognized as a complex of six discrete typing units (DTUs). Infection with T. cruzi is lifelong and can lead to a spectrum of pathological complications. However, the relationship between specific clinical outcomes and parasite genotype remains elusive. The availability of whole genome sequences has driven advances in high-resolution genotyping techniques and re-invigorated interest in exploring the genetic diversity present within the various DTUs. We developed a highly resolutive mitochondrial multilocus sequence typing (mtMLST) scheme, which was evaluated against current nuclear typing tools using isolates belonging to the oldest and most widely occurring lineage TcI. In parallel, we explored read depth data, generated by illumina sequencing of the mitochondrial genome from the TcI reference strain to investigate the existence of mitochondrial heteroplasmy (heterogeneous mitochondrial genomes in an individual cell) in T. cruzi and resolve its role as a potential source of genotyping error. Comparison of nuclear and mitochondrial data uncovered multiple novel mitochondrial introgression events among disparate geographical populations as well as between major T. cruzi DTUs. Illumina sequencing data from the TcI genome strain revealed multiple different mitochondrial genomes within an individual parasite (heteroplasmy) that were, however, not sufficiently divergent to represent a major source of typing error. mtMLST provides a powerful approach to genotyping at the sub-DTU level. This strategy will facilitate attempts to resolve phenotypic variation in T. cruzi and to address epidemiologically important hypotheses in conjunction with intensive spatio-temporal sampling. The observations of gross nuclear mitochondrial phylogenetic incongruence indicate that genetic recombination is geographically widespread and continues to influence the natural population structure of TcI, a conclusion which challenges the traditional paradigm of clonality in T. cruzi.

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

Juliana M. Sa1, Sarah R. Kaslow1, Jiangbing Mu1, Evan Kessler1, Soundarapandian Velmurugan2, Adam Richman1, Yonas F. Abebe1, Eric R. James2, B. K. Sim2, Stephen L. Hoffman2, Robert W. Gwadz1, Thomas E. Wellems1

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 × 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, pvcr1-o) resides in one of these chromosome regions.

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

Sachel Mok1, Mallika Imwong2, Kek-Yee Ljong1, Zhaoting Lin1, Wai-Hoe Chin1, Joan Sim1, Poravuth Yi4, Mayfong Mayxay2, Kesinee Chotivanich3, Bruce Russell2, Duong Socheat1, Paul N. Newton4, Nicholas P. Day6, Nicholas J. White6, Peter R. Preiser1, Francois Nosten7, Arjen M. Dondorp6, 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, 6Singapore, 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 makers or 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 UbH5a-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 Dek2, Vorleak Try2, Sokunthea Sreng3, Seila Suon1, Sivanna Mao4, Chantha Soph4, Baramey Sam3, Elizabeth A. Ashley3, Arjen M. Dondorp5, Nicholas J. White6, Jennifer M. Anderson1, Chanaki Amarasingha1, 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, 4 MAKARA 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.

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

María B. Jiménez-Díaz1, Sara Viera1, Javier Ibáñez1, Noemi Magán-Marchal1, Helena Garuti1, Lorena Cortés-Gil1, Vanessa Gómez1, Teresa Mulet1, Francisco-Javier Gamo1, Laura M. Sanz1, Didier Leroy2, 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 (PRR) 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 murine model can be a valid model to study the development of resistance caused by sub-therapeutic treatment.

**ARTEMETHER-LUMEFANTRINE SELECTS FOR MALARIA PARASITES WITH DECREASED LUMEFANTRINE SENSITIVITY ALTHOUGH PARASITES REMAIN SENSITIVE TO THIS REGIMEN IN TORORO, UGANDA**

Patrick K. Tumwebaze1, Jessica Bloome2, Oswald Byaruhanga1, Christine Nakazibwe1, Andrew Walakira1, Jaffer Okiring1, Samuel L. Nsobya1, Roland A. Cooper3, Philip J. Rosenthal2

1Makerere University-University of California San Francisco Research Collaboration, Kampala, Uganda, 2University of California, San Francisco, CA, United States, 3Dominican University, San Rafael, CA, United States

Artemisinin-based combination therapies (ACTs) may select for malaria parasites with decreased drug sensitivity. We studied the sensitivity of parasites from children enrolled in treatment and prevention trials in Tororo, Uganda from June, 2010 to February, 2012. When *Plasmodium falciparum* malaria was diagnosed, blood was obtained, parasites (286 isolates) were cultured with serial dilutions of chloroquine (CQ), monodesethylamodiaquine (AQ), quinine (QN), dihydroartemisinin (DHA), lumefantrine (LM), or piperaquine (PQ) for 72 h, and *ex vivo* sensitivities were assessed by HRP-2-based ELISA. Sensitivities (nM) to CQ (median IC50 486.2; IQR 206.5-748.8), AQ (83.3; 58.4-132.4), PQ (20.3; 7.6-47.5) and QN (126.4; 74.9-196.3) varied widely; parasites were highly sensitive to LM (2.7; 0.97-6.7) and DHA (1.7; 1.0-2.8). IC50 values for 4 successive quartiles, each with 71-73 isolates collected over ~4 months, varied little for control strains (3D7 IC50s 0.54-1.8 nM) and all drugs except LM and PQ. For PQ, sensitivity decreased after the first quartile (median IC50 6.5), but was then stable (26.6-32.0). For LM, IC50s were low, but increased consistently (successive quartiles 1.1, 2.3, 3.5, and 6.2, p<0.05 for all comparisons except 2nd-3rd quartiles). Receiving monthly DHA-PQ in a prevention trial was not associated with changes in PQ sensitivity compared to those receiving placebo. Having received artemether-LM (AL) within 60 days as treatment for a prior episode of malaria was associated with decreased sensitivity to LM (median IC50 1.59 for those without [n=84] vs. 3.24 for those with [n=119] recent AL; p=0.022 [GEE regression with log IC50 values]), but no significant change in sensitivity to the other study drugs. In summary, recent isolates of *P. falciparum* in Tororo were highly sensitive to components of AL, the national treatment regimen, but treatment with AL selected for parasites with decreased sensitivity, and overall sensitivities decreased from 2010 to 2012. Longitudinal surveillance of sensitivities of parasites under different selective drug pressures continues.
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, Francois Nosten2, Rick M. Fairhurst3, Arjen M. Dondorp4, Duong Socheat5, Anders Björkman6, Andreas Märtensson7, Steffen Borrmann7, Mayfong Mayxay8, Paul N. Newton6, Delia Bethel9, Youyi Se9, Harald Noedl10, Abdoulaye A. Djimde11, Nicholas J. White6, Philippe J. Guerin1

1WWARN, Oxford, United Kingdom, 2Shoklo Malaria Research Unit, Maesot, 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, Prom 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 Barnako, Barnako, 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 between studies. 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 vitro 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 artelnic 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 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 Jiménez2, Amel Hamad4, Betuel Siquaué2, Bijender Singh2, Llorec Quinto2, Sanjeev Kumar2, Puneet Gupta2, Virander S. Chauhan2, Carlota Dobaño1, Pedro L. Alonso1, Clara Menéndez1, Chetan E. Chitnis2

1Barcelona Center for International Health Research (CRESIB), Hospital Clinic-Universitat de Barcelona, Barcelona, Spain, 2International Centre for Genetic Engineering and Biotechnology, New Delhi, India, 3Centro de Investigación en Salud de la Manchíca (CISM), Manchíca, 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, DBL6c) and other blood stage antigens (DBLγy, DBLδ, 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, DBLγy 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 (DBL3X and DBL6c), 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-bearing 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 Anderson3, Peter Mungai1, Penny Holding1, Christopher King1, Arlene Dent1
1Rainbow Babies and Children’s Hospital, Cleveland, OH, United States, 2Center for Global Health and Diseases, Case Western Reserve University, Cleveland, OH, United States, 3Case 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), Plasmodium 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 ANTIGEN-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, Msambweni, 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 tolerogenic fetal natural (CD25hi, Fox3+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 Färnert3
1Kenya Medical Research Institute-Wellcome Trust Research Programme, Kilifi, Kenya, 2Nyamasiti 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
we have identified putative CD8+ T-cell epitopes on CSP and AMA-1, immunized HLA-A*01:01+ and HLA-B*18:01+ volunteers. We have also recorded 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 Kim4, Bjorn Peters5, Maria Belmonte1, Hanni Ganeshan6, Jun Huang7, Cindy Tamminger1, Alessandro Sette8, Carter Diggs9, Lorraine Soisson10, Michael R. Hollingdale9, Thomas L. Richie11, Martha Sedegah11

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 epoites 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 establish the total magnitude of the vaccine-induced CD8+ T cell response, we have identified putative CD8+ T-cell epitopes on CSP and AMA1, using PBMC samples from volunteers immunized with a single dose of serotype 5 adenovirus expressing two malaria antigens, CSP and AMA1 (AdCA). We next prepared a series of tetramers, each consisting of one of seven HLA-A or five HLA-B class I molecules and each presenting one of 16 distinct CSP epitopes or one of 11 distinct AMA1 epitopes. We then used the tetramer staining technique to label and thereby enumerate the total number of malaria antigen-specific CD8+ T cells in immunized HLA-A*01:01+ and HLA-B*18:01+ volunteers. We have also demonstrated that malaria tetramer-positive CD8+ T cells obtained three weeks post immunization express high levels of the CD38 and HLA-DR activation markers, raising the possibility that these markers might serve as a surrogate to detect multiple antigen-specific CD8+ T cells induced by whole parasite vaccines where the majority of antigens have not yet been identified. Work is also ongoing to carry out tetramer staining, ELISpot, and intracellular staining assays using pre-vaccination and post vaccination PBMC samples to look for comparability of results generated from the different assays.

### GENERATION OF NOVEL “HUMAN-IMMUNE-SYSTEM” HUMANIZED MOUSE STRAINS CO-EXPRESSING HLA CLASS I AND CLASS II MOLECULES IN NOD.RAGKO.II2RGKCO BACKGROUND

Sai Majji1, Rebecca Danner2, Eileen Villasante3, Thomas L. Richie1, Teodor D. Brummeanu2, Sofia A. Casares1

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.II2RgKCO 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.II2RgKCO 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, Lindsey 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 Anopheles gambiae immune system. The gene mapping was done in a F1 cross between 2 lines that differ in their survival in A. gambiae L3-5; GB4 successfully infects the mosquito while 7G8 is mostly eliminated by the mosquito 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.II2RGKCO 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.II2RgKCO 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.

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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. Vicente3, Amabelia Rodrigues1, Martin J. Donnelly1, Joao Pinto3, Alessandra della Torre1

1University of Rome Sapienza, Rome, Italy, 2Liverpool School of Tropical Medicine, Liverpool, United Kingdom, 3Institute of Hygiene and Medicine, Liverpool, United Kingdom, 41University of Rome “La Sapienza”, Rome, Italy, 5Institute of Hygiene and Tropical Medicine, Lisbon, Portugal, 6Instituto Nacional de Saúde Pública, Bissau, Guinea-Bissau, 7European Bioinformatics Institute, Hinxton, United Kingdom, 8Welcome Trust Sanger Institute, Hinxton, United Kingdom

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 westernmost 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 females (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.
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.

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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 (RexO 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 sRNAs (20-24nts) and pviRNAs (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 include miR155, miR2755, miR281 and miR277, among others. 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.

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GENETIC REGULATION OF VECTOR MOSQUITO SALIVARY GLAND DEVELOPMENT

Molly Duman Scheel1, Chiilinh Nguyen2, Zeinab Annan1, Emily Andrews1, Christy Le3, Longhua Sun2, Anthony Clemmons2, David W. Severson2
1Indiana University School of Medicine South Bend at Notre Dame, South Bend, IN, United States, 2University 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.

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

John M. Marshall1, Bruce A. Hay2
1Imperial College London, London, United Kingdom, 2California 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.

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VACCINATION WITH EXCRETORY/SECRETORY PRODUCTS CONFIERS 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 CpG/Alum and subsequently challenged with 40 infectious larvae. Mice that were vaccinated with LS antigen showed no significant protection against challenge infection compared to control; however issues arise from their potential to spread across international borders. 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.