effectiveness of this approach at estimating mosquito demographic and dispersal parameters, we develop an individual-based model of mosquito life history which labels each individual with a unique identification number. Using the dengue vector Aedes aegypti as a case study, we find the CKMR approach can provide unbiased estimates of adult census population size, adult mean dispersal distance, and adult and larval mortality rates for logistically feasible sampling schemes. Considering a simulated population of 3,000 adult mosquitoes, estimation of adult parameters is accurate when ca. 40 adult females are sampled bivieously over a three month period. Estimation of larval parameters is accurate when adult sampling is supplemented with ca. 120 larvae sampled bivieously over the same period. The methods are also effective at detecting intervention-induced increases in adult mortality and decreases in population size. As the cost of genome sequencing declines, CKMR holds great promise for characterizing the demography and dispersal of mosquitoes and other insect vectors of human diseases.

ADVANCEMENTS OF ARTIFICIAL INTELLIGENCE (AI) IMAGE RECOGNITION FOR USE IN VECTOR SURVEILLANCE OPERATIONS

Tristan Ford, Sameerah Talatha, Thomas Jenkins, Roy Fairman, Jewell Brey, Sanket Padmanabhan, Autumn Goodwin

Mosquito surveillance, the monitoring of vector abundance, distribution, and diversity, is a critical step to assess mosquito-borne disease risk. Despite its importance, few vector control organizations have access to expert taxonomists with the capacity to evaluate the significant quantities of specimens collected by routine vector surveillance programs. As a result, organizations must rely on seasonal staff with minimal taxonomic expertise to guide important downstream intervention strategies. Convolutional neural networks (CNNs) for image recognition, a deep learning method, have emerged as a promising modality with the capability to visually differentiate between species. The IDX is a system consisting of a controlled optical configuration integrated with algorithms that continue to evolve and improve based on representative specimen image data contributed by partners. In the most recent iteration, the system performed with an accuracy of 97.75±0.06% across 21 species in lab testing. In a test of the deployed algorithm performance on IDX, data from a California vector control district was analyzed, and achieved a macro averaged recall of 97.1% across the top five species. These advancements demonstrate translation of these algorithms from the lab to field practice. In the next phase of work, data collection and testing will be conducted with a wider range of partners in operational scenarios.

EVALUATION OF MYRISTICA FRAGRANS ESSENTIAL OIL AS A POTENTIAL BIOPESTICIDE FOR THE CONTROL OF AEDES MOSQUITOES IN JAMAICA

Mario Akeeno Joemaine Golding, Nadia K. Khouri, Simone L. Sandiford

University of the West Indies, Mona, Kingston, Jamaica

The use of synthetic insecticides to control Aedes mosquitoes continues to be a significant challenge in Jamaica’s fight against arboviruses due to rising resistance in mosquito populations. As a result, novel control methods are urgently needed. As the use of plant extracts as mollusccidal agents is currently being investigated worldwide, we therefore sought to assess the potential efficacy of Myristica fragrans (nutmeg), as a natural biopesticide. The nutmeg essential oil was screened against different life stages of Aedes aegypti. Formulations of the oil may be studied for prospective use as a biopesticide in Jamaica. Keywords: Biopesticides, Aedes mosquito, Jamaica

INTERIM EFFICACY REPORT OF ECO BIOTRAPS IN DHARAVI, MUMBAI, INDIA

Susanta Kumar Ghosh1, Mirgendra Pal Singh2, Prasad Phadke3, Nitin Khope3, Chetan Vijay Choubal4

1ICMR-National Institute of Malaria Research, Bangalore, India, 2ICMR-National Institute of Malaria Research, New Delhi, India, 3Ecobio Consulting Private Limited, Ahmedabad, India, 4Brihannam Municipal Corporation, Mumbai, India

We had previously presented an introductory note on the use of a green ovitrap device – Eco BioTrap for an innovative adjunct integrated vector management for vector control. This longitudinal cohort study was carried out in two areas Kumbharwada and Rajiv Gandhi Nagar covering approximately 100,000 population in 10,000 households of Asia’s largest slum Dharavi, Mumbai, India. Dharavi is endemic for dengue for several years. A set one trial with mosquito attractant and anti-larval IGR compound (Pyriproxyfen) and control was placed 10 to 15 meter apart in the study sites following the World Health Organization (WHO) guideline for mosquito larvaeicides. Two teams comprising of two trained health staff each conducted the study with a follow-up period on day 7, day 14, day 21 and day 28, respectively. Data were recorded on excel sheet and thus analysed. The three-month interim deployment study showed that Eco BioTraps are effective (93%) in attracting the gravid female mainly Aedes aegypti for ovipositing to lay eggs and is highly impactful in eliminating breeding as part of Source Reduction/Larval Source Management (LSM). This device can also be used for larval surveillance for evaluating of any intervention. Further study is underway to find out the long-term impact on the mosquito vector burden and dengue.

EVALUATION OF THE SYSTEMIC INSECTICIDAL EFFECTS OF IVERMECTIN TREATED CATTLE ON Aedes aegypti, VECTOR OF ARBOVIRUSES


1Université de Dédougou, Dédougou, Burkina Faso, 2Centre International de Recherche Développement sur l’Élevage en zone Subhumide, Bobo-Dioulasso, Burkina Faso, 3Institut de Recherche pour le Développement (IRD), Montpellier, France, 4Medincell, Montpellier, France, 5Université Nazir Boni, Bobo-Dioulasso, Burkina Faso, 6Institut de Recherche pour le Développement (IRD), Bobo-Dioulasso, Burkina Faso, 7Institut de Recherche en Sciences de la Santé, Centre National de Recherche Scientifique et Technologique (IRSS/CNFRST), Bobo-Dioulasso, Burkina Faso

The burden of Aedes-borne viral pathogens has increased over the past two decades. In the absence of specific treatments against the virus, insecticide-based vector control tools remain the best way to decrease transmission. However, the selection and spread of insecticide resistance in Aedes vectors call for the development of complementary vector control tools. In the IMPACT project funded by Unitaid Agency, we evaluated the systemic insecticidal effects of ivermectin injected to calves on the survival and fertility of A. aegypti, one of the primary vectors of arboviruses. A total
of 7,005 females of *Ae. aegypti* from two strains were used, an insecticide susceptible strain “Bora-Bora” and the “Bobo” strain, a recent colony developed from wild larva captured in Bobo-Dioulasso, Burkina Faso. Mosquitoes were directly blood fed on cattle injected with ivermectin at 1 mg/kg and 0.8 mg/kg of body weight. Survival, reproductive parameters were measured and compared between strains and in function of the dose and the delay after injection (DAI) at which blood feeding occurred. Our data show that all parameters but hatching rate were significantly decreased in Bora Bora fed on treated calves, with different magnitude in function of the ivermectin dose and the DAI: survival decreased by at least 50% until 14-21 days post treatment, mean number of females that laid eggs decreased by 45 to 50%, while the number of eggs laid by the females that remained alive decreased by 65 to 85% until 21 DAI. For the Bobo strain, survival decreased by 15% until 7 DAI for the 1mg/kg dose only, and hatching rate was the only reproduction parameter significantly impacted, with 20 to 40% eggs that were laid that didn’t hatch. These differences in ivermectin toxicity between Bora Bora and Bobo call for ivermectin differential susceptibility between strains. However, in both populations, the impacted parameters have all great importance in determining vectorial capacity. Hence, ivermectin could contribute to significantly reduce *Ae. aegypti* populations densities if the toxic effect is sustained over time, notably through the use of Long Acting Ivermectin Formulations.

**UNLEASHING NATURE’S SECRET WEAPON: TACKLING MOSQUITO MENACE WITH STREPTOMYCIES SP. KSF103**

Amelia Zheng Hua Yap, Pouya Hassandarvish, Van Lun Low
*University of Malaya, Kuala Lumpur, Malaysia*

The use of environmental-friendly techniques for mosquito control has garnered attention in recent years, and our study aimed to evaluate the potential of Streptomycies isolates for Culicidae control. The insecticidal property of Streptomycies sp. KSF103 ethyl acetate (EA) extract against mosquitoes and non-target organisms; its preliminary mode of action; and its effect on dengue virus 2 replication in mosquitoes were investigated. We used in vitro and in vivo experiments to determine the insecticidal activity of the EA extract. The results revealed that the EA extract exhibited strong cytotoxicity against C6/36 cells and considerable mortality in eggs, larvae and adults of all four important vector mosquitoes, namely *Aedes aegypti*, *Aedes albopictus*, *Anopheles crascon*, and *Culex quinquefasciatus*. However, it displayed no significant toxicity effect on non-target cellular model human fibroblasts (MRC-5) and non-target organisms such as *Chlorella spp.* and *Odontoponera denticulata*. Apoptosis induction was found to be the preliminary mode of action of the EA extract on C6/36 cells as well as the larvae and adults of *Ae. aegypti*. Moreover, biochemical assays revealed that it inhibited acetylcholinesterase activity, indicating the disruption in the insect nervous system. We also discovered the inhibiting property of the EA extract against DENV-2 replication in C6/36 cells. Pre- and post-treatment assays of the EA extract on C6/36 cells demonstrated significant inhibitions, suggesting the disruptions on virus entry and adsorption on pre-treated cells and intracellular replication of the virus on post-treated cells. Chemical profiling of the EA extract revealed the presence of pentanamide, c17 sphinganine, dicianehatin, dodemorph, and antillatoxin B, which antillatoxin B and dichamanetin were active against DENV NS2B/NS3, NS5, and envelope protein (E) of DENV-2 via in silico study. Our study suggests that Streptomycies sp. KSF103 EA extract can potentially become a promising source of environmentally benign biocidal agents for mosquito-borne diseases.

**METHODS USED FOR MALARIA AND MOSQUITO CONTROL AT THE HOUSEHOLD LEVEL IN TANZANIA. THE SCHOOL MALARIA AND NUTRITION SURVEY**

Mbaraka John Remiji1, Tajiri Laizer1, Samson Kiware1, Frank Chackya2
1*Itakala Health Institute, Dar es Salaam, Tanzania, United Republic of, 2Ministry of Health, National Malaria Control Programme, Dodoma, Tanzania, United Republic of*

Global Technical Strategy for Malaria 2016-2030 is to ensure universal coverage for all people at risk of malaria using effective vector control with either LLINs or other core prevention tools such as indoor residual spraying. These are highly effective methods of vector control, but additional interventions are needed for long-term, sustainable malaria control and elimination. This study aimed to determine factors related to methods used for malaria and mosquito control at the household level in Tanzania. A cross-sectional survey involving primary school pupils was selected for an interview and a random sample of households around the schools was interviewed on malaria prevention, treatment, and control methods. We applied a generalized linear model to determine factors associated with methods used for malaria and mosquito control at the household level. Our study involved 6,018 households from 26 regions in Tanzania. We find that the head of household engaged in business had an AOR of 0.93 (CI = 0.87 - 0.99, p = 0.032) times less likely to more methods compared to those who deal with agriculture activities. Head of household who is employed had an AOR of 0.92 (CI = 0.84 - 1.01, p = 0.066) times less likely to use methods compared to those who are dealing with agriculture activities. Head of household who is unemployed had an AOR of 0.81 (CI = 0.68 - 0.96, p = 0.015) times to use fewer methods compared to those who are dealing with agriculture activities. We found households size between 5-8 members had an AOR of 1.05 (CI = 1.00 - 1.11, p = 0.059) times to use more methods compared to a household with people between 1-4. Household sizes between 9-13 had an AOR of 1.17 (CI = 1.09 - 1.26, p < 0.001) times to use more methods compared to a household with people between 1 to 4. We find that households with wood floor structures had an AOR of 0.85 (CI = 0.72 - 0.99, p = 0.043) times less likely to use more methods compared to those households with the cement floor structure. Our results highlight the different measure used at household level to control and eliminate malaria cases in Tanzania.

**IMPACT OF CLIMATE VARIABILITY ON VECTOR-BORNE DISEASES (MALARIA) IN THE DEMOCRATIC REPUBLIC OF THE CONGO**

Christel Muteba Tshiteya
*PNLP DRC, kinshasa, Congo, Democratic Republic of the*

According to the WMO, by 2050, temperatures are predicted to rise by 2-3°C in temperate latitudes, with a 7% increase in average precipitation. In the context of these rising temperatures, there will likely be an increase in vector reproduction and high average temperatures will lead to an increase in malaria cases. In this context, in DRC, efforts have been undertaken by the National Malaria Control Program (NMCP) to understand and mitigate this scenario to identify areas that are at high risk of malaria transmission and implement targeted interventions to prevent and control the spread of the disease. Of these, the development of the integrated malaria dashboard, a visualization tool to monitor the performance of the malaria indicators, have been undertaken since 2021. This captures epidemiological (malaria weekly surveillance cases and deaths from IDES) and malaria transmission factors (including meteorological data). Through those decisions, the evolution of the malaria indicators will be monitored to have a clear picture of the influence of climate variability in the spread of malaria in DRC. The country is also setting up innovative approaches to control and eventually eliminate the disease and providing specialized trainings, to inform healthcare workers and other stakeholders. Finally, advocating for the improvement of water, hygiene and sanitation systems in DRC is an effort
undertaken by the NMCP, as these are responsible for the transmission of malaria, and they are likely to increase with rising temperatures will reduce the breeding sites of mosquitoes. As such, the rise of temperatures in DRC would lead to an increase of malaria burden if all efforts are not put in place. Climate change has the potential to exacerbate the burden of malaria in DRC, the country with the second highest burden of malaria, but efforts are being undertaken by the NMCP to mitigate this scenario and prevent a potential worsened malaria pandemic crisis in the context of changing climate conditions.

**Spatial and Temporal Distribution of Anopheles Species Across Three Different Ecological Zones in Ghana**

Terrel Sanders, Shirley Nimo-Paintsil
US Naval Medical Research Unit No. 3, Bethesda, MD, United States

Vector management of Anopheles mosquito species in West Africa is challenged by limited data on their distribution, species profile, and lack of reporting mechanisms from sentinel sites. To provide insights into the epidemiology of vector-borne diseases such as malaria and filariasis, this study investigated the distribution and species composition of Anopheles mosquitoes in three ecologically distinct zones of Ghana. We collected adult Anopheles mosquitoes using CDC light traps, UV light traps and Biogents Sentinel (BG) traps, and morphologically identified on monthly basis, between 2017 and 2021. We subsequently performed analysis using R version 4.1.0 and fit a Generalized Linear Mixed Model (GLMM) with a negative binomial distribution to depict the trapping method and month of collection as fixed effects, and the year of collection and site as random effects. Out of a total of 20,222 Anopheles mosquitoes collected, majority were from Navrongo (66.1%), Kintampo (32.1%), and Kumasi (1.8%). The most predominant Anopheles species identified was An. gambiae (67.83%), followed by An. coustani (21.39%), An. rufipes (5.12%), An. pharoensis (4.35%), and An. funestus (0.91%). There was a significant association between season and the collection of An. gambiae (p < 0.001). Additionally, significantly higher numbers of An. gambiae were collected in the wet season than in the dry season in Kintampo (p < 0.001) and Navrongo (p = 0.0023). Furthermore, there was a less likelihood of An. gambiae collected using UV light traps compared to the CDC light traps (IR = 0.8, 95%CI = 0.67-1.07, p = 0.0989). The results of this study will inform the development of evidence-based vector management strategies and contribute to the efforts to reduce the burden of vector-borne diseases in Ghana and West Africa at large.

**Relevant Dengue Transmission Risk in Non-Household Environments in Kenyan Cities Can Lead Us to Rethink the Household as the Focus of Vector Control Activities**

Victor H. Peña-García1, Francis M. Mutuku2, Bryson A. Ndenga3, Joel O. Mbakaya4, Samwel O. Ndire5, Paul S. Mutuku2, Said L. Malumbo2, Jason Andrews1, Erin Mordecai1, Desiree LaBeaud1

Dengue transmission has recently been reported in multiple African countries including Kenya, raising attention as an emerging public health concern. In endemic countries, most vector control activities are applied within households; however, recent evidence suggests that spaces other than households might have a relevant role in dengue transmission in urban areas. To evaluate the role of non-household spaces in dengue risk in Kenya, we conducted analyses based on data from extensive vector sampling carried out in Kisumu and Ukunda, two Kenyan cities with documented dengue virus circulation. Four sampling strategies targeting different vector stages were used to collect mosquitoes in both household and non-household environments. Data were analyzed to assess the differential proportion of spaces from both environments with presence of the vector and the number of individual vectors per structure. Further, taking these data as input, we simulated total vector and human populations to estimate the difference in vectorial capacity for household and non-household environments based on m, the vector-to-host density. The proportion of sampling sites with vectors present was similar in household and non-household environments. The number of vectors was higher in non-household environments in Kisumu and similar in households and non-households in Ukunda. Mirroring vector density, vectorial capacity was higher in non-households in Kisumu and similar in both environments in Ukunda, suggesting an important entomological risk in non-household environments in both cities. Our results highlight the importance of including non-household sites in vector control activities to improve city-wide disease control efforts.

**Measuring the Long-Term Public Health Impact After City-Wide WMel Deployments in Yogyakarta**

Citra Indrani
Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Sleman, Indonesia

A cluster randomised trial of wMel Wolbachia-infected mosquito releases in Yogyakarta City, Indonesia, in 2018-2020 showed a 77% reduction in dengue incidence and 86% reduction in dengue hospitalisations in wMel-treated areas. Yogyakarta now represents the first dengue-endemic setting in which wMel has been established at a high level at a city-wide scale (~400,000 people), since releases were completed throughout untreated areas in January 2021. The incidence of dengue notified to the hospital based passive surveillance system was lower in 2021 and 2022 than any two-year period in the past 20 years, but not zero. It is unknown what proportion of these remaining clinically-reported cases are true dengue cases and resident in Yogyakarta. We established a prospective enhanced dengue surveillance study in 13 primary health clinics in January 2023, to test the feasibility of achieving local dengue elimination in Yogyakarta following area-wide Wolbachia deployment. Patients aged 3-45 years with 1-4 days of undifferentiated fever and resident in Yogyakarta City are enrolled and tested for virologically-confirmed dengue (VCD) by dengue virus RT-PCR and NS1 ELISA. History of traveling outside of the city within the 14 days prior the illness onset is captured through interview. The hypothesis is that enhanced clinical surveillance will demonstrate an absence of locally-acquired virologically-confirmed dengue within 5 years post-intervention (2023). Aggregate data on notified hospitalised dengue patients from the routine surveillance system is obtained on a monthly basis and interrupted time-series analysis of notified dengue cases will be used to evaluate the long-term public health impact of Wolbachia. In January to March 2023, we have enrolled 549 participants, 2 VCD cases were found from 440 tested blood samples. This showed a low prevalence of dengue case during the dengue season. We will present the design and first 9 months of results from the enhanced dengue surveillance study, and results of the interrupted time series analysis showing the impact of Wolbachia on notified dengue incidence 2.5 years after city-wide releases.

**Modeling the Effectiveness of Attractive Targeted Sugar Baits in Reducing Clinical Malaria**

Lars Kamber, Aurelien Cavelan, Emma Fairbanks, Melissa Penny, Nakul Chitnis
Swiss Tropical and Public Health Institute, Allschwil, Switzerland

Attractive targeted sugar baits (ATSBs) are a novel vector control intervention that have shown promising results of mosquito population reduction in semi-field and field studies. Randomized control trials are currently ongoing in Kenya, Zambia and Mali to determine the effectiveness of ATSBs in reducing clinical malaria. We use an individual-based simulation model of malaria, OpenMalaria, to estimate the effectiveness of ATSBs in reducing clinical malaria incidence in children aged 1-14 in settings based

astmh.org
on western Kenya and western Zambia. Our results suggest that even with relatively high pre-existing coverage of insecticide-treated nets (ITNs), ATSBs can be very effective in reducing clinical malaria. Even relatively low daily probabilities of mosquitoes feeding on an ATSB, that lead to modest reductions in mosquito densities, can lead to a 30% reduction in clinical incidence in children. Our results suggested a greater effectiveness in reducing clinical malaria in Kenya than in Zambia, assuming equivalent coverage of sugar sources with ATSBs; and similarly suggested higher effectiveness when the main vector species was An. gambiae rather than An. funestus. Our results were most sensitive to assumptions on baseline transmission intensity (ATSBs were more effective in low transmission settings); assumptions on frequency of sugar feeding (ATSBs were less effective when mosquitoes only sugar-feed while host-seeking); and on the duration of the host-seeking stage - which is especially difficult to measure in the field (ATSBs were less effective when mosquitoes found hosts quickly). Overall, our results support the optimism behind ATSBs but suggest that settings where they are deployed should be carefully considered and particular importance should be paid to estimating the time mosquitoes require to find and feed on blood hosts.

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INSECTICIDE TREATED RESTING STATIONS REDUCE PARITY RATE OF THE ENZOOTIC MOSQUITO VECTOR OF EEE VIRUS, CULISETA MELANURA

Edward D. Walker, John B. Keven
Michigan State University, East Lansing, MI, United States

Laboratory and field experiments were conducted to develop and test the concept of insecticide-treated, resting stations to reduce the vectorial capacity of Culiseta melanura for eastern equine encephalitis (EEE) virus. Of four commercial insecticides applied to the inner surfaces of resting stations (black-painted, durable molded fiber containers used in the greenhouse industry) and presented to adult Cs. melanura, the microencapsulated formulation of lambda cyhalothrin Demand CS showed the highest mortality and longest duration of effect (more than 98% mortality for 5 weeks).

Mosquito density in treated boxes was nearly nil in the field, compared to untreated boxes. A field evaluation was implemented at six bog sites in southwestern Michigan with natural populations of Cs. melanura and history of EEE activity, where three sites each received 300 boxes treated with Demand CS and distributed at bog perimeters, and three control sites had no treated boxes. Mosquitoes were sampled once weekly for 9 weeks from mid-July to mid-September from 75 untreated boxes at each site. Results showed a statistically significant decrease in the percentage of parous, female Cs. melanura at treatment compared to control sites following distribution of treated resting boxes, with up to 50% reduction in parity rate during the course of the experiment (3,185 dissected), and further revealed a shift towards a younger population age structure when considering unfed, blood fed, half gravid, and gravid physiological categories. Survival analysis suggested that the vectorial capacity of Cs. melanura populations at the treatment sites was reduced meaningfully with regard to control of EEE virus transmission. Blood meal analysis showed an avian host selection profile, with northern cardinal, American robin, and blue jay as the most commonly selected hosts. Overall, the results of the study support a proof of concept for use of insecticide treated resting stations for targeted control of EEE virus transmission.

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IN WITH THE NEW AND OUT WITH THE OLD: INITIAL OBSERVATIONS OF THE EXTENT OF LONG-TERM USE OF OLD LLIN DESPITE THE AVAILABILITY OF NEW NETS DURING THE 2020 MASS CAMPAIGN IN BENIN

Martin Akogbéto1, Bruno Akinro1, Saïd Chitou1, Moustapha Idrissou Souler1, *Albert S. Salako1, Kéllia Th Bathirou1, Ramziath Agbanin1, André Somoinahouan1, Rock Alkpom2, Cyriaque Affoukou2, Virgile Gnanguenon2, Patrick Condé1, *Ahmed S. Hassani3, *Daniel Imponivè3, *Germain G. Padonou1
1CREC, Cotonou, Benin, 2NMCR Cotonou, Benin, 3USAID, Cotonou, Benin, CDC, Atlanta, GA, United States

From 2011 to 2020, there were four mass distribution campaigns and ancillary continuous distribution of Long-Lasting Insecticide-treated Nets (LLINs) in Benin. Although new LLINs are freely available across malaria-endemic countries, there are anecdotal and empirical reports of continued use of old LLINs, despite likely diminished bio-efficacy against malaria vectors. To quantify the number of old nets in use despite the availability of newly distributed LLINs, households were surveyed to determine which nets were hanging and the integrity of those nets after the 2020 LLIN campaign in Benin. Household surveys of 2902 randomly selected houses were conducted in 24 randomly selected villages in Benin. Observation of nets and information on the net origin, utilization, and physical integrity were recorded using a survey form on Open Data Kit (ODK) loaded on tablets. A mosquito net that was hanging over a bed was classified as in use. A total of 7576 nets were found in the surveyed houses. An average of 956% of LLINs that were hanging or laying on or near the bed were in use of which 48% of these were torn. The origin of most LLINs (71%) found in households was from the 2020 mass campaign (n=5384). A total of 11% (n=809) of LLINs were from 2011, 2014, and 2017 campaigns, and 14% (n=1036) were from either purchase, routine distribution, or other sources. Approximately 96% (765 of 809) of LLINs found in the house from 2011, 2014, and 2017 mass campaigns were still in use. Only 5.5% (n=447) of households saved their new 2020 LLINs for later use. This study provides initial insights into the persistent use of old LLINs despite the availability of new LLINs. Retaining old LLINs in the presence of new LLINs does not seem to be common practice as most people hung their new nets. However, nets from 2011 were still found hanging after the 2020 mass campaign. Still, this assessment only provides provisional results. More evaluation is needed to understand the factors leading to the persistent use of old nets to develop strategies and policies that promote maximum usage of new LLINs.

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INTEGRATED VECTOR MANAGEMENT IMPLEMENTED TO REDUCE DENV-1 POSITIVE CASES IN HUMANS AND MOSQUITOES IN MAYAGÜEZ, PUERTO RICO, 2022

Nexilianne Borroto, Raiza Alvarado, Luis Doel Santiago, Joanelis Medina, Cristhian R. Sánchez-Rolón, Verónica Rodríguez-Quínonez, Jania P. García, Luis Marrero, Tatiana Ortiz-Ortiz, Julieanne Miranda-Bermúdez, Grayson Brown
Puerto Rico Vector Control Unit, Ponce, PR, United States

In September 2022, following the passage of Hurricane Fiona through Puerto Rico, cases of DENV increased areas of Puerto Rico (PR) including the western part of the island. The Puerto Rico Department of Health (PRDH) began to report elevated DENV-1 human cases in the municipality of Mayagüez and classified this area as critical area. At that time, there were approximately 54 human cases. Most confirmed cases occurred within public housing complexes of this municipality. The Puerto Rico Vector Control Unit (PRVCU) established entomological surveillance using nearly 100 Autodich Gravid Ovitraps (AGO) distributed over seven zones of active DENV1. The PRVCU deployed additional AGOs to control the mosquito population through saturation trapping. Our surveillance traps captured 8,773 females Aedes aegypti females, the principal vector of dengue, Zika and chikungunya in Puerto Rico. All these females were tested for dengue,
Zika and chikungunya viruses, yielding a total of 42 mosquito pools were positive for DENV-1, but no other serotypes were found. Neither were there any pools positive for chikungunya or Zika viruses. The PRVCU attempted to treat each of the seven zones with our standard regimen of Wide Area Larvicide Spraying (WALS). However, due to major security challenges, we could only complete about 1/3 of the total larvicide applications although one zone did receive all required applications. In that zone, other control strategies were also used such as community outreach, distribution of educational materials and repellents, installation of control traps, etc. The number of trapped female Ae. aegypti decreased in all treated zones but the decrease was greater in the zone with the largest number of treatments. In that zone, the number of female Ae. aegypti was reduced by about 70%, falling from a pre-treatment average of 17.5 of female Ae. aegypti mosquitoes per trap per week to 5.1/trap/week. Given that Ae. aegypti in Puerto Rico has very high insecticide resistance, this was an important success here. The results are encouraging, and we hope to be able to implement this approach in other, more secure areas of Puerto Rico in the future.

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THE SPREAD OF CHIKUNGUNYA VECTORS: A POTENTIAL THREAT TO GLOBAL HEALTH

Giorgia Tiozzo1, Amber Tiemes1, Gerard T. Vondeling2, Adrienne de Roo2

1Department of Health Sciences, University Medical Center Groningen, Groningen, Netherlands, 2Valneva, Vienna, Austria

Chikungunya is a viral disease caused by the chikungunya virus (CHIKV), a re-emerging arthropod-borne virus transmitted to humans through infected Aedes aegypti and Aedes albopictus mosquitoes. Chikungunya is characterized by acute febrile polyarthralgia and inflammatory arthritis, the latter of which can be severe, debilitating, and long-lasting. This targeted literature review was performed to identify evidence on CHIKV vectors and their role in the global spread of the disease. CHIKV was first identified in Tanzania in 1952 and isolated in Africa and Asia over the following decades, causing occasional outbreaks in those regions. Since 2004, CHIKV has spread to over 110 countries throughout Africa, Asia, the Americas, and Europe. CHIKV often causes large explosive outbreaks with high attack rates. One of the most recent major outbreaks occurred in Colombia in 2014, affecting roughly 106,000 people. As of 2014, CHIKV cases have been identified among U.S. travelers returning from areas at risk of transmission. Furthermore, because of globalization and international trade, CHIKV vectors can cover vast distances in a short time. The rapid spread of CHIKV is also influenced by global climate change. Temperature and precipitation changes, in particular, affect the proliferation, survival, and abundance of Aedes mosquito vectors, ultimately increasing the worldwide spread of habitat regions for the Aedes genus of mosquitoes. Consequently, this increase exposes more areas to the risk of local autochthonous transmission if the travelers should import the virus, as observed in Italy over the last 15 years, where outbreaks occurred within 10 years of vector introduction. All in all, climate change and an increase in globalization will cause distributional shifts, and likely expansion, in the climatically suitable areas for CHIKV transmission. This has global public health implications and highlights the need for a preventive vaccine against chikungunya.

HOST PREFERENCES OF ANOPOPHES GAMBIAE S.L. AND THEIR IMPLICATIONS FOR MALARIA TRANSMISSION IN FOUR INDOOR RESIDUAL SPRAYING INTERVENTION DISTRICTS IN GHANA

Kwame Desewu, Nicholas Ato Egyir, Gilbert Dossah, Grace Oppong, Francis Ofori Amankwah, Joseph Mwinkukubu Vulgate, Agyei Kumi, Matilda Kaabo, Ignatius Williams, Samuel Asiedu

AngloGold Ashanti Malaria Control (AGA mortal), Obuasi, Ghana

The risk of exposure to infections transmitted by mosquitoes is influenced by their host preference. In Ghana and other parts of sub-Saharan Africa, Anopheles gambiae s.l. is a significant vector of malaria, with a high propensity to feed on humans. The AngloGold Ashanti Malaria Control program implemented indoor residual spraying (IRS) in sixteen (16) districts of Ghana in 2021. As part of broader entomological surveillance activities to track the impact of the indoor residual spraying on the local vector populations, pyrethrum spray collections were carried out from January to December 2021, to sample indoor-resting mosquitoes from randomly selected houses at four (4) sentinel sites located in Obuasi, Wa, Lawra, and Sissala East. Mosquito samples were identified morphologically and also tested for the source of blood-meal using direct enzyme-linked immunosorbent assays (ELISAs). A total of 451 Anopheles species were collected from the sentinel districts, out of which 85% (n=383) were morphologically identified as An. gambiae s.l. Two hundred and twenty-six (226) of the An. gambiae s.l. were blood-fed and were tested for the source of blood-meal. The results showed that across the four (4) IRS districts, An. gambiae s.l. fed on five (5) different hosts that comprised of human blood-meal (55-89%), bovine (3.6-5%), goat (1.8-2.8%), pig (1.4-3.6%) and dog blood-meals (1.4-3.6%). There were also mixed blood-meals of two sources recorded at all the sites. Human blood-meal was the predominant source in all districts, with Obuasi having a human blood index (HBI) of 0.76, Wa 0.87, Lawra 0.91 and Sissala East 0.98. These findings have important implications for malaria transmission and the design and implementation of vector control measures. The high level of anthropophagy observed indicates that the vectors have a strong preference for human blood-meal. Therefore, targeted interventions that focus on reducing human-vector contact could be effective in tackling the transmission of malaria in the study areas.

OPTIMAL CONTROL OF DENGUE WITH EXISTING AND FORTHCOMING INTERVENTIONS

Alex Perkins1, Hannah Clapham2, Oliver Brady3

1University of Notre Dame, Notre Dame, IN, United States, 2National University of Singapore, Singapore, Singapore, 3London School of Hygiene and Tropical Medicine, London, United Kingdom

Progress towards controlling dengue has proven to be difficult, with clear examples of successful control being few and far between and typically not sustained over time. At the same time, evidence from trials indicates that a range of interventions should be capable of reducing transmission. This contradiction raises the possibility that there is scope to improve how interventions are used. We addressed this possibility using a mathematical model of seasonally varying dengue virus transmission in nearly 2,000 cities. The model was informed principally by Aedes aegypti occurrence maps, temperature and its effects on mosquito and virus traits, and spatial estimates of dengue virus force of infection. We applied optimal control theory to models for each city, resulting in estimates of the frequency with which each of several interventions should be deployed if cost-effectiveness is to be maximized. While our results indicate that some combinations of interventions may be more cost-effective than others, especially in some settings, there are challenges that all interventions face. Namely, limits to intervention coverage impair effectiveness, and increased intervention effort is required over time to counterbalance the effect of rising susceptibility, particularly for more effective interventions. We also found that cities with more seasonally marginal levels of transmission and higher costs incurred
by dengue morbidity and mortality have greater scope to engage in cost-effective control programs. Our results offer a novel piece of information that decision makers could use to inform rational choices about efforts to control dengue within their communities.

### Changing Assumptions about Mosquito Habitat Availability Drive Variation in Seasonal Dengue Dynamics When Behavioral Control is Present

Marya L. Poterek, Mauricio Santos-Vega, T. Alex Perkins

1University of Notre Dame, Notre Dame, IN, United States, 2Universidad de los Andes, Bogotá, Colombia

Though vector control strategies for dengue can vary significantly in efficacy and scale of implementation, human behavior is an important component of their success. Behavior can influence compliance patterns with spraying campaigns and participation in mosquito habitat reduction, namely the elimination of standing water. The nature of the relationship between that influence and the outcomes we observe in behavioral studies is difficult to pinpoint, though, since several factors influence the presence of standing water in a home besides behavioral control alone. Specifically, the connection between rainfall and mosquito habitat availability is uncertain, as increased rainfall has been associated with both greater and lesser habitat availability—while it can directly increase standing water presence outdoors, it can also discourage intentional water storage. To explore the possible consequences that this relationship may have on the efficacy of behavioral interventions, we developed a deterministic, coupled-contagion model for the spread of dengue virus and associated container-management behaviors. Preliminary analyses suggest that the effects of changing assumptions regarding rainfall are most notable under temperate climate regimes, where mosquito population dynamics are subject to more seasonal variation than in tropical climes. In a temperate setting, when mosquito habitat availability is positively correlated with monthly rainfall patterns, we observe a higher, later seasonal peak in dengue incidence than when there is an inverse relationship between habitat availability and rainfall. This effect is dampened by the transmission of container control behaviors, driven by mosquito presence, disease incidence, and social conformity, which produce a mosquito population that is more stable over time. In this work, we found that the impact of dengue control behaviors is largely dependent on assumptions about what drives mosquito reproduction in a given setting, and suggest this as a focus when tailoring interventions to community-specific conditions and concerns.

### INFLUENCE OF THERMAL AND INSECTICIDE GRADIENTS ON IMMATURE Aedes aegypti PERFORMANCE

Patrick M. Heffernan, Jason Rohr

University of Notre Dame, Notre Dame, IN, United States

Mosquito-borne diseases are ubiquitous, with mosquitoes vectoring parasites, arboviruses, and bacteria to wildlife, domestic animals, and humans. Temperature is a well understood, fundamental factor that influences both mosquito and pathogen traits, altering R₈, an important proxy for the population growth rate of pathogens. Consequently, global climate change is expected to alter incidence and seasonal dynamics of many mosquito-borne diseases, and ecologically relevant predictive models are essential to mitigating these changes to disease risk. In recent years, eight mosquito and pathogen traits have been shown to exhibit unimodal responses with temperature, and these thermal performance curves (TPCs) have been implemented into a generalized R₈ equation. Temperature is the sole abiotic factor considered, however, and little is known about how other abiotic factors, such as insecticides, impact each parameter. To begin understanding the extent to which insecticides fit into the temperature-dependent R₈ equation, we implemented a response-surface design on Aedes aegypti mosquitoes in temperature-controlled incubators to examine if temephos (a widely used organophosphatase larvicide) interacts with temperature to influence larval and pupal mortality and development time and adult body size. Initial analysis using generalized additive models show a significant interaction between temperature and temephos dose for larval and pupal mortality and pupal development time. These results demonstrate the significance of incorporating abiotic gradients into temperature-dependent R₈ models to better understand disease spread and implement vector control.

### FROM THE LAB TO THE FIELD: LONG-DISTANCE TRANSPORT OF STERILE MALE MOSQUITOS

Hamidou Maiga, Mame Thierno Bakhournou, Nanwintoum Sèverin Bimbilé Somda, Wadaka Mama, Jeremy Bouyer

1Institut de Recherche en Sciences de la Sante., Bobo-Dioulasso, Burkina Faso, 2Laboratoire National de l’Élevage et de Recherches Vétérinaires, Institut Sénégalais de Recherches Agricoles (ISRA), BP 2057., Dakar, Senegal, 3Unité de Formation et de Recherche en Sciences et Technologies (UFRTST), Université Norbert Zongo (UNZ), BP 376, Kouadougou, Burkina Faso, 4Institut de Recherche Agricole pour le Développement (IRAD), Yaoundé P.O. Box 2123, Cameroon, Yaoundé, Cameroon, 5CIRAD, Montpellier, France

Pilot programme of the sterile insect technique (SIT) may rely on importing significant and consistent numbers of high-quality sterile males from a distant mass-rearing facility. As such, long-distance mass-transport of sterile males may contribute to meeting the requirement if their survival and quality are not compromised. This study therefore aimed to develop and assess a novel method for long-distance shipments of sterile male Aedes aegypti mosquitoes from the laboratory to the field. In addition, different types of mosquito compaction boxes and a simulated transport of marked and unmarked sterile males was assessed in terms of survival rates/recovery rates, flight ability and morphological damage to the mosquitoes. The novel mass-transport protocol allowed long-distance shipments of sterile male mosquitoes for up to four days with a non-significant impact on survival (> 90% for 48h of transport and between 50 and 70% for 96h depending on the type of mosquito compaction box), flight ability and damage. In addition, a one-day recovery period for transported mosquitoes post transport increased sterile male escape ability by more than 20%. This novel system for long-distance mass-transport of mosquitoes may therefore be used to ship sterile males worldwide for journeys of two to four days. This study demonstrated that the protocol can be used for standard routine mass-transport of marked or unmarked chilled male mosquitoes required for the sit or other related genetic control programmes.

### BACTERIAL COMMUNITIES ASSOCIATED WITH ANOPHELES GAMBAEAE LARVAL HABITATS IN SOUTHERN GHANA.

Akua Obeng Forson, Isaac Kwame Sraku, Idan Baah Banson, Kwabena Obeng Duedu,Yaw Asare Afrane

1Centre for Vector-borne Diseases Research, Department of Medical Microbiology, University of Ghana Medical School, University of Ghana, Korle-Bu, Accra, Ghana, 2Department of Biomedical Sciences, University of Health and Allied Sciences., Ho, Ghana, 3Department of Biomedical Sciences, University of Health and Allied Sciences, Ho, Ghana

Mosquito breeding habitats is an ecosystem that comprises of a complex, intimately associated micro-organisms. Understanding the bacterial community structure, and its dynamics on mosquito larval productivity is a pre-requisite for comprehending mosquito habitat selection for oviposition. Sequencing of the 16S rRNA using Oxford Nanopore’s MinION platform was used to identify and compare the bacterial communities in Anopheles positive breeding habitats (productive and semi-productive habitats) and negative habitats (non-productive) from Southern Ghana. A total of 12 bacterial taxa were identified in all the samples tested. Significantly, mosquito positive breeding habitats (productive and semi-productive) had more bacterial diversity compared to mosquito negative habitats (non-productive). Comparisons of bacterial composition revealed that Epsilonproteobacteria was more common (P < 0.05) in...
unproductive habitats, Gammaproteobacteria, Actinobacteria and Sphingobacteria were more common (P < 0.05) in productive habitats, and Gammaproteobacteria, Betaproteobacteria, and Alphaproteobacteria were the most abundant bacterial class in Anopheles larvae. Only two taxa, belonging to the phyla Gammaproteobacteria and Betaproteobacteria were common to both larvae and mosquito positive breeding habitats. These results suggest a higher bacteria composition may play a role in Anopheles mosquitoes’ attractiveness to a breeding habitat. These findings contribute to the understanding of which bacteria, directly or indirectly, can be linked to absence or presence of mosquitoes larvae in breeding habitats, and set the basis for the identification of specific bacterial taxa that could be harnessed for vector control in the future.

A VOLATILE PYRETHROID SPATIAL REPELLENT (VPSR) USING TRANSFLUTHRIN AS AN INTERVENTION FOR REDUCING OUTDOOR MALARIA TRANSMISSION

Tim Burton1, Limonty Simubali2, Lewis Kabinga2, Lepa Syahrami3, Dendi Fermania3, Ismael Rozi3, Jennifer Stevenson1, Monicah Mburu4, Edgar Simulundu5, Puji Ashi6, Din Syafuddin7, Neil Lobo8

1University of Notre Dame, Notre Dame, IN, United States, 2Macha Research Trust, Macha, Zambia, 3Eijkman Institute, Jakarta, Indonesia

Presently, the most common malaria control tools - i.e. long lasting insecticide-treated nets (LLINs) and indoor residual spraying (IRS) are limited to targeting indoor biting and resting behaviors of Anopheles mosquito species. Few interventions are targeted towards malaria control in areas where transmission is driven or persists due to outdoor biting behaviors. A volatile pyrethroid-based spatial repellent (VPSR) using a transfluthrin active ingredient was designed to address this gap in protection. A collection of one semi-field and three field trials were conducted in communities vulnerable to outdoor biting in Zambia and Indonesia, assessing the protection provided by the VPSR in outdoor spaces where biting is known to occur. The product provided significant protection to users during semi-field trials by preventing host-seeking activity by roughly 40% per night and increasing mortality among exposed mosquitoes. Host-seeking was significantly reduced in structures protected by the VPSR device across the remaining three field trials, with significant nightly reductions of around 70% observed in these trials. Individual hourly protection between 60% and 80% was observed across each trial. This study aims to compare the results of these VPSR trials and leverage additional information from the study areas - possibly including household density, road networks, and surveyed human behavior - to further assess the efficacy and cost-effectiveness of the VPSR intervention in each study area and across possible scenarios. Both nightly and hourly protection were considered based on the transmission setting and in terms of possible improvements to semi-field trial design to measure possible repellency or mortality effects on mosquitoes in a confined space.

EVIDENCE-BASED DESIGN OF ENHANCED VECTOR SURVEILLANCE FOR LARVAL SOURCE MANAGEMENT ON BIOKO ISLAND, EQUATORIAL GUINEA

David Galick1, Carlos A. Guerra2, Wolfgang Ekoko Eysap3, Olivier Tresor Donrack1, Michael Von Ficken4, Nestor Rivas Bela1, Wonder P. Phiri1, David L. Smith1, Guillermo A. Garcia1

1MCD Global Health, Malabo, Equatorial Guinea, 2MCD Global Health, Silver Spring, MD, United States, 3George Mason University, Fairfax, VA, United States, 4Institute of Health Metrics and Evaluation, University of Washington, Seattle, WA, United States

From 2004-2018, sustained intensive vector control on Bioko Island reduced malaria prevalence from over 40% to around 10%, but since 2018 there has been a resurgence in prevalence despite continued vector control. To supplement the core interventions of indoor residual spraying and insecticide-treated net distributions, larval source management (LSM) was added to the intervention package. LSM has been implemented on a small scale on Bioko Island at various times from 2013-2021, and in 2022 a breeding site survey was conducted to support an expanded LSM implementation in 2023. However, to enable evaluation of the impact of LSM, it was necessary to enhance the vector surveillance system on Bioko. Since 2004, human landing catch (HLC) in 14 sentinel surveillance sites has been the primary vector surveillance activity, but expanding HLC to evaluate LSM impact is cost-prohibitive. In 2017, CDC light traps were also placed indoors in sentinel sites, but especially since 2018 have been highly inefficient in capturing Anopheles mosquitoes. Thus, two new trapping methods were selected to implement for LSM evaluation in 2023 based on their operational suitability and cost: resting sticky boxes (RSB) and oviposition traps (OT). Sites for trap placement were selected primarily based on the 2022 larval habitat survey data. For each 1km x 1km grid cell targeted for LSM, at least five sites were selected for RSB and at least one for OT. Site selection was based on a statistical analysis of the breeding survey data, which incorporated information about breeding site characteristics and environmental covariates to rank potential sites locations. Final sites were selected from a subset of the highest ranked potential sites based on operational considerations such as accessibility, trap integrity and frequency of trap revisit in relation to the LSM deployment. The resulting surveillance framework continues to monitor adult mosquito density in an urban area.

COMBINING A SCHOOL-BASED AND COMMUNITY-BASED EDUCATIONAL INTERVENTION IN URBAN KENYA FOR LARVAL SOURCE REDUCTION

Prathik Kalva1, Jenna E. Forsyth1, Arielle Kempinsky1, Helen O. Pitchik1, Catharina J. Alberts1, Francis M. Mutuku2, Lydia Kibe3, Nicole M. Ardoin4, Desiree A. LaBeaud1

1Stanford University School of Medicine, Stanford, CA, United States, 2Stanford Woods Institute for the Environment, Stanford, CA, United States, 3University of California at Berkeley School of Public Health, Berkeley, CA, United States, 4Technical University of Mombasa, Mombasa, Kenya, 5Kenya Medical Research Institute, Nairobi, Kenya

Aedes aegypti mosquitoes are the primary vectors for many arboviral diseases including chikungunya (CHIKV) and dengue (DENV), and they primarily breed in domestic containers. Previous studies have shown that there is a severe lack of knowledge about non-malaria arboviral diseases in Kenya, so we proposed a household and school-based educational intervention in Urban Kenya to determine whether it could bring about long-term improvements in knowledge, attitudes, and behaviors related to source reduction of Ae. aegypti vectors. In this cluster-randomized controlled trial, there were 243 households from five control villages and 249 households from five intervention villages. In each household, at least one child (11-14 years) and his/her primary caregiver were enrolled. The intervention used printed informational posters, live demonstrations, and videos to focus on source reduction techniques for the most productive mosquito breeding sites found in urban Kenya. Data on the participants’ knowledge, attitudes, and behaviors were collected at baseline, 3-, and 12-months post-intervention as well as counts of immature mosquitoes in containers in the participants’ households. Caregivers in the intervention group significantly outperformed the control group in the knowledge and behavior questions at the 3- and 12-month follow-up (p<0.01). Children in the intervention group showed similar performance to the control group in knowledge and behavior questions at the 3-month follow-up, but at the 12-month follow-up, the intervention group significantly outperformed the control group (p<0.01). Although the total number of immature mosquitoes did not differ between the control and intervention groups, there was significantly less mosquito breeding in intervention household containers - a focal point of the intervention - at the 12-month follow-up compared to baseline (26.19% to 1.93%, p<0.01). Paired educational interventions at the school and household level can bring about positive changes in knowledge, attitudes, and behaviors that protect against arboviral diseases and may be relevant in other urban Kenyan communities.
ADVANCEMENTS TOWARD COMMERCIAL-SCALE PRODUCTION OF YEAST RNAi INSECTICIDES FOR MOSQUITO CONTROL

Majidah Hamid-Adiamoh1, Corey Brizzea, Teresa Njoroget, Akiah Stewart1, Jack Crawford2, Keshava Mysore1, Molly Dumran-Scheel1
1Indiana University School of Medicine, South Bend, IN, United States, 2DeMeoTr AgBio, Lexington, KY, United States

The development of new mosquito control interventions, such as RNAi-based yeast insecticides, is a multi-staged process. Efforts to move RNA yeast technology from the bench to the field began with proof of concept experiments to demonstrate yeast efficacy and modes of action in the lab. These studies, which were performed with a laboratory yeast strain, were followed by semi-field and small-scale field trials that involved the development of delayed-release formulations, as well as scaling yeast production from shake cultures to bioreactors. The pursuit of large-scale field trials and ultimately the global deployment of a commercial product requires substantial scale-up of yeast production and the transition from laboratory yeast strains to more robust commercial-ready strains suitable for scaled fermentation. Cas-CLOVER, an RNA guided dimeric nuclease system, was used in combination with piggyBac transposase to generate a robust yeast strain containing multiple integrated copies of an insecticidal shRNA expression cassette. This enabled production of shRNA that targets a sequence which is conserved in mosquito Shaker genes, but which is not found in non-target organisms, during yeast propagation. The yeast performed well in laboratory trials conducted on Aedes spp., Culex spp., and Anopheles gambiae larvae. Delivery of the yeast to adult mosquitoes as an attractive targeted sugar bait (ATSB) also induced high levels of adult mortality. Large-scale fermentation facilitated kilogram-scale production of the yeast, which was subsequently heat killed and dried. 150 levels for the yeast insecticide produced at scale, which correlated with shRNA levels that were several-fold higher than laboratory yeast strains, were reduced with respect to the lab strains. Efforts to begin to evaluate the yeast in additional species of mosquitoes, including Anopheles stephensi, a substantial threat to malaria prevention, and to confirm the efficacy of the yeast in field trials will be discussed. These studies indicate that yeast RNAi insecticide production can be successfully scaled for large field trials and global product distribution.

SHOULD WE BE SPRAYING 80% OF THE HOUSES? AN OPERATIONAL, CLUSTER RANDOMIZED TRIAL ON BIOKO ISLAND TESTING THE NON-INFERIORITY OF A LOWER COVERAGE

Carlos A. Guerra1, David Galick2, Liberato Motobe Vaz2, Lucas Ondo Nze3, Jeremia Nzamio Mba Eyono2, Restituto Mba Nguema Avue2, Teresa Ayirongono Ondo Mfumu2, Matilde Rlhoa Rivas2, Olivier Tresor Donfack2, Wonder P. Phiri2, David L. Smith1, Guillermo A. Garcia1
1MCD Global Health, Silver Spring, MD, United States, 2MCD Global Health, Malabo, Equatorial Guinea, 3Ministry of Health and Social Welfare, National Malaria Control Program, Malabo, Equatorial Guinea, 4Institute of Health Metrics and Evaluation, University of Washington, Seattle, WA, United States

Indoor residual spraying (IRS) has been used annually on Bioko Island for 20 years. By 2023, 30 rounds had been completed for high coverage. Throughout IRS’s history, the question of what constitutes high coverage and how to balance it with limited resources has arisen. We present an operational, cluster randomized trial of IRS investigating the non-inferiority of 50% coverage against the canonical 80% recommended. The alternative hypothesis of non-inferiority posits that the effect size of 50% coverage is at least as large as the effect size of 80% coverage. Two arms of 57, randomly allocated clusters were sprayed for two consecutive years. Malaria indicator surveys were used as benchmarks to measure malaria prevalence at baseline (2020) and following each of the two rounds (2021 and 2022). The outcomes of interest were malaria infection at individual level and malaria prevalence and change in prevalence at the cluster level compared to the baseline. The intervention (50%) arm proved challenging given that IRS cannot be denied to the population, resulting in over spraying (above the target) of some rural clusters. In the control (80%) arm, spray teams struggled with under spraying (below the target), particularly in urban areas where achieving high coverage is challenged by low adherence and high numbers of houses to spray. Analyses were conducted in both intention-to-treat (74) and per-protocol clusters (27 clusters in the control and 23 in the intervention arm, after exclusions given by over and under spraying) using cluster-level summaries and mixed effects logistic regression models. Spillover effects were handled in two ways. First, analyses were conducted only at cluster cores, which were separated from the boundaries by a distance of 300 m. Second, coverage was estimated at each house within the cores by Using 100, 200 and 300 m circular buffers around it and dividing the number of neighboring houses that were sprayed by the total number within each buffer. The question at hand is critical given IRS is labor intensive and resource expensive, and support of a non-inferiority hypothesis would open new possibilities for vector control programs.
reached the hundreds, highlighting the urgent need for improved measures to curb WNV transmission. Certain species of mosquitoes, primarily Culex, serve as vectors to transmit WNV from birds to humans. Effective targeting of mosquito eradication techniques such as insecticide larvicide and bug traps remains challenging, and judicious selection of deployment location is one way to improve their effectiveness. Therefore, accurate short-term forecasting of WNV disease can aid mosquito control efforts. Machine learning methods have seen increased application to geospatial environmental problems, such as precipitation nowcasting, haze forecasting, and crop yield prediction. However, many of the machine learning methods applied to mosquito population and disease forecasting do not inherently take into account the underlying spatial structure of the given data. We apply a spatially-aware graph neural network model consisting of GraphSAGE layers to forecast the presence of West Nile virus in Illinois, which may aid mosquito surveillance and abatement efforts within the state. More generally, we show that graph neural networks applied to irregularly sampled geospatial data can exceed the performance of a range of baseline methods including logistic regression, XG-Boost, and fully-connected neural networks.

TARGETING OF VECTOR CONTROL INTERVENTIONS TO MOBILE, MIGRANT, ETHNIC, AND VULNERABLE POPULATIONS IN MALARIA ELIMINATION SETTINGS: A COMPARISON OF APPROACHES IN THE GREATER MEKONG SUBREGION AND MESOAMERICA REGION

Yang Hu1, Edric Luci2, Tara Seethaler3, Elijah Filip1, Julia Dunn2, Lucia Fernandez Montoya2
1Clinton Health Access Initiative, Phnom Penh, Cambodia, 2Clinton Health Access Initiative, Vientiane, Lao People’s Democratic Republic, 3Clinton Health Access Initiative, Minneapolis, MN, United States, 4Clinton Health Access Initiative, Manchester, United Kingdom, 5Clinton Health Access Initiative, Panama City, Panama

Vector control is a key component to malaria control and elimination. About 78% of all clinical cases averted in Sub-Saharan Africa from 2000 and 2015 have been attributed to ITNs and IRS. Compared to high burden settings, Greater Mekong Subregion (GMS) and Mesoamerica Region are approaching malaria elimination, share similar socio-economic statuses, and have increasingly focalized transmission in hard-to-reach areas among mobile, migrant, ethnic, and vulnerable populations (MMEVPs). While the GMS is an early adopter of a mix of ITNs and novel interventions, contrastingly, deployment of ITNs is relatively nascent in the Mesoamerica Region. This presentation compares how GMS and Mesoamerican countries target vector control interventions to MMEVPs differently, and explores potential factors that contribute to these differences. In the GMS, alternative ITNs and novel interventions are deployed to protect MMEVPs against malaria, especially in outdoor settings. Cambodia is distributing forest packs containing LLINs (hammock nets) and topical repellents to forest-goers, while Vietnam is targeting LLI-Ns to MMVEPs in the forested Central Highland (Ph hotpot) and single-sized LLINs to their counterparts in the mountainous North (Ph hotspot), accounting for 25.7% and 30.5% of populations in targeted communes, respectively. In the Mesoamerica Region, Panama is distributing LLINs to indigenous populations, Dominican Republic is covering Bateyes inhabited by migrant agriculture workers with LLINs, and Honduras is conducting IRS for Garifuna communities with proven poor LLIN use. Meanwhile, Guatemala is faced with the challenge of covering all active foci with LLINs due to limited funding and registered products. Moving forward, both regions are looking to continuously improve targeting of vector control interventions by addressing similar challenges. These include complex logistics for reaching unsafe or hard-to-reach malarious areas, inadequate protection against outdoor biting, and insufficient monitoring and evaluation among MMEVPs in order to optimize ITN usage and to select the most suitable interventions.
Articles were then filtered by removing duplicates, after which the title and abstracts underwent an eligibility check for inclusion in the review. Eligible articles contained mappable tick collection data, such as descriptions of collection event localities, map-displayed data, or GPS coordinates. Remaining papers then underwent a data extraction process. Additional sources were captured from reference sections of extracted articles representing articles which were not captured with the initial search terms. Information extracted from articles included tick and host taxonomy, geographic collection information, and pathogen detection information. Each unique collection event was georeferenced using the point-radius method and uploaded to VectorMap (vectormap.si.edu). The final novel dataset product consisted of data extraction events from nearly 300 articles, representing ticks from numerous genera, including Amblyomma, Aponomma, Argas, Carolis, Dermacentor, Haemaphysalis, Hyalomma, Ixodes, Margaropus, Ornithodoros and Rhipicephalus. This data provides an oversight into surveillance coverage within the region, while also allowing for initial disease acquisition risk mapping.

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SPOTLIGHT REPORT: TICK SURVEILLANCE IN NIGERIA

Graham Matulis¹, Abigail Lilak¹, David B. Pecor², Alexander M. Potter², Dustin Rodriguez³, Regina M. Jobson¹, Michael E. von Fricken¹, Yvonne-Marie Linton²
¹George Mason University, College of Public Health, Fairfax, VA, United States, ²Walter Reed Biosystems (WRBU), Smithsonian Institution Museum Support Center, Suitland, MD, United States, ³James Madison University, Harrisonburg, VA, United States

A systematic literature review was conducted to establish a baseline dataset of tick surveillance coverage of Nigeria within the VectorMap online database. The literature review made use of nineteen search terms, using PubMed, Scopus, and WOS search engines to capture articles published between 1901-2022. Over 2,000 articles were initially captured with these search terms, after which article titles and abstracts were assessed for relevance to tick and tick-borne disease surveillance. A total of 150 articles met the final inclusion criteria, while 53 additional articles were sourced from searches of the reference sections of articles that met the final inclusion criteria, for a total of 203 articles that underwent data extraction. Information captured during the data extraction process included tick genera and species, tick collection source, collection location, and instances of microbial species detection. Ten genera of ticks were reported published between 1901-2022, while 53 additional articles were sourced from searches of the reference sections of articles that met the final inclusion criteria, for a total of 203 articles that underwent data extraction. Information captured during the data extraction process included tick genera and species, tick collection source, collection location, and instances of microbial species detection. Ten genera of ticks were reported published between 1901-2022, while 53 additional articles were sourced from searches of the reference sections of articles that met the final inclusion criteria, for a total of 203 articles that underwent data extraction. Information captured during the data extraction process included tick genera and species, tick collection source, collection location, and instances of microbial species detection.

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SPOTLIGHT REPORT: TICKS AND TICK-BORNE DISEASE THREATS IN TUNISIA

Regina M. Jobson¹, Abigail Lilak¹, Graham Matulis¹, David B. Pecor², Alexander M. Potter², Dustin Rodriguez³, Michael E. von Fricken¹, Yvonne-Marie Linton²
¹George Mason University, College of Public Health, Fairfax, VA, United States, ²Walter Reed Biosystems (WRBU), Smithsonian Institution Museum Support Center, Suitland, MD, United States, ³James Madison University, Harrisonburg, VA, United States

A systematic review was implemented to establish a dataset with tick geographic distribution within Tunisia. Establishing this dataset allows for it to be incorporated into VectorMap and potentially be used to inform vector-borne disease risk assessments. Over 1,070 peer-reviewed articles from 1901-2022 were systematically screened to meet the inclusion/exclusion criteria. A total of 35 articles met final inclusion criteria with data extraction and geo-referencing, before being combined into a single database. Tick species identified were from seven different genera including five hard ticks (Dermacentor, Haemaphysalis, Hyalomma, Ixodes and Rhipicephalus) and two soft tick genera (Argas and Ornithodoros). Eight species of Hyalomma ticks were reported, followed by 5 different species of Rhipicephalus ticks. The most commonly survey tick species were Hyalomma dromedarii (Koch, 1844) and Ixodes ricinus (Linnaeus, 1758), with approximately 52% of articles detecting these species; with Hyalomma marginatum (Koch, 1844) and Hyalomma excavatum (Koch, 1844), detected in 46% of studies. However, Rhipicephalus turanicus, Pomerantsev, 1936, was detected only in approximately 20% of studies. There were 21 pathogens were reported in Tunisian tick records, including: species of Anaplasma, Borrelia, Coxiella, Ehrlichia, Francisella, Rickettsia, Theileria and Trypanosoma. A lone record of Tick-borne encephalitis virus (TBEV) was also recovered. Hyalomma and Rhipicephalus ticks were the most reported genera with confirmed pathogens. The data collected from this systematic review provides valuable information on the diversity of tick species and can be used in part with surveillance to examine transmission of tick-borne diseases within Tunisia.

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SUCCESSFUL BARTONELLA HENSELAE INFECTION BY AN INCIDENTAL VECTOR IN IMMUNOCOMPROMISED AND IMMUNOCOMPETENT MOUSE MODELS

Rebekah Bullard, Monica Embers
Tulane National Primate Research Center, Covington, LA, United States

The role of ticks in the transmission of Bartonella spp. has become an important topic in understanding Bartonella infections. The overlap of Bartonellosis diagnoses and Lyme disease patients implicates the black-legged deer tick (Ixodes scapularis) as a possible vector. Additionally, the identification of Bartonella spp. in wild I. scapularis with and without the presence of Borrelia spp. requires a systematic approach to determining vector competence. In this study, we compare the frequency and dissemination of bacteria in immunocompetent and immunocompromised mouse models by evaluating a wide variety of tissues for infection using nested PCR. The ability of Bartonella to infect and persist in the tick vector was determined using artificial and natural forms of infection. Pathological findings of the liver, kidney, brain, and heart were examined using H&E staining. The data give insight into the role of I. scapularis as a vector of B. henselae as well as perspective on the requirements for infection and animal model development.
SPOTLIGHT REPORT: HISTORICAL RECORD OF TICK DIVERSITY IN ALGERIA

Abigail Lilak1, Graham Matulis1, David B. Pecor2, Alexander M. Potter3, Dustin Rodriguez2, Regina M. Jobson1, Michael E. von Fricken1, Yvonne-Marie Linton2

1George Mason University, College of Public Health, Fairfax, VA, United States, 2Walter Reed Biosystematics Unit (WRBU), Smithsonian Institution Museum Support Center, Suitland, MD, United States, 3James Madison University, Harrisonburg, VA, United States

Tick-borne diseases remain a concern to the well-being of wildlife, livestock, and humans yet often lack essential data pertaining to tick diversity and distribution within countries. Conducting a systematic review allows for the geographic characterization of tick distribution within a country which can be helpful in informing about potential pathogen presence and provide a dataset for VectorMap, an online collection database. Utilizing PubMed, Scopus, and WoS databases, MeSH terms were used to capture literature. A total of 711 peer-reviewed articles from the years 1901-2022 were systematically screened for data meeting the specific inclusion and exclusion criteria. After removing duplicates and filtering based on title, abstract, and relevant data, 63 articles qualified for extraction and geo-referencing. Nine different genera were the most species with individual records. From the collection records with host information, 76% of tick collection events were linked to animals, of which 84% were from livestock. There were 34 reported pathogens, which 84% were from livestock. There were 34 reported pathogens, which included Crimean-Congo hemorrhagic fever, Anaplasma spp., Babesia spp., Borrelia spp., Ehrlichia spp., Rickettsia spp., and Theileria spp. This systematic review provides important information about tick diversity and distribution within Algeria over the past 100 years. Additionally, this data can be utilized to assist with surveillance and help identify research knowledge gaps on ticks and tick-borne diseases in Algeria through manipulation of this geographic dataset.

SPOTLIGHT REPORT: TICS AND TICK-BORNE DISEASES OF NIGER

Dustin Rodriguez1, David B. Pecor2, Alexander M. Potter3, Graham Matulis1, Abigail Lilak1, Regina M. Jobson1, Michael E. von Fricken1, Yvonne-Marie Linton2

1James Madison University, Harrisonburg, VA, United States, 2Walter Reed Biosystematics Unit (WRBU), Smithsonian Institution Museum Support Center, Suitland, MD, United States, 3George Mason University, College of Public Health, Fairfax, VA, United States

Despite ticks and tick-borne diseases continuing to be a threat to the health of humans and livestock throughout West Africa, there continues to be a lack of data for adequate risk assessment within the country of Niger. Determining the distributions of various tick species and identifying associated pathogens can provide valuable information regarding at risk areas for TBDs. To supplement tick collection data already documented in VectorMap, a systematic review of literature published between 1901 and 2022 was conducted. Over 300 articles were pulled from three databases, PubMed, Web of Science, and Scopus. Standardized search terms were used, and the resulting articles went through a series of title, abstract, and full review focusing on our inclusion criteria. This was followed by extraction and greferencing of relevant data. Of the articles found, 40 met the inclusion criteria for Niger and six contained extractable data. A total of 22 individual species were identified from six genera, with Rhipicephalus most frequently reported. Pathogens in two genera were also documented including several newly sequenced genotypes of Ehrlichia and Rickettsia, Hyalomma truncatum (Koch, 1884) and Amblyomma variegatum (Fabricius, 1794) were found to be the most common hosts of pathogens. The current dataset, while limited, identify ticks as disease vectors in Niger and highlights the need for expanding current surveillance efforts. Further examination and analysis of these findings may aid in the selection of future sampling sites.

ENHANCING EHRLICHIOSES RISK DETERMINATION THROUGH THE SOUTHEASTERN TICK-BORNE EMERGENT PATHOGEN SURVEILLANCE (STEPS) PROGRAM IN TENNESSEE

Abelardo C. Moncayo1, Jeff Gruntmeir2, Rhoei Dinglasses2

1Tennessee Department of Health, Nashville, TN, United States, 2University of Florida, Gainesville, FL, United States

Ehrlichiosis is a tick-borne disease caused by bacteria of the genus Ehrlichia and transmitted by the lone star tick (Amblyomma americanum), an aggressive human-biter widespread in the southeastern United States. This illness affects for a substantial burden of tick-borne disease cases in Tennessee. Field collections of questing ticks were conducted in the summer of 2021 in thirteen Tennessee counties with varying ehrlichiosis incidences to evaluate the utility of tick pathogen prevalence data to augment reported human disease incidence data to enhance understanding of tick-borne disease risk. Two sites per county were sampled by a dragging method 2-3 times between June and August, resulting in over 1,400 ticks collected. Samples were identified, pooled, processed, and tested for relevant human pathogens by real-time PCR. A. americanum ticks were tested for Rickettsia spp., Rickettsia rickettsii, R. parkeri, Ehrlichia chaffeensis, E. ewingii, Panola Mountain Ehrlichia sp., Bourbon virus, and Heartland virus; Dermacentor variabilis ticks were tested for Rickettsia spp. and R. rickettsii; Amblyomma maculatum ticks were tested for R. parkeri; and...
Ixodes scapularis ticks were tested for Borrelia spp., B. burgdorferi s.s., and Anaplasma phagocytophilum. All A. americanum (n=1,307) were collected from four Middle Tennessee counties surveyed. Thirteen percent of A. americanum tick pools were positive for an Ehrlichia spp.; E. ewingii and the novel Panola Mountain Ehrlichia species (PME) were detected at higher rates than expected. Counties with higher ehrlichiosis incidences generally had higher infection rates of Ehrlichia spp. in A. americanum ticks. Based on our findings in tick populations, more ehrlichiosis cases may be due to E. ewingii infection than are being reported. Physicians should consider testing for E. ewingii in addition to E. chafeensis when ehrlichiosis is suspected. The human pathogenicity of PME is uncertain, but the high prevalence of this pathogen in host-seeking ticks supports the need for further study to determine the public health risk posed.

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EFFECTIVENESS OF FLURALANER TREATMENT REGIMENS FOR THE CONTROL OF CANINE CHAGAS DISEASE. A MATHEMATICAL MODELING STUDY

Edem Fiasou1, Rachel E. Busselmang1, Gabriel L. Hamerg1, Sarah A. Harnerg1, Martial L. Ndeffo-Mbasa1, Brian Herrin3, W. Brian Herrin3, William K. Herrin3
1Texas A&M University-College Station, College Station, TX, United States, 2Texas A&M University-College Station, College Station, TX, United States, 3Kansas State University, Manhattan, KS, United States

Canine Chagas disease is caused by the protozoan parasite Trypanosoma cruzi and transmitted by insect triatomine vectors known as kissing bugs. The agent can cause cardiac damage and long-term heart disease and death in humans, dogs, and other mammals. In laboratory settings, treatment of dogs with systemic insecticides has been shown to be highly efficacious at killing triatomines that feed on treated dogs. We developed compartmental vector-host models of T. cruzi transmission between the triatome and dog population accounting for the impact of seasonality and triatome migration on disease transmission dynamics. We considered a single vector-host model without seasonality, and model with seasonality, and a spatially coupled model. We used the models to evaluate the effectiveness of the insecticide fluralaner with different durations of treatment regimens for reducing T. cruzi infection in different transmission settings. In low and medium transmission settings, our model showed a marginal difference between the 3-month and 6-month regimens for reducing T. cruzi infection among dogs. The difference increases in the presence of seasonality and triatome migration from a sylvatic transmission setting. In high transmission settings, the 3-month regimen was substantially more effective in reducing T. cruzi infections in dogs than the other regimens. Our model showed that increased migration rate reduces fluralaner effectiveness in all treatment regimens, but the relative reduction in effectiveness is minimal during the first years of treatment. However, if an additional 10% or more of triatomines killed by dog treatment were eaten by dogs, treatment could increase T. cruzi infections in the dog population at least during the first year of treatment. Our analysis shows that treating dogs every three to six months could be an effective measure to reduce T. cruzi infections in dogs and triatomines in peri-domestic transmission settings. However, further studies at the local scale are needed to better understand the potential impact of routine use of fluralaner treatment on increasing dogs’ consumption of dead triatomines.

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MOLECULAR DETECTION OF YERSINIA PESTIS AND BARTONELLA SPP. IN RODENT FLEAS FROM PIURA, PERU

Magdalena Mallqui1, Diego Cuicapuza2, Cusi Ferradas3, Marco Risco4, Luis Mosto4, Victor Pacheco4, Andres G. Lescano4, Winnie Contreras5
1Emerge, Emerging Diseases and Climate Change Research Unit, School of Public Health and Administration, Universidad Peruana Cayetano Heredia, Lima, Peru, 2Laboratorio de Genómica Microbiana, Facultad de Ciencias y Filosofía, Universidad Peruana Cayetano Heredia, Lima, Peru, 3Universidad Nacional Mayor de San Marcos, Natural History Museum, Lima, Peru

Fleas are arthropod vectors of emerging and re-emerging pathogens, including bacteria of the genus Yersinia and Bartonella. In the Andean region of Piura, sporadic human cases of plague and bartonellosis had occurred. Thus, it is necessary to use vector surveillance to characterize the risk of zoonotic disease transmission. We conducted a cross-sectional study in Piura to determine the presence of Yersinia pestis and Bartonella spp. among fleas infecting rodents. DNA was extracted from 127 fleas collected from 130 rodents. A conventional multiplex PCR targeting the ypo2088 and plg genes was used for Yersinia pestis, while a conventional PCR targeting the gltA gene was used for Bartonella spp. Overall, 5 flea species were taxonomically identified: Xenopsylla cheopis (109), Plocopsylla hector (13), Leptopsylla segnis (3), Pulex irritans (1), and Ctenocephalides felis (1). Of rodents, Akodon molly was the most abundant (46.1%, 56), followed by Rattus rattus (38.4%, 50) and Mus musculus (13.1%, 17), and four other species. Bartonella spp. DNA was detected in 15.6% (17/109) of Xenopsylla cheopis and 61.5% (8/13) of Plocopsylla hector. Of the flea samples that were positive for Bartonella, 96% (24/25) were collected from Akodon molly. Yersinia pestis DNA was detected in two specimens of Xenopsylla cheopis 1.6% (2/127) that were collected from one Rattus rattus and one Akodon molly. We conclude that the presence of Bartonella spp. and Yersinia pestis in fleas of peridomestic and wild rodents in Piura represent a potential zoonotic risk. The molecular detection of Bartonella spp. in Plocopsylla hector is described for the first time and the need to study its vectorial capacity in the transmission of Bartonella to human populations is highlighted, as well as the role of Akodon molly
as a reservoir. These pathogens represent important public health risks, especially in rural areas where humans and domestic animals are in close contact with peridomestic and wild rodents. It is necessary to propose ectoparasite control strategies due to the presence of *Yersinia pestis* in *Xenopsylla cheopis* because of previous reports in the area of bubonic plague cases.

CHARACTERIZATION OF EPSTEIN BARR VIRUS INFECTION IN TONSILS OF CHILDREN RESIDING IN MALARIA HOLOENDEMIC REGION OF WESTERN KENYA

Emmily Jepkemboi Koech
Kenya Medical Research Institute, Kisumu, Kenya

Epstein Barr virus (EBV) is one of the co-factors linked to endemic Burkitt lymphoma (eBL) development, which remains the most common pediatric cancer in sub-Saharan Africa. We have previously shown that children in malaria-endemic areas acquire EBV infection by 6-months of age but there is little knowledge about EBV infection in tonsils, a secondary lymphoid organ in the source of virus transmission. In this cross-sectional study, we characterized EBV infection in blood, plasma, saliva and tonsillar mononuclear cells (TMCs) in children aged 1-14 years undergoing tonsilllectomy from western Kenya, a malaria holoendemic region. EBV viral loads and EBV type were determined by PCR in blood, plasma, saliva and tonsillar mononuclear cells (TMCs). We found that the mean EBV viral load in TMCs was significantly higher compared to levels in blood (p=0.002). In addition, higher mean EBV viral loads was observed in saliva as compared to mean viral loads in plasma (p=0.0465). Although there was no correlation between age and EBV DNA copies in plasma, saliva and blood, a significant negative effect of age on EBV loads was demonstrated in TMCs (r=-0.6875, p=0.0001), indicating that there is reduction of EBV viral particles as age increases. Children coinfected with both EBV type 1 (EBV-1) and EBV-2 had significantly higher viral loads as compared to those infected with only EBV-1 (p=0.0024) and not EBV-2 in saliva compartment, no difference was observed in the other compartments. This data suggests that, children residing in malaria endemic regions have elevated viral loads in tonsils which decreases as age progresses. In addition, coexistence of both EBV-1 and EBV-2 may favor increase of EBV infected cells hence increase in EBV viral loads.

RECONCILIATION OF ADVERSE PREGNANCY OUTCOME RISKS BETWEEN FOUR ZIKA VIRUS COHORTS IN LATIN AMERICA

Olivia Pluss1, Vivian Avelino Silva2, André Cabie3, Benoit Tressieres4, Mathieu Nacher5, Patricia Brasil6, Leo Pomar7, Mauricio Rio de Janeiro, Brazil, 7Materno-fetal and Obstetrics Research Unit, à-Pitre, Guadeloupe, France, 5Centre Hospitalier de Cayenne - Centre Chagas National Institute of Infectious Diseases, Oswaldo Cruz Foundation, Switzerland, 8Virology Research Laboratory, Sao José do Rio Preto School during pregnancy, using individual patient data meta-analysis to reconcile findings. The studies recruited participants between September 2015 and November 2016 in Brazil and the French Territories in the Americas (Guadeloupe, Martinique, French Guiana). All studies included pregnant women at any gestational age, with confirmed ZIKV infection, and provided systematic follow-up until the pregnancy outcome. However, important methodological differences were identified, including season of enrollment, further inclusion criteria applied, and modes and times of collection of mother and infant examinations. The overall pooled standardized risk estimate of any adverse fetal or infant outcome reported by the studies was 28% [95%CI: 16-41], with high heterogeneity (I2=91%). There was high heterogeneity in pooled risk for many specific adverse outcomes, both overall and by trimester of maternal ZIKV infection diagnosis, such as spontaneous pregnancy loss and clinically apparent abnormalities at birth. We saw agreement (I2=25%) between studies for some adverse outcomes by subgroups, including the risk of structural brain abnormalities after 1st trimester ZIKV infection diagnosis (7%, 95%CI: 3-12), and the risk of microcephaly after 1st or 3rd trimester ZIKV infection diagnosis (4%, 95%CI: 1-8, & 3%, 95%CI: 0-6, respectively). Our study suggests that the variability of initial risk estimates published for adverse fetal and infant outcomes following maternal infection during pregnancy can be partially explained by methodological differences between studies. Standardization of early investigation protocols and pooling of findings in real-time during emerging disease events is recommended.

EVALUATING BARRIERS AND FACILITATING FACTORS AROUND COVID-19 VACCINATION IN WESTERN UGANDA: A SURVEY OF COMMUNITY MEMBERS

Stephen Asiimwe1, Mastulah Nakalule1, Azfar D. Hossain2, Amir M. Mohareb3, Louise C. Ivers4, Kilande Esther Joan5, Richard Hasunira5, Clifft Abenaitwe5, Kenneth Mwehonge6
1Mbarara University of Science and Technology, Mbarara, Uganda, 2Harvard Medical School, Boston, MA, United States, 3Massachusetts General Hospital, Boston, MA, United States, 4Harvard Global Health Institute, Harvard University, Boston, MA, United States, 5Coalition for Health Promotion and Social Development (HEPS-Uganda), Kampala, Uganda

More than two years since COVID-19 vaccines were first administered, striking global disparities in COVID-19 vaccination rates continue to persist. While media reports have highlighted stagnating COVID-19 vaccination rates in Uganda — where only 28 percent of people have completed the primary vaccination series — specific drivers of low vaccination uptake at the community level remain unclear. We aimed to understand perceived barriers and facilitating factors around COVID-19 vaccination in Mbarara, Western Uganda. A survey (offered in both English and in Runyankole, the local language) was administered to community members 18-years and older passing by temporary COVID-19 vaccination tents set up in public areas. Individuals who approached the tents were invited to participate regardless of their plans to receive COVID-19 vaccination. Chi-square analysis was used to test relationships between prior COVID-19 vaccination status and other variables measured. 1006 community members completed the survey between 30 September 2022 to 11 October 2022. 765/992 (77 percent) had received at least one prior COVID-19 vaccine dose, while 227/992 (23 percent) had never received COVID-19 vaccination. 741/990 (75 percent) of respondents agreed that “A permanent Covid-19 vaccination site is located close to my home or workplace,” and 946/990 (96 percent) said they could reach the nearest COVID-19 vaccination site by foot or motorcycle taxi. Previously unvaccinated individuals were more likely to be younger (p = 0.02), be male (p = 0.03), have never been previously turned away from a vaccination site (p = 0.0001), and say that confidence in vaccines should be improved in order to improve local COVID-19 vaccination rates (p = 0.001). Although vaccine supply remains a challenge in western Uganda, these results suggest that many community members can conveniently access COVID-19 vaccination sites, and local vaccination programs should prioritize demand creation including among young men.
The global outbreak of MPOX (MPXV) in 2022 highlights the need to better understand the ecology and distribution of this historically uncommon viral zoonosis. Yet, extensive antigenic cross-reactivity among orthopoxviruses limits specificity of serological assays used to assess MPXV seroprevalence in humans and wildlife. Here, we develop an antigen-based multiplex immunocassay to discriminate MPXV-positive IgG from vaccinia (VACV) and smallpox (VARV). First, 21 MPXV, VACV, and VARV immunodominant surface proteins produced from a variety of sources and expression systems were coupled to magnetic microspheres and tested in multiplex microsphere-based immunocassays with hyper-immunized polyclonal rabbit sera raised against VACV and VARV. To qualify homologous antigens and establish cross-reactions, we tested multiplex panels incorporating homologous MPXV, VACV, and VARV surface proteins. Antigen-antibody cross-reactions were observed in each of these panels and four MPXV antigens with lower cross-reactivity to VACV polyclonal antisera were identified. Two antigens, A35 and A29L, were selected as potential targets for sensitive binding of MPXV IgG and two antigens, E8L and L1R, were selected as potential targets for specific antibody-binding. Sera from non-human primates challenged with Modified vaccinia Ankara (MVA) were tested with this four-antigen multiplex to further characterize cross-reactions against MVA. Interestingly, A35 and E8L were cross-reactive against MVA while A29L and L1R were less cross-reactive, potentially conferring a higher degree of specificity. The performance of a protein and peptide antigen multiplex panel for specific MPXV antisera detection will be evaluated with convalescent sera from MPOX-infected individuals to further investigate observed antibody-binding patterns. Future work will focus on establishing signal ratios, thresholds for seropositivity, and determining sensitivity and specificity of the assay.

A SCOPING LITERATURE REVIEW OF GLOBAL DENGUE AGE-STRATIFIED SEROPREVALENCE: ESTIMATING DENGUE FORCE OF INFECTION IN ENDEMIC COUNTRIES

A SCOPING LITERATURE REVIEW OF GLOBAL DENGUE AGE-STRATIFIED SEROPREVALENCE: ESTIMATING DENGUE FORCE OF INFECTION IN ENDEMIC COUNTRIES

DEVELOPMENT OF A SURFACE PROTEIN-BASED MULTIPLEX IMMUNOASSAY FOR MPOX SEROLOGICAL TESTING AND SURVEILLANCE

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DETERMINATION OF REQUIREMENTS TO ENSURE EFFECTIVE INACTIVATION OF POWASSAN VIRUS AS A SURROGATE BIOLOGICAL SELECT AGENTS AND TOXINS (BSAT)

DETERMINATION OF REQUIREMENTS TO ENSURE EFFECTIVE INACTIVATION OF POWASSAN VIRUS AS A SURROGATE BIOLOGICAL SELECT AGENTS AND TOXINS (BSAT)

ASSOCIATION OF FOREST RELATED ACTIVITIES WITH MADARIAGA VIRUS INFECTION IN THE COMMUNITY OF ARUZA IN PANAMA

ASSOCIATION OF FOREST RELATED ACTIVITIES WITH MADARIAGA VIRUS INFECTION IN THE COMMUNITY OF ARUZA IN PANAMA
the risk of MADV infection vary geographically within communities the Darin province. For example, MADV risk of infection appears to increase in communities with extensive agriculture and cattle ranching activities, while this risk appears to be dismissed in communities with forest proximity. We intend to evaluate whether livestock or agricultural activities are associated with the seroprevalence of the Madariga virus in the community of Aruza, Panama, the community with the highest MADV seroprevalence. A cross-sectional population serosurvey was undertaken in Aruza during 2019. Characteristics of the study population was obtained. The association with MADV seroprevalence and outcome variable was evaluated at the univariate and multivariate level and expressed as prevalence ratios (PR) using a Generalized Linear Model (GLM), poisson family Log link function. Independent variables include forest related activities, sex, age, among others. An epidemiological based strategy was used to obtain the most parsimonious model. A total of 251 participants were enrolled in the study, of these, 28.5% reported carrying out livestock or agricultural activities. The estimated seroprevalence of MADV in Aruza was 23.4 %. People who carry out livestock or agricultural activities have a prevalence of MADV of 1.95 (PR=1.95; IC95% 0.58 - 6.55; p=0.282) times compared to those who do not do these activities, adjusting for sex, age and other occupations. Working in the forest has a seropositive for MADV of 4.47 (FP=4.47; IC95% 1.89 - 10.54; p=0.001) times compared to those who do not carry out this type of activity in the forest, adjusting for sex, age and other activities. Our results suggest that people who work in the forest are at higher risk of MADV infection, these results contrasts with previous findings and highlights the need for future evaluations of MADV risk factors.

### Describing the Implications of Temperature on the Transmission Potential of Rift Valley Fever Virus in Culex Tarsalis and Aedes Aegypti Mosquitoes

Shelby Cagle, Arielle Glass, Corey Campbell, Olivia Martinez, Emma Harris, Rebekah C. Kading

Colorado State University, Fort Collins, CO, United States

Disease-transmitting mosquitoes are constrained by environmental conditions in which they live. The impacts of temperature on mosquito development has been characterized, but few investigations have elucidated the effects of the larval rearing temperature on virus dissemination and transstadial persistence in naturally-infected mosquito vectors. This is of considerable significance as global climate change shifts the habitat boundaries of mosquito vectors, allowing global transmission of infectious diseases to previously unaffected geographical regions. One such emerging infectious disease of concern is Rift Valley fever virus (RVFV), a zoonotic disease endemic to sub-Saharan Africa that is poised to expand the habitat boundaries of mosquito vectors, allowing global transmission of infectious diseases to previously unaffected geographical regions. The persistence of RVFV RNA in a large proportion of patients with Rift Valley fever has important clinical and infection control implications.

### Persistence of Viral RNA in Hospitalized Patients with Lassa Fever in Liberia

Carwolo Pewu1, Emmanuel Kerkula1, Martha Gaylouw1, Nukai Doetein1, Alfred Flomo1, Amara Fofana1, Stanley Kerkula1, Thomas Sumo1, Rosi Wanst1, Marta Zizek1, Catherine Nimley1, McKenzie Colt1, David A. Woh1, William A. Fischer2, Jeffrey Sibley2

1The University of North Carolina at Chapel Hill, Chapel Hill, NC, United States, 2Phebe Hospital, Bong County, Liberia

Lassa virus (LASV) is a persistent regional and global public health threat that causes annual outbreaks in Liberia, Sierra Leone, and Nigeria. Despite the large numbers of individuals infected annually and high rates of mortality among hospitalized patients (25-50%), little is known about viral replication kinetics or the association between host and virus-related factors and clinical outcomes. Plasma samples from patients with suspected LASV infection admitted to Phebe Hospital in Bong County, Liberia were tested for LASV using the semiquantitative Altona 2.0 polymerase chain reaction assay. Among those with confirmed LASV infection serial sampling of a subset of patients occurred on days 3, 5, 7, 10 following diagnosis and 3-, 6-, 9-, and 12-months following discharge along with the collection of demographic and clinical information at each time point. Host- and virus-related factors including sex, age, time from symptom onset and viral loads assessed by cycle threshold (CT) values at the time of diagnosis were compared between patients who survived and died. Clinical data and CT values from 76 patients (mean age 24 years; 50% female; mean 6 days from symptom onset) with confirmed Lassa fever were analyzed including 38 with serial sampling. Of the 76 patients, 66 (87%) survived to discharge. Of 38 patients with more than 1 sample, all of whom received Ribavirin treatment, 90% (26/29) on day 3, 85% (22/26) on day 5, 77% (20/26) on day 7, and 87% (20/23) on day 10 had detectable plasma LASV RNA. The proportion of patients in which LASV RNA was still detectable declined at 3 months post-discharge to 18% (2/11) and 0% (0/7) at 6 months post-discharge. LASV L CT values were significantly greater in survivors (mean CT 31.3) compared with non-survivors (mean CT 26.0; p=0.0184) while LASV GP CT values demonstrated a trend in the same direction (mean CT 30.6 and 27.3 respectively; p=0.059). While PCR positivity indicates that LASV RNA is detectable, it is not known if this represents infectious virus. The persistence of LASV RNA in a large proportion of patients with Lassa fever despite Ribavirin treatment has important clinical and infection control implications.

### Significant Gaps in Knowledge and Attitudes Towards Vaccination in a Highly Affected Population by the Monkeypox Epidemic: Case of Kumba Health District, Cameroon, September 2022

Akenji B. Mboringong1, Hervé Gaël Ndalle1, Wafo Tochis Elvise1, Pauline Akosung2, Ernadj Ines Nguidjol3, Audrey Lacroix4, Nadine Lamare3, Mba Flaubert Djonzo5, Makang Severine Ndifor6, Linda Esso7, Etoundi Alain Mballa8, Armel Evouna1

1Cameroon Field Epidemiology Program, Yaoundé, Cameroon, 2Regional Delegation for Health, South-West Region, Cameroon, Buea, Cameroon, 3Directorate for the Control of Disease, Epidemics and Pandemics, MOH, Cameroon, Yaoundé, Cameroon, 4University of Montpellier, France, Montpellier, France, 5Centre de Recherches sur les maladies Émergentes et Ré-émergentes, MINRESI, Cameroon, Yaoundé, Cameroon, 6Kumba District Health Service, MOH, Cameroon, 7Kumba, Cameroon, 8Kumba Health District (KHD),prompting a field investigation during which we evaluated knowledge, attitudes, and practices (KAP) among HCWs and determined seroprevalence among inhabitants. We conducted a KAP study among HCWs in KHD from September 9-14, 2022. We used questionnaires to collect socio-demographic data, knowledge, attitudes toward vaccination impact.

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and perceived threat posed by monkeypox. We collected blood in EDTA-tubes from persons visiting health facilities for evaluation of seroprevalence. Samples were tested using an in-house Luminex™ anti-Monkeypox IgG detection kit. We interviewed 104 HCWs (38% nurses, 21% nurse assistants, 9%, laboratory personnel, 4% physicians). Half of them (51/102) had previous knowledge on monkeypox, 13.6% (14/102) had received university or jobsite monkeypox training. The percentage-standardized score for knowledge on prevention, transmission, clinical presentation, and management of monkeypox was 31.2/100. There was no difference between the knowledge score and vaccination attitudes of the four categories of HCWs studied. Sixty-two percent (63/101) were accepting of monkeypox vaccination, but only 42.4% (42/99) were ready to pay for it. Monkeypox was perceived as less of a health threat than tuberculosis, hepatitis B, HIV, and COVID-19. We tested 166 inhabitants for anti-monkeypox IgG with median age of 25 years (5 months - 73 years) and male/female sex ratio of 0.8. The prevalence of anti-monkeypox IgG was 12.7% (21/166). There were significant knowledge gaps and unsatisfactory attitudes toward vaccination. Monkeypox was overlooked as a serious pathogen. The seroprevalence of monkeypox was high among inhabitants. This findings stress the importance of appropriate training of HCWs and strengthening of the monkeypox surveillance system.

EVALUATION OF THE LEVEL OF KNOWLEDGE, ATTITUDES, PRACTICES OF PREVENTIVE MEASURES AGAINST COVID-19 DISEASE AMONG MEDICAL STUDENTS AT THE UNIVERSITY OF HEALTH SCIENCES IN GABON

Ornella Anaïse Mbang Nguema
Université des Sciences de la Santé, Gabon, Owendo, Gabon

In Gabon, during the acute phase of the COVID-19 pandemic, medical students were involved in response activities. This has contributed to their exposition to infection. The objective of this study was to assess the level of knowledge, attitudes and practices of preventive measures related to COVID-19 by medical students of Faculty of Medicine, in Gabon. A cross-sectional survey was conducted among undergraduate and postgraduate medical students aged over 17 years, at the Faculty of Medicine, in Gabon from February to June 2022. Socio-demographics characteristics, knowledge, attitude, and practice toward COVID-19 were collected through a self-administered questionnaire under the supervision of the team. The results were analysed using statview 5.0 software and A p-value of <0.05 indicated statistical significance. Of the 711 survey participants, 55.3% were female and the majority were under 25 years of age (63.6%). A majority of medical students had good knowledge (87.9%, n= 625/711), positive attitudes (80.3%; n= 571/711) and only 40.5% (n= 289/711) had good practices. The majority of the participants (n= 683; 96.4%) knew that COVID-19 is a viral disease. More than 85% knew that preventive measures such as wearing mask, frequent hand washing and social distancing are effective. Attitude analysis highlighted that 86.4% of surveyed population considered that COVID-19 is a serious illness and 93% (n= 659) stated that it can be cured. Although 75.3% of the students reported frequent hand washing and 87.9% regularly wore masks, 75.5% reported high frequency of crowded places. Men and those under 25 years had better knowledge, attitudes and practices (p= 0.3). In addition, the rate of good knowledge (96% Vs 86.6%) was higher among postgraduate medical students than undergraduate students. In conclusion, this study demonstrated satisfactory knowledge, positive attitudes regarding COVID-19 among medical student; however, effective good practice was low. There is therefore a need to intensify education and to perform training sessions on COVID-19.
ENDEMIC VENEZUELAN EQUINE ENCEPHALITIS VIRUS ACTIVITY IN RURAL AND URBAN SETTINGS OF PANAMA

Carlos A. Lezcano, Jose Francisco Jose Galue, Xaciel Rodriguez, Yelissa Nicole Juarez, Jean Paul Carrera
Gorgas Memorial Institute of Health Studies, Panama, Panama

Venezuelan equine encephalitic (VEEV) viruses (Alphavirus genus, Togavirus family) are arthropod-borne zoonotic RNA viruses associated with human and equine disease throughout Central and South America. Panama, like many other countries, reports annually humans VEEV infections, which indicates its active circulation in rural enzootic areas. Symptoms of VEEV infection in humans can range from mild symptoms similar to those caused by other arboviruses to severe encephalitis, approximately 1% of cases are fatal. Using a population cross-sectional survey undertaken in urban areas of Panama and Panama Oeste during the COVID-19 outbreak, we aimed to detect evidence of VEEV circulation in urban areas. A total of 2198 participants were enrolled from November 30 to December 4, 2020, in 10 townships of Panama and Panama Oeste provinces. Serum samples from participants were used to detect antibodies against VEEV using a plaque reduction neutralization test. The association between VEEV seroprevalence and the exposure variables was evaluated at the univariate and multivariate level using a logistic regression model and a likelihood-ratio test as a variable selection method. A total 60.3% of the population were women, while 25.5% were adults over 60 years old, 63.7% of the population had mixed background, 48.4% had elementary studies. A total of 1951 participants reports living for years in the investigate area. The VEEV seroprevalence was 2.9% . Adults with more than 60 years old were at higher risk of VEEV infections compared to the other age groups. Antibodies against VEEV were found in Juan Demostenes Arosemena, Vista Alegre, Ernesto Cordoba Campos and Chillibre townships. The risk of living in Juan Demostenes Arosemena township in Panama Oeste province was 4.1 times compared to the other townships. Higher education was a protective factor for VEEV infection when compared with having at least primary or elementary studies. Our results suggest an endemic circulation of VEEV in urban settings of Panama and Panama Oeste provinces.

TWO-DOSE VACCINE EFFECTIVENESS FOLLOWING THE FIRST REACTIVE MASS VACCINATION CAMPAIGN AGAINST HEPATITIS E IN BENTIU, SOUTH SUDAN

Robin Nesbitt, John Rumunu, Vincent Kinya Asilaza, Priscilla Gitahi, Patrick Nkemenang, Melat Haille, Jetske Duncker, Zelle Antier, Etienne Gignoux, Manuel Alberte, Primitive Gakima, Joseph F. Wamala, Kediende Chong, Catia M. Alvarez, Isabella and conducted a case-control study to estimate two-dose vaccine effectiveness (VE). All suspected cases presenting to the MSF hospital who were eligible for vaccination and provided consent were enrolled in the study, comprising a questionnaire, laboratory tests and a follow-up visit after 2-4 weeks. Vaccine-eligible suspect cases were matched to community controls. We estimated VE against probable (anti-HEV IgM & elevated ALT, or &gt;4-fold IgG rise) and confirmed (HEV RNA+) hepatitis E using conditional logistic regression. From 11 May to 30 December 2022, we enrolled 287 vaccine-eligible suspect cases, including 1 probable and 16 confirmed. Among these, 2 (11.8%) were vaccinated with &gt;2 doses compared to 40 (40%) of 100 matched controls. We estimate a VE of 86.5% (95%CI 36.3-97.1) for 1-2 doses and 93.9% (95%CI -33.1-98.1%) for 2 doses. In addition to this direct protection, we observed a 5.5-fold decrease in the incidence rate of probable/confirmed cases after the second dose campaign. Lab confirmation is ongoing, and we will revise VE estimates and incidence based on these results. Following the first mass reactive vaccination campaign against hepatitis E, incidence declined. Preliminary VE estimates suggest that the short-term protection provided by this reduced dose-regimen may be high and potentially sufficient for outbreak response.

DIAGNOSTIC ACCURACY OF THE ZIKV DETECT™ 2.0 IGM CAPTURE ELISA AND THE ZIKA IGM RAPID TEST PROTOTYPE FROM INBIOS INTERNATIONAL INC

Moyra Machado Portilho, Juliana Gois Costa, Carolina Sacramento Gomes, Patricia Moreira, Leile Camila Jacob-Nascimento, Rosangela Oliveira Anjos, Mariana Kikuti, Laura Tauro, Mitermayer Galvão Rei, Guilherme Sousa Ribeiro, Instituto Gonçalo Moniz, Salvador, Brazil, Instituto Gonçalo Moniz, Universidade Federal da Bahia, Salvador, Brazil

Serological ZIKV diagnosis is challenging due to cross-reactivity with other flaviviruses. We evaluated the accuracy of two IgM-based serological tests from Inbios International Inc: the ZIKV Detect™ 2.0 IgM Capture ELISA and a Zika IgM Rapid Diagnostic Test (RDT) prototype, using a panel of sera from Brazil. Sensitivity was evaluated in 48 acute (<7 days post-fever onset, DFPO) and 26 convalescent (10-60 DPFO) paired samples from RT-PCR-positive Zika cases. Specificity was evaluated in paired acute and convalescent samples from 39 RT-PCR-positive dengue cases, 25 RT-PCR-positive chikungunya cases, 50 acute febrile illness (AFI) cases with negative RT-PCR for ZIKV, DENV and CHIKV, and 23 blood donor samples; these samples were collected outside ZIKV epidemic year, except for samples from 6 dengue cases. We also evaluated the tests on paired samples from 47 AFI cases with negative RT-PCR for ZIKV, DENV and CHIKV from ZIKV epidemic year, aiming to verify whether the tests could detect ZIKV infections despite a negative ZIKV RT-PCR. The RDT results were blindly assessed. Sensitivity increased from &lt;10% in the acute phase for both tests to 96% and 73% during the convalescent phase for ELISA and RDT, respectively, and was highest after 15 DPFO (100% and 73.7%, respectively). Specificities among the dengue samples were 87.2% and 92.3%, respectively; excluding the samples from the epidemic year, they were 96.6% and 94.4%, respectively. Specificities were 84% for both tests among the chikungunya samples; 96% and 92% in the non-epidemic AFI samples, respectively; and 91.3% and 100% among the blood donor samples, respectively. Among RT-PCR-negative AFI cases from the ZIKV epidemic, the tests’ positivity increased from the acute to convalescent samples (from 40.4% to 70.2% and 17% to 44.7%, respectively), suggesting that the RT-PCR missed some Zika cases. This was more evident for the subgroup with DENV IgM seroconversion (from 38.5% to 84.6% and 7.7% to 83.8%, respectively). The ELISA had excellent sensitivity during convalescence and high specificities during years non-epidemic for ZIKV. The RDT prototype had high specificity and moderate sensitivity.
EPIDEMIC TRANSMISSION OF CHIKUNGUNYA VIRUS DURING COVID-19 PANDEMIC LOCKDOWN MEASURES: A COHORT STUDY IN AN URBAN INFORMAL SETTLEMENT IN BRAZIL

Jaqueline Silva Cruz1, Meng Xiao2, Juan Aguilar2, Nivison Nery Jr3, Emília M. M Belitardo1, Daiana de Oliveira2, Mitermayer Reis1, Albert Ko1, Guilherme Ribeiro1, Federico Costa1

1Gonçalo Moniz Institute - Fiocruz, Salvador, Brazil, 2Department of Epidemiology of Microrganisms, Yale School of Public Health, New Haven, CT, United States, 3Instituto de Saúde Coletiva, Universidade Federal da Bahia, Salvador, Brazil

The observed decrease in CHIKV incidence globally during the pandemic has been speculated to be due to COVID-19-related disruption of human movement or alternatively, disruption of natural arboviral surveillance. We evaluated the incidence of CHIKV infection in community-based cohort from Salvador, Brazil was followed prior to and during the initial phase of the COVID-19 pandemic when lockdown measures were implemented and identified risk factors associated with transmission. We prospectively investigated a cohort of residents from an informal urban settlement by conducting three serosurveys from Sep 21 to Dec 16, 2018, Sep 09 to Nov 11, 2019 and Nov 18 to Feb 26, 2021. We performed interviews during surveys to obtain information on demographics, self-reported symptoms, and environmental risk factors. CHIKV seroconversion was ascertained by evaluated paired samples in the anti-CHIKV ELISA IgG. Among 1,038 participants of the 1st survey, 608 (59%) were followed in the 2nd and 3rd surveys. Among the 608 participants, the incidence of CHIKV seroconversion was 61.6% (37/608) between Survey 1 and 2 (pre-pandemic) and 31.1% (178/571) between Survey 2 and 3 (post-pandemic period). Residents whose households are located more than 50 meters away from an open refuse deposit are at a lower risk of CHIKV seroconversion (OR= 0.44, 95% CI= 0.2 - 0.7). However, reporting mosquitoes at home in the past 7 days is a risk factor for CHIKV seroconversion during the post-pandemic period (OR= 1.60, 95% CI= 1.1 - 2.3). Self-reported CHikungunya-associated symptoms were significantly higher (47.2% vs 30.9%, p<0.001) in participants who seroconverted compared to those who did not. Our findings indicate that epidemic CHIKV transmission can occur and impart high (30%) attack rates despite pandemic-associated interventions that restricted human movement and that low incidence of reported CHikungunya cases in similar settings was likely due to disruption of surveillance systems. The findings, although limited by recall bias, suggest that the symptomatic illness-to-infection ratio attributable to CHIKV exposure may be lower than traditionally believed.

SARS-COV-2 SEROPREVALENCE AND PREECLAMPSIA MARKERS AMONG UNVACCINATED MOZAMBIAN PREGNANT WOMEN WITH FETAL LOSS

Maureen Chileshe1, Tacilia Nhampassa2, Carla Carrilho3, Anete Mendes2, Elvira Luis4, Jahit Sacaríal5, Jaume Ortí3, Natalia Rakislova4, Clara Menendez5, Raquel González6

1Barcelona Institute for Global Health, Hospital Clinic-Universitat de Barcelona, Barcelona, Spain, 2Manhiça Health Research Center (CISM), Maputo, Mozambique, 3Department of Anatomic-pathology, Maputo Central Hospital, Maputo, Mozambique, 4Obstetrics and Gynecology Department, Maputo Central Hospital, Barcelona, Spain, 5Eduardo Mondlane University, Maputo, Mozambique

SARS-Cov-2 infection in pregnancy has been associated with increased risk of poor pregnancy outcomes, including preeclampsia and perinatal deaths. However, there is limited information on the burden of SARS-CoV-2 infection among pregnant women and their offspring in most African countries. We estimated SARS-CoV-2 seroprevalence among unvaccinated Mozambican pregnant women with fetal losses. This is a descriptive cross-sectional study including pregnant women delivering a stillborn or an infant dying in first hours of birth (early neonatal death) at Maputo Central Hospital (MCH), Mozambique. SARS-CoV-2 immunoglobulins (Ig) were determined in maternal and fetal blood. Biomarkers of preeclampsia (sFlt-1/PIGF) were also assessed in maternal blood. A total of 100 women were enrolled in the study between March 2021 to April 2022. All were COVID-19 unvaccinated women and had a mean age of 29 years (SD ±6.7). Overall, SARS-CoV-2 Ig were detected in 69 [69%] [95% CI (0.58 - 0.76)] women and 55 [55%] [95% CI (0.45 - 0.74)] fetuses. 60 women had SARS-CoV-2 IgM [95% CI (0.81 - 0.96)] and 53 had SARS-CoV-2 IgG [95% CI (0.69 - 0.88)]. Levels of sFlt-1/PIGF were significantly increased in women with SARS-CoV-2 Ig. In conclusion, SARS-CoV-2 seropositivity among Mozambican unvaccinated pregnant women with fetal loss was high and it was associated with increased preeclampsia markers.

MORTALITY FROM CHRONIC HEPATITIS C IN BRAZIL: ANALYSIS OF THE MULTIPLE CAUSES OF DEATH IN THE PERIOD 2000 TO 2019

Larissa Festa, Gerusa Maria Figueiredo

University of São Paulo, São Paulo, Brazil

Chronic hepatitis C is the leading cause of death among viral hepatitis in Brazil. The relevance of working with multiple causes of death (basic cause and associated causes) constitutes a breakthrough in the analysis of mortality statistics. To describe deaths from chronic hepatitis C, as the underlying cause and multiple causes of death, and to analyze the temporal and spatial distribution of these deaths in Brazil, from 2000 to 2019. This is an ecological study, with data from the Mortality Information System (SIM).

All analyses will be performed comparing mortality rates for chronic hepatitis C as the underlying cause of death and as multiple causes of death. Descriptive analysis of deaths from chronic hepatitis C will be performed, according to sociodemographic aspects. For the time series analysis, chronic hepatitis C mortality rates will be calculated by macro-region, and the temporal trend will be analyzed by Praiss-Winten regression. The spatial distribution by states of Brazil of mortality rates for chronic hepatitis C will be carried out, in the five-year period 2000 - 2004; 2005 - 2009; 2010 - 2014; 2015 - 2019. Work is still in progress, so as preliminary results the database consisted of 33.115 deaths from chronic hepatitis C in any line of the death
COVID-19 is an acute respiratory syndrome caused by the new coronavirus, SARS-CoV-2, the origin of which is still debated, which emerged in December 2019 in the city of Wuhan, Hubei Province, China. The spread of SARS-CoV-2 in China led to a pandemic, declared on 11 March 2020 by the WHO. From 25 March 2020, Mali recorded its first two outbreaks with two (2) confirmed cases. We conducted a prospective study from 1 April to 31 August 2021 to assess the level of knowledge, attitude and practice of the population in relation to the Coronavirus disease in the largest market of Bamako. We surveyed 400 people. The average age was 25.96±8.95 with extremes of 18 and 72 years; the male sex was the most represented with 67.3%. The modes of transmission were well known in our sample, 57.9% spoke of greeting with the hands. The majority of the participants had practiced hand washing with soap and chlorinated water with 86.3%. To improve prevention, participants recommended information, education and communication to the population. More than half of our surveys (64.5%) had poor knowledge of covid-19 followed by 33.3% who had average knowledge and only 2.3% had good knowledge of covid-19. More than half of our surveys (54%) had poor practice of barrier measures against covid-19 followed by 43% who had acceptable practice and only 3% who had good practice. We found a statistically significant relationship between the participant’s level of education and the level of knowledge (P=0.000). We found a statistically significant relationship between the participant’s level of education and the practice of covid-19 barrier measures (P=0.005).

WORLD HEPATITIS DAY, 2023 - COMMUNITY ENGAGEMENT ACTIVITIES

Diana Asandem1, Philip S. Segbefia2, Lutrodt Bentum-Enin2, Rawdat Baba-Adam2, Bright Asare1, Frank Osei2, Georgina Agyekum2, Linda E. Amoah2, Kwadwo A. Kusi2, Joseph H.K Bonney3

1West African Centre for Cell Biology of Infectious Pathogens (WACCBIP), Accra, Ghana, 2Immunology Department, Noguchi Memorial Institute for Medical Research, Accra, Ghana, 3Virology Department, Noguchi Memorial Institute for Medical Research, Accra, Ghana

We commemorated World Hepatitis Day 2022 with awareness activities, medical screening, and free vaccination against Hepatitis B Virus (HBV) aiming to draw public attention to hepatitis, garner stakeholder support and identify cases for management. Screening activities took place at the Maamobi General Hospital (MGH) and the Noguchi Memorial Institute for Medical Research (NMIMR), in Ghana. Participants went through pre-counselling encompassing hepatitis causes, transmission, prevention, and management. After consenting, blood samples were collected and screened for HCV antibodies and HBV markers (surface antigen/antibody-HBsAg/HBsAb, envelope antigen/antibody-HBeAg/HBeAb, and core antibody-HBcAb). In total, 351 were screened- 281 from the Maamobi community and 170 from the NMIMR. From the NMIMR participants, 4 (3.7%) were positive for HbsAg indicating HBV infection whilst 20 (18.7%) had HBsAb indicating immunity from either previous infection or vaccination. All four infected people also tested positive for HBeAb and HBcAb indicating non-replicative viral phase. A total of 21 (7.5%) and 28 (10.0%) HbsAg and HBsAb positives respectively were recorded at Maamobi. All those positive for HbsAg were also HBeAb positive except one, who was rather HBeAg positive. This individual could be highly infectious compared to the rest. HbsAg positive individuals were referred for clinical management at the MGH. In all 315 participants were eligible and received first dose of HBV vaccines. However, only 204/315 (64.8%) returned for all 3 doses within the 6-month span, 146/204 (71.6%) from Maamobi and 58/204 (28.4%) from NMIMR. A total of 111/315 (35.2%) were lost to follow-up. Only one person was positive for HCV antibodies. Our findings show a high HBV prevalence within the Maamobi community. This calls active surveillance within communities to find cases that may serve as transmission reservoirs for management. Furthermore, house-to-house vaccination approaches may reduce losses to follow up.

WHO ZIKV INDIVIDUAL PARTICIPANT DATA META-ANALYSIS: PRELIMINARY FINDINGS FROM A CONSORTIUM-WIDE INITIATIVE

Ronaldo Silva, ZIKV IPDMA Consortium

World Health Organization, Geneva, Switzerland

Zika virus (ZIKV) infection during pregnancy is a known cause of microcephaly and other congenital and developmental anomalies. To better understand the relationship between ZIKV infection during pregnancy, and adverse fetal, infant, or child outcomes, international leaders in ZIKV research established the ZIKV Individual Participant Data (IPD) Consortium in 2017. Datasets from 33 of 64 participating studies (21 cohorts and 12 surveillance-based) of contributors from Brazil, Colombia, French Guiana, Grenada, Guatemala, Honduras, Kenya, Puerto Rico, Spain, Trinidad and Tobago, and the USA were included in this preliminary analysis. Selection bias was assessed using meta-data and a four-question survey; data from 24 retained studies were pooled to estimate absolute (AR) and relative risk (RR) using a meta-analytic approach to account for heterogeneity. According to the study definition, 6,655 mothers (59.6%) had evidence of ZIKV infection, 3,597 (32.2%) were ZIKV-negative, and this information was missing in the remaining 8.1%. 41.2% of participants had arbovirus-related symptoms. The median age of participants was 27 years, the median gestational age at birth was 39 weeks, and the median gestational age at ZIKV infection was 18 weeks. The preliminary estimates with a 95% confidence interval showed the AR of microcephaly, miscarriage, and fetal loss in ZIKV-positive women was 3.43 (0.95-11.68), 0.15 (0.01-2.08), and 0.06 (0.00-1.36), respectively. In ZIKV-negative women, the AR was 0.06 (0.00-1.36), 0.02 (0.00-1.29), and 0.17 (0.01-2.69). Congenital zika syndrome (CZS) using the study definition was 4.43 (1.96-9.68) and 0.29 (0.01-7.24) for ZIKV-negative women. When comparing ZIKV-positive and -negative women, the RR of microcephaly, miscarriage, fetal loss, and CZS was 1.36 (0.70-2.65), 1.24 (0.54-2.87), 1.81 (0.78-4.19) and 0.84 (0.11-6.39), respectively. The consortium’s goal is to identify, collect and synthesize IPD from longitudinal studies to inform the development of recommendations for pregnant women, couples planning a pregnancy, and public health practitioners.


JH Kofi Bonney1, Stephen Ofori Nyarko1, Deborah Pratt1, Esinam Agbosu1, Yaw Awuku Larbi1, Abigail Abankwa1, Takaya Hayashi2

1Noguchi Memorial Institute for Medical Research, Accra, Ghana, 2Tokyo Medical and Dental University, Tokyo, Japan

World Health Organization estimates that a third to half of the world’s population is at risk of getting infected with Chikungunya. This infection...
DENGUE VIRUS SEROEPIDEMIOLOGY IN KINSHASA, DEMOCRATIC REPUBLIC OF CONGO

Rachel Sendor1, Daniel O. Espinoza2, Kristin Banek1, Melchior M. Kashamuka1, Joseph A. Bala1, Marthe Nkalani3, Georges E. Mahilu4, Georges Khuma5, Joseph Atibu6, Sam J. White6, Kyaw L. Thwai6, Theinny L. Bobanga7, Tommii Niseka8, Jeffrey A. Bailey9, Michael Emch10, Rhoecl Dinglasan11, Jonathan J. Juliano12, Matthew H. Collins3, Alexandre K. Tshéfu13, Jonathan B. Parr14

1University of North Carolina at Chapel Hill, Chapel Hill, NC, United States, 2Emory University, Atlanta, GA, United States, 3Kinshasa School of Public Health, Kinshasa, Congo, Democratic Republic of the, 4Brown University, Providence, RI, United States, 5University of Florida, Gainesville, FL, United States

Dengue virus (DENV) presents a major public health threat across tropical climates, yet little is known about its epidemiology within sub-Saharan Africa. In highly malaria-endemic countries such as the Democratic Republic of Congo (DRC), underreporting and misdiagnosis of DENV cases are thought to be common in the absence of readily available diagnostic tests, limiting prevalence estimation. We screened a convenience sample of dried blood spots (DBS) from a 2021 cross-sectional survey of children and adults ≥6 months old in Kinshasa Province to estimate the seroprevalence of DENV. This household survey was the final follow-up visit of a 4-year DENV surveillance study. Residents of Greater Accra and the Northern Region are more likely to have evidence of at least one DENV infection. The high DENV prevalence detected indicates a need for additional studies to determine the burden of infections in the DRC and sub-Saharan Africa.

CLINICAL FEATURES OF RESURGENT DENGUE 2 AS A MAJOR CAUSE OF ACUTE FEBRILE ILLNESS IN NICARAGUA FOLLOWING THE ZIKA EPIDEMIC

Alexis Domeracki1, Armando Matute2, Hernán Vargas3, Elana Horwitz4, Edwing Cuadra5, Omar Zepeda5, Tianchen Sheng6, Demetrios L. Samaras7, Lakshmanane Premkumar8, Aravinda de Silva10, Fílemón Bucardo3, Megan Reilly7

1Duke University School of Medicine, Durham, NC, United States, 2Hospital Escuela Oscar Danilo Rosales Arangue, National Autonomous University of Nicaragua at León, León, Nicaragua, 3National Autonomous University of Nicaragua at León, Nicaragua, León, Nicaragua, 4University of North Carolina, Chapel Hill, NC, United States, 5Duke Global Health Institute, Durham, NC, United States, 6University of North Carolina School of Medicine, Chapel Hill, NC, United States, 7Duke Global Health Institute; Division of Infectious Diseases and International Health, Department of Medicine; Durham Veterans Affairs Health Care System, Durham, NC, United States

Dengue virus has been endemic to Nicaragua for decades with periodic re-emergence of 1 or >1 dengue virus serotypes (DENV-1/4). Symptomatic dengue ranges from undifferentiated fever to dengue shock syndrome. We collected epidemiological and clinical data and acute and convalescent-phase sera to identify and characterize dengue as a cause of acute febrile illness (AFI) in post-Zika (2019) Western Nicaragua. We assessed clinical features of acute primary (1°) and secondary (2°) dengue vs other AFI in children (age <18) and adults. Among 820 subjects enrolled (80% with convalescent follow-up), 193 (29%) had acute dengue (all DENV-2) confirmed by paired serology and/or reverse transcriptase (RT)-PCR. Most (170, 88%) was 2° dengue. Dengue accounted for a greater proportion of AFI (31.7% vs 26.0%) in children vs. adults. Those with acute dengue were less likely than others to have rhinorrhea (5.2% vs 11.3%, p=0.02), dry cough (19.7% vs 27.0%, p=0.04), productive cough (7.8% vs 18.3%, p=0.001), chest crackles (0.5% vs 4.0%, p=0.02), and diarrhea (12.4% vs 24.5%, p=0.001) but more likely to have lower WBC and platelet counts (p=0.001). Features associated with 2° rather than 1° dengue were chills (80.0% vs 50.0%, p=0.004) and headache (90.6% vs 68.2%, p=0.002), whereas rash was more frequent in 1° dengue (63.6% vs 14.1%, p<0.001). Absence of rhinorrhea, cough, and sore throat and presence of headache and rash were associated with acute dengue in children but absence of diarrhea in adults. Lower WBC and platelet counts were associated with acute dengue vs other AFI. Headache was more frequent in 2° dengue and rash in 1° dengue in all age groups. Twenty-nine patients with acute dengue were treated with antibiotics; one died. We identified acute DENV-2 as a major cause of AFI in Western Nicaragua post-Zika. Compared with our pre-Zika AFI study at the same hospital, we observed exclusively DENV-2 vs >1 serotype and a higher proportion of AFI attributable to dengue (29% vs 5%).
Mosquito-borne La Crosse virus (LACV) is the leading cause of arboviral encephalitis among children in the United States. Most infections are reported from socioeconomically disadvantaged communities in the Appalachian region, but despite decades of focal endemicity in the area, LACV disease remains neglected with minimal public health infrastructure to prevent, detect, or respond to cases. We recently conducted a national study of geographic LACV disease clustering where we found that eastern Tennessee and western North Carolina form a persistent hotspot for LACV disease. Here, we increase the spatial resolution of our previous research by incorporating the sites of all reported LACV disease in Tennessee and North Carolina from 1997-2020. Our objectives were to investigate fine-scale spatial clustering of LACV disease clusters and to identify environmental predictors of disease risk. The results of our study show that LACV disease is heterogeneously distributed; most cases are consistently reported from a subset of geographic clusters with elevated risk relative to the study area overall. We also found that a key set of environmental, demographic, and economic predictor variables were associated with disease risk in the region. To evaluate targeted public health response to reported infections, we used random forest models to predict the likelihood of a location being a site of LACV disease. Our approach can help guide targeted public health interventions. In our presentation, we will contextualize our findings within the epidemiology of LACV disease and suggest approaches to reduce the disease burden in Appalachia.

MULTIPLEX SAMPLE-SPARING IMMUNOASSAY TO MEASURE SEROPREVALENCE OF CHIKUNGUNYA, DENGUE, AND ZIKA VIRUSES

Izabella N. Castillio1, Edwin G. Cuadra1, Yerun Zhu2, Sharon Taft-Benzi3, Fileenon Bucardo3, Aravinda M. de Silva1, Brian D. Byrd3, Abelardo Moncayo3, Michael Doyle4, Carl Williams4

1University of North Carolina, Chapel Hill, NC, United States, 2Emory University, Atlanta, GA, United States, 3National Autonomous University of Nicaragua, Leon, Nicaragua, 4Emory University, Atlanta, NC, United States

Chikungunya, dengue, and Zika viruses (CHIKV, DENV, ZIKV) co-circulate and cause similar clinical symptoms. We previously developed a multiplex assay to detect past DENV and ZIKV infections. However, due to antibody cross-reactivity, current serological assays for CHIKV using an inactivated virus or the E1/E2 envelope protein are unsuitable to distinguish between alphaviruses. To address this, we developed a multiplex immunoassay utilizing the antigenically distinct regions of the E1 protein for six medically relevant alphaviruses. To address this, we created a multiplex immunoassay utilizing the antigenically distinct regions of the E1 protein for six medically relevant alphaviruses, including CHIKV, ONVV, RRV, MAYV, VEEV, and EEEV. We tested the assay's ability for specific and sensitive detection of past CHIKV infection using 69 reference samples (24 CHIKV-infected and 45 CHIKV-uninfected). The multiplex assay's CHIKV antigen was 100% sensitive to CHIKV-positive sera and 100% specific to CHIKV-negative, flavivirus-positive sera. There was no cross-reactivity between the MAYV, RRV, and EEEV antigens to CHIKV-positive sera, but the closely related OVV antigen showed low cross-reactivity that could be resolved at higher sample dilution. In comparison, CHIKV-immune sera reacted strongly in CHIKV and MAYV E2 ELISA assays. However, MAYV E2 binding was not supported by MAYV neutralization. Next, we combined the CHIKV antigen coupled bead into the multiplex bead set configured with DENV and ZIKV EDIII antigens to assess the seroprevalence of these viruses among 172 febrile illness patients in 2015 (n=83) and 2018 (n=89) in Leon, Nicaragua. In 2015, 62.7% of patients reported fever had CHIKV, 53.1% had DENV, and 3.6% had ZIKV. The 2018 seroprevalence for CHIKV (56.2%) and DENV (73.3%) in age-matched patients with fever was comparable to 2015. The proportion of primary and secondary DENV prevalence in 2015 (50.7% vs. 49.2%) and 2018 (47.7% vs. 52.3%) were similar. However, ZIKV seroprevalence in these patients increased sharply from 3.6% in 2015 to 42.7% in 2018 due to the 2016 ZIKV outbreak in Nicaragua. This multiplex immunoassay provides a significant advantage over traditional assays to accurately measure seroprevalence of CHIKV, ZIKV, and the four DENV serotypes using a small blood sample.

EPIDEMIOLOGICAL CHARACTERISTICS OF MEASLES CASES IN LIBERIA, 2018-2021

Bode I. Shoibayo1, Julius S M Gilayeneh1, Emmanuel Dwalu1, Sunor L. Flomo2, Ralph W. Jeth1, Alberta B. Corvah1, Momo Teg2, Fahn M. Tarweh1, Obateemi J. Babalola2, Chukwuma D. Umekonkwo2, Jane A. MaCauley1

1National Public Health Institute of Liberia, Monrovia, Liberia, 2Africa Field Epidemiology Network, Monrovia, Liberia

Measles remains a disease of public health concern in Liberia, with annual outbreaks since 2017. It is an immediately reportable vaccine-preventable disease. We described measles’ epidemiological characteristics and associated factors in Liberia from 2018 to 2021. We reviewed Measles case-based surveillance data from 2018 to 2021 obtained from the National Public Health Institute of Liberia (NP-HIL). All cases that met Measles case definition for the period as clinically compatible, lab confirmed, and epi-linked were included. The data was extracted from the weekly surveillance reports, cleaned and analysed using Epi Info version 7.2. We calculated frequency, proportion, and rates. The median age of the 5905 measles cases reviewed was 7 (IQR:3-13) years. Majority of the cases, 72% (4238) were clinically compatible, while epi-linked, 15% (884) and lab confirmed 13% (783) were the least. The majority of the cases were females, 51% (2991). About 34% (1996) of the cases were vaccinated, while 50% (2972) had unknown vaccination status. Montserrado County accounted for the highest, 20% (1184) followed by Nimba, 12% (715). Pleebo District (Maryland) accounted for the highest, 9% (537) followed by Bushroad District, 6% (325) in Montserrat. Vaccination status (vaccinated) (OR=0.22, 95%CI: 0.05-0.99, p=0.031) was associated with measles outcome. Those vaccinated were less likely to die from Measles compared to others. Measles burden remained generally high in Liberia with low vaccination rates observed among cases. This decrease in vaccination coverage needs to be further investigated. The need to increase measles vaccination coverage to improve measles outcome is recommended. Measles, vaccination, Liberia, clinically compatible, lab confirmed, epi-linked

SARS-COV-2 INFECTION IN BROWN-HEADED SPIDER MONKEYS (ATELES FUSCICEPS) AT A WILDLIFE RESCUE CENTER ON THE COAST OF ECUADOR SOUTH AMERICA

Veronica Barragan

Universidad San Francisco de Quito, Quito, Ecuador

Human populations can be affected in unpredictable ways by the emergence and spread of zoonotic diseases. The COVID-19 pandemic was a reminder of how devastating these events can be if left unchecked. However, once they have spread globally, the impact of these diseases is heterogeneously distributed; most cases are consistently reported from a subset of geographic clusters with elevated risk relative to the study area overall. We also found that a key set of environmental, demographic, and economic predictor variables were associated with disease risk in the region. To prevent the continued persistence of LACV disease in resource-deprived areas, the risk predictors we identified should be used to guide targeted public health interventions. In our presentation, we will contextualize our findings within the epidemiology of LACV disease and suggest approaches to reduce the disease burden in Appalachia.
three zookeepers responsible for feeding and deworming the monkeys were also positive, suggesting human-animal transmission. Whole genome sequencing using Oxford Nanopore Technologies identified the omicron clade 22B BA.5 lineage in most samples. These findings highlight the threat posed by an emerging zoonotic disease in wildlife species and the importance of preventing spillover and spillback events during epidemic or pandemic events.

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MEASLES SURVEILLANCE SYSTEM EVALUATION IN THE FATICK REGION IN 2022

Ndiaye Faly DIOP1, Mamadou Sarifou BA2, Bouna NDIAYE2, Babacar NDOYE2, Issane NDIAYE2

1District sanitaire, Foundioungne, Senegal, 2MSAS, Dakar, Senegal

Measles surveillance is done on a case-by-case basis and aims at elimination. The Fatick region recorded an outbreak measles in 2021 and performances of its surveillance system is unknown. The study objective was to evaluate the measles surveillance system in the Fatick region. We conducted a descriptive cross-sectional study based on the CDC's guideline for evaluating surveillance systems. The health facilities to be visited were selected on a purposive basis. Data were collected using questionnaire administration and literature review. Usefulness, simplicity, acceptability and data quality attributes were assessed. Data were entered and analyzed using Epi Info 7.2.1.0 software. The score for each attribute was calculated based on the averages of the scores for each level. A score below 50% was classified as poor, a score between 50% and 80% was considered fair and a score of 80% or higher was good. A total of 24 (89%) providers were surveyed out. The scores were 96%, 88% and 82% respectively for usefulness, simplicity and acceptability. Promptness of weekly reports was 59% (n=27). The proportion of archived notification forms was 100% (n=27) at the level of the health posts and centers. The compliance rate between the registers and the data entered in the DHIS2 was 100% (n=27). The proportion of missing data was 12.2% (n=156) and 15.4% (n=26) respectively at the health center and health post levels. In conclusion, the measles surveillance system in the Fatick region was useful, simple and acceptable. The quality of the data was affected by missing data in the filling of the notification forms. It is necessary to train health hut actors and make the IDSR guide available to improve the measles surveillance system.

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THE EFFECT OF PRIOR ZIKA VIRUS INFECTION ON MARKERS OF MALE FERTILITY

Filemon Bucardo1, Viviana Pinedo Cancino2, Gustavo Natvio1, Jayrintzina Palacios1, Hernan Vanegas3, Donayre Marilly2, Rafael Saavedra Langer1, Maria Vasquez Chasnamote1, Oksana Kharabora1, Brenda Vasquez Martinez2, Ayla Bullock2, Edwing C Cuadra1, Lakshmanane Premkumar1, Aravinda de Silva1, R. Matthew Coward1, Natalie M. Bowman1

1Universidad Nacional Autonoma de Nicaragua-Leon, Leon, Nicaragua, 2Universidad Nacional de las Amazonas, Iquitos, Peru, 3University of North Carolina, Chapel Hill, NC, United States, 4Atlantic Reproductive Medicine, Raleigh, NC, United States

Zika virus (ZIKV) swept through the Americas in 2015 and 2016. Previously considered a mild illness of little clinical importance, unusual features of ZIKV were identified during the American epidemic - prolonged shedding in semen, sexual transmissibility, and congenital anomalies. In mouse models, ZIKV caused profound decreases in testicle size and sperm count, causing significantly impaired fertility. It is unknown if ZIKV has this effect in humans. One case series of 15 men showed decline in sperm count and testosterone after acute ZIKV that recovered after several weeks. To investigate the long-term effect of ZIKV infection on human male fertility, we recruited a cohort in two sites that experienced intense ZIKV transmission 1-2 years before the study. Healthy men aged 18-40 years were enrolled July 2018-March 2019. We recorded demographic, clinical, and epidemiological data. Men provided semen and blood samples quarterly for 12 months. Blood was tested for ZIKV EDIII IgG, dengue IgG and IgM, testosterone, and follicle stimulating hormone (FSH). Fresh semen analysis was performed on-site. Markers of fertility were compared between ZIKV-seropositive and seronegative men. Data were analyzed with Student's t-test and Wilcoxon rank-sum test for continuous data and Chi-squared test for categorical data. We enrolled 110 men (50 in Peru, 60 in Nicaragua). Median age was 23 years (IQR 19-27) and 55% were students. 35/97 (36%) reported vaccination to yellow fever. More than 90% were seropositive for dengue IgG. 39% were seropositive for ZIKV infection at enrollment (42% in Peru, 37% in Nicaragua, not significantly different). There was no association between age and odds of ZIKV positivity; 26% of men reported having impregnated a partner, with no difference by ZIKV status. There were no differences at enrollment by ZIKV exposure in semen pH, total sperm count, percent abnormal sperm, total motile sperm count, vitality, or round cell count. Further analyses are in progress, but these preliminary data reassuringly suggest that any effect of Zika virus infection on male fertility is likely short-lived.

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CLINICAL AND EPIDEMIOLOGICAL DIFFERENCES BETWEEN THE FIRST AND SECOND COVID-19 WAVES IN PATIENTS OF A HOSPITAL IN NORTHERN PERU

Virgilio E. Failloc-Rojas1, Mario J. Valladares-Garrido2, Alicia Torres-Mera3, Rubi Plasencia-Dueñas4, Jorge Hernandez-Cordova4

1Universidad Cesar Vallejo, Piura, Peru, 2Universidad San Martin de Pormes, Chiclayo, Peru, 3Universidad Nacional Pedro Ruiz Gallo, Lambayeque, Peru, 4Universidad Privada Norbert Wiener, Lima, Peru

Peru reported the second highest number of COVID-19 cases in Latin America, after Brazil. The first COVID-19 wave occurred during March December in 2020, and the second wave occurred during January-September in 2021. The differences between these waves remain largely unknown, and there is yet no comparison between them in Peru. We evaluated the differences in the clinical and epidemiological characteristics of COVID-19-affected patients during the two waves in northern Peru. We conducted a retrospective study using the clinical follow-up database of Lambayeque and the epidemiological notification form database of NotiWeb. The COVID-19-associated factors during the two waves were determined using a simple and multiple regression analysis, and the prevalence ratio (PR) was estimated. During the second wave of COVID-19 there was an increase in the symptoms of cough in 12.1%, odynophagia in 5.0%, chills in 16.0% compared with the first wave. The frequency of nasal congestion in the second wave was 2.17 times of that in the first wave (PR: 2.17). The second wave was marked by a higher proportion of affected children and adolescents and a greater percentage of respiratory symptoms than the first wave.

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CLINICAL SYMPTOMS OF CONFIRMED CASES OF CRIMEAN CONGO HEMORRHAGIC FEVER IN SENEGAL

Gamou Fall1, Allou Barry2, Ndeye Saïka Bob Niang2, Ousmane Faye3

1Institut Pasteur de Dakar, Dakar, Senegal

Emerging infectious diseases are considered major challenges for human survival. Among them, diseases caused by arboviruses such as Rift Valley fever virus (RVFV) and Crimean Congo hemorrhagic fever virus (CCHFV) have great economic, medical and veterinary impact. However, their epidemiology still need to be better understand in Africa. Indeed, despite significant efforts in capacity building, many countries are still not well prepared to detect these viruses, probably due to the lack of appropriate surveillance systems. In Senegal, a new surveillance system named 4S for Syndromic Sentinel Surveillance in Senegal, has been implemented in 2015. In the sentinel sites, blood sample is collected for any arbovirus and VHF suspected case and shipped to Institut Pasteur de Dakar for laboratory diagnosis. Next Generation Sequencing is also conducted on PCR positive
samples for genetic characterization. From 2015 to now, about 10,000 samples have been tested and several RVF and CCHF cases have been detected in 9 different regions while only few human cases were detected in 4 regions before 2015. Indeed, an RVF outbreak in 2020 and 3 CCHF fatal cases in 2022 and 2023 in Northern Senegal and Dakar have been detected. Our results also showed that apart from these fatal CCHF cases, clinical symptoms of the confirmed cases are usually mild with no death and large outbreaks are still not detected in Senegal. Viral genome sequence analyses highlighted virus introductions in Senegal from other countries including Mauritania and high viral genetic diversity for CCHF with probable reassortment events. The implementation of this syndromic sentinel surveillance in Senegal has improved CCHF and RVF detection in humans in Senegal. However, for a better understanding of their epidemiological profile in Senegal, implementation of sentinel surveillance in animals and arthropods as well as seroprevalence studies in humans and animals are needed. Our results, also showing virus introductions from other countries, called for collaborative interventions between African countries for a better disease surveillance and control.

**EVALUATION OF THE SURVEILLANCE SYSTEM FOR ACUTE FLACCID PARALYSIS BEFORE AND DURING COVID-19 IN PALESTINE, 2014-2021**

Ayyam A. Sawalmeh¹, Kostas Danis², Emily White Johansson², Dia’a Hajieh³

¹Ministry of health, Primary healthcare directorate, Preventive medicine department, Nablus, Palestinian Territory; ²Mediterranean and Black Sea Programme in Intervention Epidemiology Training (MedPIET), European Centre for Disease Prevention and Control (ECDC), Solna, Sweden; ³Ministry of health, Primary healthcare directorate, Preventive medicine department, Ramallah, Palestinian Territory

A nationwide Acute Flaccid Paralysis (AFP) surveillance system is the gold standard for detecting cases of Poliomyelitis and a main component of the WHO’s Global Polio Eradication Initiative (GPEI). Since the COVID-19 pandemic could potentially have a negative impact on surveillance systems, we compared the performance of the Palestinian national AFP surveillance during 2020-2021 with earlier years by making a descriptive retrospective study from the surveillance dataset provided by Palestinian ministry of health and we evaluated the attributes of the AFP surveillance in Palestine from 2014 to 2021 for completeness, timeliness and sensitivity by comparing it to the WHO AFP-surveillance standards for certification. Timeliness was measured as samples reaching the laboratory within three days of sample collection; completeness was measured as having no missing data on the investigation form, collecting “adequate samples” and performing a follow up investigation after 60 days; sensitivity was measured as having two or more AFP samples per 100,000 population per annum. The Results were a total of 286 AFP cases (57% Male) reported in 2014-2021 in the West Bank (65%) and Gaza (35%); (54%) were 0-5 years old. Completeness was >98%. Of all samples, (70%, SD=13) reached the laboratory in three days or less of being taken. Sensitivity of the system met the WHO criteria before the COVID-19 pandemic in 2014-2019 (2.4 per 100,000) but dropped in 2020-2021 during the pandemic to (0.9 per 100,000). We concluded that sensitivity was affected by the pandemic and made recommendations to the ministry of health to bolster it to reach the WHO-set criteria, such as training new public health staff and enforcing the reporting mandate more vigorously.

**INNOVATIONS FOR ENCOURAGING MEMBERS OF THE LOCAL COMMUNITY TO LIVE SAFELY WITH BATS IN THE MOUNTAIN ELGON AREA, EASTERN UGANDA**

Lillian Nalukenge¹, Robert M. Kityo¹, Betty Nalikka¹, Bernd Matovu¹, Michael J. Mutebi¹, Siya Aggrey¹, Rebekah Kading¹, Natalie Wickenkamp¹, Kalani Williams², Emma Harris², Tanya Dewey², Kevin Castle³

¹Makerere University, Kampala, Uganda, ²Colorado State University, Colorado, CO, United States, ³Wildlife Veterinary Consulting, Colorado, CO, United States

Bats are an important element of our environment. Currently, there is an increased Bat-Human interaction as humans move closer to bat habitats like caves and forests. Bats have been linked to numerous Emerging Infectious Disease (EID) events (Ebola and Murburg) and have been recognized as important reservoir hosts for viruses that cross to humans and domestic animals. In this regard, bats are seen as harmful by most of the people in various communities and therefore usually killed at sight. One objective in our bio-surveillance project in the Mt Elgon area is to create awareness among local communities especially where people are living in close proximity to bat habitats about the benefits as well as the risks of associating with bats. One of our approaches has targeted the primary school going age group at Kaptete Primary school as it is a few metres from one of our cave sites for activities that include information exchange in a classroom setting, quizzes and knowledge acquisition assessment using fine art and puzzles. We anticipate that the knowledge transfer to this age group would be impactful in changing the misconceptions & myths associated to bats and educating them on how to safely live with bats. In this presentation we shall present our tentative results that have so far; Engaged 55 pupils who all indicated awareness about the existence of bats around them. About 70% of them indicated they had ever visited a bat cave. 50% of them were aware that bats are said to cause them diseases like Ebola & Murburg. From this engagement, we also established that 10% of them had ever eaten a bat. Close to 80 % of them were interested in being part of a “Bat protection program” if initiated to them.

**OPTIMIZING TRAP PLACEMENT TO PREDICT WEST NILE VIRUS**

Anwesha Chakravarti¹, Rebecca Smith¹, Bo Li², Dan Bartlett³, Patrick Irwin⁴

¹University of Illinois Urbana Champaign, Urbana, IL, United States, ²NorthWest Mosquito Abatement District, Wheeling, IL, United States

Since its emergence in the New York Metropolitan area in the fall of 1999, the West Nile Virus (WNV), which is the leading cause of mosquito-borne disease in the continental United States, has been spreading rapidly. With over 52,000 cases nationwide to date, among which around 2,700 are from Illinois, taking adequate measures for the control of WNV has become vital. The lack of vaccines or medications to treat WNV makes prevention through mosquito control the only solution for controlling the spread of the infection. Mosquito traps that are used to test for the presence of the virus in mosquito populations play a crucial role in monitoring WNV and informing response. But how do you decide where to place a mosquito trap for WNV surveillance? And what makes a good trap, anyway? We present a statistical approach to determine the value of a mosquito trap in predicting human WNV cases in the next two weeks within a 1500m radius of the trap. We then use that value to understand what landscape, demographic and socioeconomic factors cause a mosquito trap to have the ability to predict correctly when human cases will occur, and when they will not, in the Chicago metropolitan area and its suburbs. This approach enables resource-limited mosquito control programs to identify better locations for their trap-based surveillance to increase trap efficiency while reducing the number of traps needed.
GENETIC AND PHYLGENETIC ANALYSIS OF INFLUENZA A VIRUSES & EVIDENCE OF AVIAN INFLUENZA INFECTION IN PIGS IN SENEGAL

Mamadou Malado Jallow1, Ndiené Koba Ndiaye1, Mamadou Aliou Barry2, Armary Fall1, Marie Pedepa Mendy1, Sara Sy1, Déborah Goudiaby1, Mbayame Ndiaye Niang1, Malick Fall1, Ndongo Dia1

1Virology Unit, Institute Pasteur, Dakar, Senegal, 2Epidemiology Unit, Institute Pasteur, Dakar, Senegal, 3Department of Biologie Animale, Faculté des Sciences et Techniques, Université Cheikh Anta DIOP, Dakar, Sénégal, Dakar, Senegal

The threat of an influenza pandemic is looming, with new cases of sporadic avian influenza infections in humans frequently reported. Pigs are considered important intermediate hosts and possible ‘mixing vessels’ for genetic reassortment of influenza viruses. However, epidemiological information on influenza infection of pigs in tropical and resource-limited countries remains sparse. So far, we investigated the epidemiology and genetic characteristics of IAVs as well as serological evidence of avian influenza virus infections in pigs at interfaces with human populations in Senegal. This study was carried out from September 2018 to December 2019 in the single pig slaughterhouse in Dakar, which receives animals from different regions of Senegal. Influenza A viruses were diagnosed by qRT-PCR and a subset of positive samples were selected for NGS sequencing. Serum samples were tested by HI assay using four reference viruses including H9N2, H5N1, H7N7 and H5N2. Influenza A Virus was detected in 30.7% of 1691 samples tested and H1N1pdm09 virus (38.07%) was the most commonly identified subtype followed by H1N2 (16.3%) and H5N2 (5.2%). Year-round influenza activity was noted in pigs, with the highest incidence between June and September. Phylogenetic analyses of all eight gene segments revealed that the isolated IAVs were closely related to human IAV strains belonging to H1N1pdm09 and seasonal H3N2 lineages. Genetic analysis revealed that Senegalese strains possessed several key amino acid changes, including D204 and N241D in the receptor binding site, S31N and P560S in the PA protein. Serological analyses revealed that 83.5% of the 1636 sera collected were positive for the presence of antibodies against either H9N2, H5N1, H7N7 or H5N2. Influenza H7N7 (54.3%) and H5N2 (53.6%) were the dominant avian subtypes detected in Senegalese pigs. Results of this study show the co-circulation of multiple subtypes of influenza viruses among Senegalese pigs. Therefore, regular monitoring and frequent surveillance of influenza viruses with one health approach are essential.

SOCIOECONOMIC VALUES ATTACHED TO CAVES AND BATS IN ELGON REGION OF UGANDA: IMPLICATIONS FOR BAT-BORNE PATHOGEN SPILLOVER

Aggrey Siya1, Robert M. Kiyo1, Nallikia Betty1, Matovu Wamala Benard1, Nakugene K. P. Lilian1, Mutebi J. Michael1, Natalie Wickenkamp2, Kalani Williams2, Emma Harris2, Kevin Castle3, Tanya Dewey4, Rebekah C. Kading4

1Makerere University, Kampala, Uganda, 2Colorado State University, Department of Microbiology, Immunology, and Pathology, Fort Collins, CO, United States, 3Wildlife Veterinary Consulting, LLC, Fort Collins, CO, United States, 4Colorado State University, Department of Biology, Fort Collins, CO, United States

Understanding the nature of the human-wildlife interactions provides an opportunity for designing the dynamic nature of the host-pathogen interfaces and determining the risk of zoonotic disease occurrence. Considering bat reproductive and roosting strategies, caves are critical to interfaces and determining the risk of zoonotic disease occurrence. These activities were majorly pursued to access material and immaterial assets including food, guano, rock salt and tourism. Security and safety related values were more pronounced in lower altitude areas where civil conflicts were common. Bat hunting was more pronounced in high altitude areas evidenced by fire events and tree branches with hooks. Although these are preliminary records, this data ought to be included in designing the human-bat interface to explicitly understand points of potential pathogen spillover.

WEST NILE VIRUS SEROPREVALENCE IN HUMANS LIVING IN THE TAMPA BAY REGION OF FLORIDA

Emma C. Underwood
University of South Florida, Tampa, FL, United States

West Nile Virus (WNV) is an arbovirus spread primarily by Culex mosquitoes with humans being an accidental dead-end host. The virus is endemic to the continental United States, having arrived in Florida in 1999, WNV was introduced to Florida in 2001, with 460 confirmed cases and resulted in 29 deaths since. It is estimated that eighty percent of cases are asymptomatic, with mild cases presenting as a non-specific flu-like illness. The Centers for Disease Control estimate that about 1 in 150 people infected with WNV develop a neuroinvasive form of the disease with 10% leading to death. Recent reports have shown that WNV can persist in the kidney for years following infection and has been linked with the development of chronic kidney disease. Other reports have shown that patients who’ve had WNV experience depression and mood disorders years following infection leading to significantly reduced quality of life. Currently, detection of WNV in humans occurs primarily in healthcare via PCR when patients present with severe manifestations of disease. This strategy is problematic given the long window of detectable viremia meaning that most WNV infections never receive an official diagnosis. While there are a few chicken-based and mosquito-based WNV surveillance programs in the United States, there is no human surveillance for WNV. Thus, the true burden of WNV in humans residing in the United States is unknown and could be a contributing factor into the rise in mental health problems and kidney disease in the United States during the last 20 years. This study utilized enzyme linked immunosorbent assay (ELISA) for IgG antibodies to test serum and plasma samples collected at Tampa General Hospital during 2020 and 2021. Plaque reduction neutralization tests were performed to confirm positive results. We found that nearly half of samples were positive for WNV antibodies, indicating a gross underestimation of WNV infection in humans. There was no statistically significant relationship between the presence of WNV antibodies and COVID-19 status.

IDENTIFYING THE VERTEBRATE HOSTS WITH POSSIBLE ASSOCIATION TO JAPANESE ENCEPHALITIS VIRUS DISPERSAL IN NATURE BY BLOOD MEAL SOURCE IDENTIFICATION AND RNA VIROME DETERMINATION IN JAPANESE ENCEPHALITIS VIRUS VECTORS

Astri N. Faizah, Daisuke Kobayashi, Ryo Matsumura, Mamoru Watanabe, Yukiko Higa, Kyoko Sawabe, Haruhiko Isawa
National Institute of Infectious Diseases, Shinjuku, Japan

In Asia, Culex mosquitoes are of particular interest because of their role in maintaining endemic mosquito-borne viral diseases, including the Japanese encephalitis virus (JEV). Nonetheless, host-feeding preferences, along with naturally infecting RNA viruses in certain Culex species, remain understudied. In this study, selected blood-fed mosquitoes were processed...
also reinforces the importance of surveillance in the management of the science gaps necessary for development of more effective vaccines, and Our findings provide data on the transmission dynamics of DENV serotypes DENV-1, genotype V and all clustered with genotype V isolates from China. Hospital=129) that were sequenced, we obtained complete sequences for DENV1 positive samples, (Mtongwe Naval Base=122 and Lamu County muscle pain 73.6% (304/413) and eye pain 56.4% (233/413). Of the 251 years (55%) and the most common clinical symptoms included headache 84.5% (349/413), chills 74.6% (308/413), joint ache 74.5% (313/413), infections. DENV1 cases were detected across the entire period with peaks in January-April 2020 (lowest=13 cases, highest=41 cases) and September 2022 to December 2022, cases of DENV3 increased gradually (lowest=2 cases, highest=7 cases). The burden of infection was highest in males (68%) between 19-39 years (55%) and the most common clinical symptoms included headache 84.5% (349/413), chills 74.6% (308/413), joint ache 74.5% (313/413), muscle pain 73.6% (304/413) and eye pain 56.4% (233/413). Of the 251 DENV1 positive samples, (Mtongwe Naval Base=122 and Lamu County Hospital=129) that were sequenced, we obtained complete sequences for 135 samples. Phylogenetic analysis showed that the genomes belonged to DENV-1, genotype V and all clustered with genotype V isolates from China. Our findings provide data on the transmission dynamics of DENV serotypes observed over a 3-year study period. Such data is useful in bridging the science gaps necessary for development of more effective vaccines, and also reinforces the importance of surveillance in the management of the infection to mitigate public health disruptions associated with outbreaks of dengue.

DENGUE SEROTYPE DYNAMICS AT THE KENYAN COAST

Josphat N. Nyataya, Kimita Gathii, Erick Muthanje, John Njenga Kenya Medical Research Institute/Medical Research Unit, Kisumu, Kenya

Dengue fever (DF), caused by four distinct serotypes of dengue virus (DENV-1-4) is transmitted by the mosquito vector Aedes Aegypti and Aedes albopictus. DF is responsible for more than 390 million infections globally. In Kenya, DENV is an emerging public health concern, especially at the Kenyan coast where DF is a common occurrence. In this study, we determined the heterotypic infections of DENV serotypes from November 2019 to September 2022 from study subjects presenting with febrile illness at Mtongwe Naval Base hospital in south coast and Lamu County Hospital in the north coast. DENV serotypes were identified by qPCR. Our data shows cyclical transmission of three dengue serotypes. DENV1 was the most dominant serotype accounting for 85% (350/413) of all DENV infections. DENV1 cases were detected across the entire period with peaks in January-April 2020 (lowest=13 cases, highest=41 cases) and September 2021 to May 2022 (lowest=10 cases, highest=70 cases). DENV2 was reported between December 2019 to April 2020 (lowest=4 cases, highest=12 cases). For DENV3, few cases were reported in December 2019 to March 2020 (highest=2 cases). From July 2022 to December 2022, cases of DENV3 increased gradually (lowest=2 cases, highest=7 cases). The burden of infection was highest in males (68%) between 19-39 years (55%) and the most common clinical symptoms included headache 84.5% (349/413), chills 74.6% (308/413), joint ache 74.5% (313/413), muscle pain 73.6% (304/413) and eye pain 56.4% (233/413). Of the 251 DENV1 positive samples, (Mtongwe Naval Base=122 and Lamu County Hospital=129) that were sequenced, we obtained complete sequences for 135 samples. Phylogenetic analysis showed that the genomes belonged to DENV-1, genotype V and all clustered with genotype V isolates from China. Our findings provide data on the transmission dynamics of DENV serotypes observed over a 3-year study period. Such data is useful in bridging the science gaps necessary for development of more effective vaccines, and also reinforces the importance of surveillance in the management of the infection to mitigate public health disruptions associated with outbreaks of dengue.

DENGUE VIRUS SURVEILLANCE IN Aedes Aegypti

David P. Tchouassi, Josephine Osalla, Gilbert Rothic International Centre of Insect Physiology and Ecology (icipe), Nairobi, Kenya

Arboviral diseases are increasingly important contributors to global human mortality and morbidity. This is exemplified by unprecedented emergence and re-emergence of epidemic arboviral diseases in the recent past such as dengue, chikungunya, Rift Valley fever and yellow fever. In many African countries, the impact of arboviral diseases is underestimated due to paucity of active surveillance, poor disease reporting systems, and lack of appropriate diagnosis. As a result, the possibility of the causative viruses circulating unnoticed, causing unresolved disease and/or outbreaks in humans, cannot be underestimated. Surveillance targeting vectors is essential to provide an early warning of the presence of viruses to reduce the potential for human disease. In this presentation, I will describe the use of cost-effective monitoring tools to assess the population dynamics of Aedes aegypti mosquito, and application of molecular (PCR, RT-PCR, sequencing) and virological (cell-culture) approaches to determine vertebrate hosts and viral associations in an urban setting in Kenya. The study findings will inform predictive risk assessment in support of preparedness plans and disease control operations.
Zika virus, a disease transmitted to humans by the bite of infected Aedes mosquitoes, emerged in the Americas in 2015 and caused large epidemics. In Colombia alone there were 72,031 Zika cases reported in the national surveillance system (SIVIGILA) between an epidemiological weeks (EWs) 22/2015 and 39/2016. Using these data, we aimed at identifying socioeconomic and environmental factors associated with the emergence and persistence of Zika across the 1,121 Colombian municipalities and 70 EWs. We fitted a zero-state Markov-switching space-time model under the Bayesian framework assuming that Zika switches between periods of presence and absence in each municipality according to spatially and temporally varying probabilities of emergence and persistence. These probabilities are assumed to follow a series of mixed multivariate logistic regressions. We present the mean odds ratio (OR) and the 95% credible interval (CI). Our results suggest that Zika emerged sooner in more densely populated areas (1.27, 1.18-1.37) and with or without weekly maximum temperatures (1.08, 1.04-1.12, lagged one week). On average, the odds of Zika emergence decreased by 6% with a 10 mm increase in the weekly accumulated precipitation lagged four weeks (0.94, 0.91-0.96), by 11% with a 0.1 increase in the Normalized Difference Vegetation Index (0.88, 0.80-0.95), and by 3% with 100 meters increase in the elevation (0.97, 0.95-0.99). More densely populated areas also presented higher odds of Zika persistence (1.32, 1.20-1.46). Zika was more likely to persist in a municipality where/when more cases were registered in the previous week (2.81, 2.21-3.66). The estimated probability of Zika presence increased weeks before cases started being registered. This is an important result because an emerging disease may circulate unnoticed for some time. The environmental factors found to be associated with the emergence of Zika may be proxies of the presence of the vector. The population density was the main driver of the first Zika epidemic in Colombia, indicating that a more densely populated areas should be prioritized for the prevention of emerging Aedes-borne diseases.

VirScan, a high throughput assay, Phage ImmunoPrecipitation Sequencing (PhIP-Seq), is a PhIP-Seq assay that allows for the testing of antibodies to approx. 200 human viruses using a small sample volume. Peptide tiles, derived from virus sequences, are presented on the phage surface and antibodies from samples bind these phages, which will be subsequently immunoprecipitated and sequenced to determine their identity. Using VirScan, we examined 130 plasma samples from 5 different Penan villages, and 20 samples from urban Sarawak as a comparator. We sought to determine whether members of the Community would have roughly a similar antibody exposure profile to that of their urban counterparts. Our preliminary analysis - subject to secondary verification - shows that human endemic viruses, such as the human herpesviruses and others, are common in the Penan population. Incidentally, we also note some individuals with antibodies to blood-borne pathogens such as Hepatitis C, where we are following up with serological verification. We are optimistic that this technology has a potential in the field of sero-epidemiology as it allows relatively hypothesis-free and high throughput testing of samples. We believe the PhIP-Seq assay will have value in helping identify specific viruses for surveillance or study.

VirScan serological profiling of the Penan tribe, an indigenous group in Sarawak, Malaysia

Charles Kevin Dee Tiu1, Ivan Yapi2, Mong Haw Ooi3, King Aik Wong2, Anand Mohan6, Samuel Leong Kheng Wong1, David Perera7, Lin-Fa Wang1

1Duke-National University of Singapore Medical School, Singapore, 2Singapore, 3Sarawak Infectious Disease Centre, Kuching, Malaysia, 4Institute of Health and Community Medicine, Universiti Malaysia Sarawak (UNIMAS), Kota Samarahan, Malawi, 5Department of Pediatrics, Bintulu Hospital, Bintulu, Malaysia, 6Petrajaya Community Clinic, Kuching, Malaysia, 7Institute of Health and Community Medicine, Universiti Malaysia Sarawak (UNIMAS), Kota Samarahan, Malaysia

Understanding the serological and exposure profile of a population, especially those residing in the animal-human interface, is of importance in understanding disease spillover and emergence risks. However, serological surveillance is often limited by the requirement of having a hypothesis. One needs to know what to test for to be able to identify antibodies to that pathogen. Here, we report our experience applying a highly multiplexable, high throughput assay, Phage ImmunoPrecipitation Sequencing (PhIP-Seq), to determine the serological profile of members of the Penan Tribe, an indigenous community in Sarawak, Malaysia. These communities are largely rural and live in very remote areas of Borneo. VirScan, developed by the Elledge group at Harvard University (and was kindly provided to us by Prof Steve Elledge), is a PhIP-Seq assay that allows for the testing of antibodies to approx. 200 human viruses using a small sample volume. Peptide tiles, derived from virus sequences, are presented on the phage surface and antibodies from samples bind these phages, which will be subsequently immunoprecipitated and sequenced to determine their identity. Using VirScan, we examined 130 plasma samples from 5 different Penan villages, and 20 samples from urban Sarawak as a comparator. We sought to determine whether members of the Community would have roughly a similar antibody exposure profile to that of their urban counterparts. Our preliminary analysis - subject to secondary verification - shows that human endemic viruses, such as the human herpesviruses and others, are common in the Penan population. Incidentally, we also note some individuals with antibodies to blood-borne pathogens such as Hepatitis C, where we are following up with serological verification. We are optimistic that this technology has a potential in the field of sero-epidemiology as it allows relatively hypothesis-free and high throughput testing of samples. We believe the PhIP-Seq assay will have value in helping identify specific viruses for surveillance or study.
Monitoring SARS-CoV-2 in wastewater is a valuable approach to track COVID-19 transmission. Designing wastewater surveillance (WWS) with representative sampling sites and quantifiable results requires knowledge of the sewerage system and virus fate and transport. We developed a multi-level WWS system to track COVID-19 in Atlanta using an adaptive nested sampling strategy. From March 2021 to April 2022, 868 wastewater samples were collected from influent lines to wastewater treatment facilities and upstream community manholes. Variations in SARS-CoV-2 concentrations in influent line samples preceded similar variations in numbers of reported COVID-19 cases in the corresponding catchment areas. Community sites under nested sampling represented mutually-exclusive catchment areas. Community sites with high SARS-CoV-2 detection rates in wastewater covered high COVID-19 incidence areas, and adaptive sampling enabled identification and tracing of COVID-19 hotspots. This study demonstrates how a well-designed WWS provides actionable information including early warning of surges in cases and identification of disease hotspots.

**T-CELL RESPONSES AS CORRELATES OF DIFFERENT DISEASE OUTCOMES OF DENGUE VIRUS INFECTION**

Rosa Isela Gálvez1, E Alexandar Escarrega1, Tulika Singh2, Angel Balmaseda3, Eva Harris1, Daniela Weiskopf4

1La Jolla Institute for Immunology, La Jolla, CA, United States, 2University of California, Berkeley, Berkeley, CA, United States, 3National Virology Laboratory, Managua, Nicaragua, 4La Jolla Institute for Allegy and Immunology, La Jolla, CA, United States

Dengue virus (DENV) circulates in the tropics worldwide, causing an estimated 400 infections, 100 million clinical cases, and at least 22,000 deaths from dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS). Dengue is a growing public health problem worldwide, and climate change is increasing the transmission intensity of dengue and allowing Aedes aegypti to spread to new regions. Currently, there is no comprehensive understanding of the role of adaptive immunity in the context of multiple DENV infections. In our study, we analyzed samples from children with a history of natural DENV infection from an ongoing prospective cohort study of dengue in Managua, Nicaragua, established in August 2004. After a first natural infection, children have different amounts of DENV-specific T cells; analyzing samples collected prior to a subsequent DENV infection allows us to determine if the T cell response has an influence on the clinical outcome of the next DENV infection. We measured DENV antigen-specific T cells in healthy children having experienced a DENV infection 4 ± 2 years before a subsequent infection using an Activation Induces Markers (AIMs) assay. We found that pre-existing DENV-specific CD8+ T cell profiles significantly predicted the outcome of the subsequent infection. Deciphering mechanisms of DENV-specific T cell responses is important to understand immunopathology and to define correlates of protection against clinical disease and is an important contribution towards dengue vaccine development and evaluation.

**ACUTE IMMUNOLOGICAL PROFILE AND PROGNOSTIC BIOMARKERS OF PERSISTENT JOINT PAIN IN THE CHIKUNGUNYA FEVER: A SYSTEMATIC REVIEW**

Anyela Lozano-Parra1, Víctor Herrera2, Silvio Urququi-Inchima3, Rosa Margarita Gélvez Ramirez4, Luis Ángel Villar Centeno5

1Centro De Investigaciones Epidemiológicas, Universidad Industrial De Santander, Bucaramanga, Colombia, 2Department Of Public Health, Universidad Industrial De Santander, Bucaramanga, Colombia, 3Departamento De Microbiología Y Parasitología, Universidad De Antioquia, Medellín, Colombia, 4Centro De Atención Y Diagnóstico De Enfermedades Infectiosas-Cdi, Fundación Inofida, Bucaramanga, Colombia, 5Centro De Atención Y Diagnóstico De Enfermedades Infectiosas-Cdi, Fundación Inofida, Bucaramanga, Colombia

Chikungunya virus infection (CHIKV) increases the risk of persistent arthralgia; however, there is no consistent evidence regarding prognostic biomarkers of progression to chronic arthropathy. This systematic review, following the PRISMA guidelines, provides an overview of currently available literature about the potential role of the acute immunologic response for predicting long-term joint pain in patients with a diagnosis of CHIKV. We searched for observational studies using the terms “chikungunya”, “cytokines”, “biomarkers”, and “joint pain” in PubMed/MEDLINE, LILACS, Cochrane Library Plus, and SCOPUS databases, restricting to articles published in English and up to January 2023. We excluded reviews and studies in which biomarkers were measured after an in vitro stimulation of human cells. The risk of bias was evaluated using the Newcastle-Ottawa Scale. The PROSPERO registration number for this review is CRD42021279400. Thirty-seven studies were selected for qualitative synthesis with a maximum duration from diagnosis to clinical evaluation of 60 months. The sample sizes ranged from 8 to 346 participants (age’s range: 0-90 years). Most of the studies (73.0%) were rated as at high risk of bias. We identified an immunologic profile during the acute phase of CHIKV...
that includes increased levels of proinflammatory cytokines (IFN-α, IFN-γ, IL-2R, IL-6, IL-7, and IL-8), anti-inflammatory cytokines (IL-1Ra, and IL-4), chemokines (MCP-1, MIG, and IP-10) and growth factors (VEGF, G-CSF, and GM-CSF). Only one out of two studies reported differences in the cytokine levels during the acute phase which predicted persistent joint pain at 20 months of follow-up. Also, persistence of anti-CHIKV IgG seemed to be a potential prognostic marker. The evidence suggests the existence of an inflammatory response in the acute phase of CHIKV that persists during its chronic phase; however, there is no unequivocal candidate set of biomarkers of progression toward long-term articular sequelae. This may be due to the heterogeneity of the studied populations, the definition of outcomes, and the timing for quantification of biomarkers during disease.

EBOLA VIRUS -SPECIFIC NEUTRALIZING ANTIBODY PERSISTS AT HIGH LEVELS IN EBOLA VIRUS DISEASE SURVIVORS TWO YEARS AFTER RESOLUTION OF DISEASE IN A SIERRA LEONEAN COHORT

Neil G. Bond1, Kayla R. Shore2, Emily J. Engel1, Erin E. Conoan1, Foday Alhassan1, Michael A. Gbakie4, Fatimah K. Kamara1, Lansana Kanneh2, Mamibu Momo1, Ibrahim M. Kanneh1, John D. Sandi1, Debra H. Elliott1, Samuel C. Ficenec1, Ashley R. Smira1, William A. Fischer4, David Wohl1, James E. Robinson2, Jeffery G. Shaffer2, Robert F. Garry1, Robert Samuels3, Donald S. Grant3, John S. Foday Alhasan3, Michael A. Gbakie3, Fatima K. Kamara3, Lansana Nell G. Bond4, Paula Rahal6, Rafaella F. Q. Grenfell2, Maurício L. Nogueira1

1Tulane University SOM, New Orleans, LA, United States, 2Tulane University School of Public Health, New Orleans, LA, United States, 3Kenema Government Hospital, Kenema, Sierra Leone, 4UNC School of Medicine, Chapel Hill, NC, United States

Ebola virus (EBOV) infection results in Ebola Virus Disease (EVD), an often-severe acute viral disease with a non-specific presentation, hemorrhagic manifestations, and death. Since its recognition, periodic outbreaks of EVD continue to occur in Sub-Saharan Africa. The 2013-2016 West African EVD outbreak was the largest recorded, resulting in a substantial cohort of EVD survivors with persistent health complaints and variable immune responses. Ebola virus spp. continue to emerge in often surprising ways including geographically (SUDV in Uganda), and rarely from reinfection or recrudescence in EVD survivors. In this study, we characterize humoral immune responses in EVD survivors and their contacts in Eastern Sierra Leone with the aim of understanding the natural course of humoral immunity and transmission in the context of EVD. We found high levels of EBOV IgG in EVD survivors and lower, yet substantial, antibody levels in household contacts indicating subclinical transplantation (55.2% and 59.4% seropositivity, respectively). Neutralizing antibody function was prevalent but variable in EVD survivors—greater than 80% of GP-IgG positive survivors had neutralizing antibody but of those individuals over 20% had weakly neutralizing antibody responses—bringing questions about the durability of immune responses from natural infection with EBOV. Indeed, this finding may also have implications for the long-term durability of EBOV vaccination. These data are particularly interesting in the context of rare cases of re-emergent EBOV from EVD survivors and requires further investigation, specifically into the non-neutralizing Fc-mediated antibody functional responses to natural EBOV infection. Additionally, we found that certain discrete symptoms—ophthalmologic and auditory—are associated with EBOV IgG seropositivity while a wide array of symptoms are associated with presence of neutralizing antibody.

PREVALENCE OF DENGUE AND CHIKUNGUNYA ANTIBODIES AMONG CHILDREN IN GRENADA, WEST INDIES

Melanie Kiener1, Roberta Evans2, Nikita Cudjoe2, Calum MacPherson2, Trevor Noel1, Randall Waechter3, A. Desiree LaBeaud1

1Stanford University School of Medicine, Stanford, CA, United States, 2Windward Islands Research and Education Foundation WINDREF at St. George’s University, True Blue, Grenada

The country of Grenada, West Indies, has experienced several arbovirus outbreaks in the last decade: chikungunya virus (CHIKV) in 2014 and Zika virus (ZIKV) in 2016. Additionally, dengue virus (DENV) is endemic to Grenada, circulating at low levels, particularly during the rainy season. Given the persistence of the mosquito vectors on the island, Grenada is at risk of future outbreaks, highlighting the importance of sero-surveillance in the population. In this study, we performed CHIKV and DENV immunoglobulin G (IgG) antibody testing on a cohort of children who were born during the ZIKV epidemic and have been followed for 5 years to assess neurodevelopmental outcomes. Children underwent approximately yearly neurologic assessments, at which time a blood sample was drawn. Using an in-house IgG enzyme-linked immunoassay (ELISA), we tested samples collected from children greater than 12 months of age, by which time we would expect maternal passive antibodies to have waned. We tested 295 samples from 237 children ranging from 12-29 months of age (median 21.0 [IQR 17.3-24.5]). Among 18/237 (7.6%) children with a positive ELISA (median 21.3 months [IQR 17.2-24.3]), 14 had both DENV and CHIKV antibodies detected, 3 had DENV antibodies, and 1 had CHIKV antibodies. Using maternal surveys, we found no significant differences in race, income, housing and floor composition, latrine type, presence of window screens, rainwater collection and storage, and history of maternal CHIKV and DENV exposure between children with and without detected antibodies. In this cohort of children, there were significantly higher than expected rates of CHIKV and DENV antibodies detected (prevalence of 7.6%), suggesting that arboviruses likely circulate at higher than both predicted and diagnosed levels, but no clear risk factors for childhood infection were identified. ELISA testing on years 4 and 5 from this cohort of children will be completed shortly, providing additional confirmation that antibody detection represents primary infection rather than maternal antibodies, and allowing for additional risk factor analysis.

ANTIBODY INDUCED RESPONSE AGAINST SARS-COV-2 OMICRON SUBLINEAGES IN A COHORT VACCINATED WITH CORONAVAC FOLLOWED BY A TWO BOOSTER DOSES PROTOCOL WITH BNT162B2 AND AD26.COV2.S

Guilherme R. F. Campos1, Nathalie B. F. Almeida2, Priscilla S. Filgueiras2, Camila A. Corsini2, Sarah V. C. Gomes2, Daniel A. P. Miranda1, Jéssica V. de Assis2, Thais Bárbara S. Silva4, Pedro Augusto Alves4, Gabriel R. Fernandes2, Jaqueline G. de Oliveira5, Paula Rahali6, Rafaela F. Q. Grendel7, Mauricio L. Nogueira1

1Faculdade de Medicina de São José do Rio Preto (FAMERP), Sao Jose do Rio Preto, Brazil, 2Diagnosis and Therapy of Infectious Diseases and Cancer, Oswaldo Cruz Foundation (Fiocruz), Belo Horizonte, Brazil, 3Faculdade de Medicina de São José do Rio Preto, Sao Jose do Rio Preto, Brazil, 4Laboratório de Imunologia de Doenças Virais, Instituto Rene Rachou - Fundação Oswaldo Cruz, Belo Horizonte, Brazil, 5Laboratório de Imunologia Celular e Molecular, Instituto Rene Rachou-Fundação Oswaldo Cruz, Belo Horizonte, Brazil, 6Laboratório de Estudos Genômicos, Departamento de Biologia, Instituto de Biodiversidades, Letras e Ciências Exatas (IBILCE), Universidade Estadual Paulista (Unesp), Sao Jose do Rio Preto, Brazil

The development of vaccines against SARS-CoV-2 has enabled a gradual return to normalcy across the world. However, the production and distribution of vaccines was not fast enough and allowed the emergence of variants with high transmissibility and capacity to evade immune response
induced by prior infections and vaccination. Thus, our study evaluated the antibody response of a Brazilian cohort vaccinated with a primary protocol of two doses of CoroNAvac, followed by two booster doses with BNT162b2 or Ad26.COV2.S, against Omicron sublineages BA.1, BA.5 and BQ.1.1. A total of 160 individuals were included and divided into 3 time points: 9, 12 and 18 months after the primary protocol. At each time point, individuals were divided equally into 3 subgroups: No booster, 1 booster and 2 boosters (except for the 9 months group, where second booster was not available). Samples were subjected to a virucidal neutralization assay, against Omicron sublineages, to evaluate the neutralization titers and the sernocversion rate. For the BA.1, 9 months after primary protocol, the first booster significantly increased the VNT50 mean (133.1 to 575.8) and the sernocversion rate (33.3% to 76.6%) when compared to the no booster subgroup. In contrast, in the 12 months group, a reduction in the VNT50 mean was observed as the first and second booster doses were administered (1292.3, 1048.1 and 949.1 for no booster, 1 booster and 2 boosters groups, respectively). However, the sernocversion rate increased as the booster doses were distributed (85%, 90% and 100%, respectively). For the 18 months group, the VNT50 mean decreased between no booster and 1 booster groups, but the neutralization mean after second booster increased significantly (1881.4, 1402.5 and 2361.5, respectively). The sernocversion rate, at this time point, was maintained at 100% for all the subgroups. Our data shows the positive impact, over time, of the booster doses in the serological response against BA.1. The same process will be performed with sublineages BA.5 and BQ.1.1 to evaluate the antibody neutralization against sublineages that already showed capacity to evade immune response.

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**ENGINEERING THE SURFACE OF DENGUE VIRUS 2 ENVELOPE PROTEIN TO SELECTIVELY ELICIT DIMER-SPECIFIC ANTIBODIES**

Thanh Thanh N. Phan, Devina J. Thiono, Shaomin Tian, Aravinda M. de Silva, Brian Kuhlman

UNC Chapel Hill, Chapel Hill, NC, United States

Dengue viruses (DVs) cause millions of infections globally each year and yet there is not a vaccine that provides complete protection for all individuals. DVs are antigenically distinct but can evoke cross-reactive immunity to other DVs. Among this cross-reactive response, weakly neutralizing antibodies can exacerbate secondary infections through antibody-dependent endocytosis of the new viruses, which proves to be a challenge for vaccine development. A major target of neutralizing antibodies against DV is the envelope (E) glycoprotein that exists as head-to-tail homodimers on the surface of the virus. Within the E-specific antibodies, a subset bind across the dimer interface, including type-specific antibody 2D22 and cross-reactive E-dimer Epitope antibodies, and are strongly neutralizing. However, these antibodies are challenging to elicit as this dimer epitope is not immunodominant. Here, we engineer a resurfaced DV2 E dimer (RS1) to only display the dimer region. Starting with a stabilized version of the soluble E dimer, we used molecular modeling to identify mutations on the surface of the protein which do not affect thermostability or secretion and are not within the epitope of interest. Through biochemical and biophysical characterizations, we determined that RS1 expresses 50 times better than wildtype E, is thermostable, and remains dimeric at low nanomolar concentration. Additionally, RS1 binds to antibodies targeting the dimer epitope, but not to antibodies that interact with other regions of the E protein. To test if immunization with RS1 focuses the antibody response to the cross-dimer region, we primed and boosted mice with WT stable E dimer alone, RS1 alone, or sequential administration of WT E dimer and RS1. Overall, we observed that RS1 elicits fewer anti-DV2 antibodies than WT E dimer, and we are currently testing if sequential administration of WT E and RS1 elicits a greater fraction of antibodies targeting the dimer epitope. Our study highlights a structure-guided protein design approach to generate better dengue vaccine antigens that can focus the immune response to epitopes of importance on the envelope protein.

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**CHARACTERIZING THE IMMUNE RESPONSE TO ZIKA VIRUS USING EPITOPE MAPPING, REPORTER VIRUS PARTICLES, AND ANTI-ZIKV ANTIBODIES**

Edgar Davidson1, J. Charles Whitbeck1, Lewis J. Stafford1, Ross Chambers1, James E. Crowe Jr1, Benjamin J. Doranz1

1Integral Molecular, Inc., Philadelphia, PA, United States, 2Vanderbilt University, Nashville, TN, United States

To characterize the immune response to Zika virus infection and vaccines we have epitope mapped over 100 anti-ZIKV MAbs at amino acid-resolution, using a comprehensive ZIKV prm/E library of 672 single alanine mutants expressed in human cells. We identified epitopes of patient MAbs, including highly neutralizing MAbs protective in animal models of ZIKV fetal disease. Epitope locations suggest that some MAbs act by binding across adjacent E proteins, preventing rearrangements necessary for ZIKV infectivity. Mapping also reveals epitopes specific for ZIKV or common to DENV, information that can help create better vaccines and therapeutics. We have also identified mutations that increased ZIKV RVP budding, which may aid the design and production of anti-ZIKV vaccines, by screening each individual ZIKV library prm/E variant for ZIKV particle budding and infectivity. To provide critical reagents, we developed ZIKV reporter virus particles (RVPs) capable of one round of infectivity, with luminescent or fluorescent readout, and demonstrated reproducible neutralization titer data (NT50 values) across different RVP production lots, volumes, time frames, and laboratories. We also isolated anti-ZIKV MAbs for ZIKV-specific immunodetection, diagnostic applications, and ZIKV neutralization studies. After immunization with DNA and sub-viral particles, and phage library panning with RVPs, we isolated 48 ZIKV-specific conformational MAbs against prm/E, including one that potently neutralized ZIKV RVPs (IC50 45 ng/ml) with a quaternary epitope spanning adjacent E proteins.

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**COMPREHENSIVE MUTAGENESIS OF DENGUE VIRUS ENVOLPE PROTEINS TO MAP ANTIBODY EPITOPIES AND IDENTIFY RESIDES ESSENTIAL FOR FUNCTION**

Edgar Davidson, Benjamin J. Doranz

Integral Molecular, Inc., Philadelphia, PA, United States

To characterize the immune response to dengue virus (DENV) infection, we have epitope mapped over 200 anti-DENV monoclonal antibodies (MAbs), using high-throughput, rapid screens of MAb binding to DENV prm/E comprehensive mutation libraries for all four DENV serotypes, 3,380 mutations in total. Each library of individual mutant expression plasmids was transfected into human cells to achieve native protein expression and folding, and immunoreactivity of MAbs to each individual prm/E variant was quantified by high-throughput flow cytometry. The epitopes obtained were both conformational (including quaternary) and non-conformational, were spread across prM and E domains I–III, and many have been correlated with their abilities to protect against DENV infection. A number of anti-DENV MAbs cross-reacted with ZIKV prm/E, predominantly within the fusion loop but also within Domain II of the E protein, identifying critical immunogenic residues shared by DENV and ZIKV. We have also produced DENV virions from all four DENV mutation libraries using a previously developed DENV reporter virus particle (RVP) system, allowing us to screen each individual DENV Env variant protein for DENV particle budding and infectivity. For DENV3, we identified residues whose mutation eliminated virus infectivity but did not impact E protein expression, antigenicity, virion assembly, or particle budding. We identified variants that showed increased DENV virion budding, up to 5-fold above wild-type, indicating the ability to engineer highly expressed, non-infectious DENV variants for use in vaccine design.
IMMUNOLOGICAL INSIGHTS FROM EPITOPE MAPPING ON THE CHIKUNGUNYA VIRUS ENVELOPE

Edgar Davidson1, Hong F. Rachel1, Rebecca Wright1, Lewis J. Stafford1, Jing Jin2, Graham Simmons2, Michael Diamond3, James E. Crowe Jr.1, Benjamin J. Doranz1

1Integral Molecular, Inc., Philadelphia, PA, United States, 2Vitalant Research Institute, San Francisco, CA, United States, 3Washington University, St. Louis, MO, United States; 4Vanderbilt University, Nashville, TN, United States

To characterize the immune response to chikungunya virus (CHIKV), we have epitope mapped over 70 monoclonal antibodies (MAbs) against the CHIKV envelope glycoprotein E2/E1, using a shotgun mutagenesis library of 910 E2/E1 alanine-scan mutants. Published studies used epitope maps to characterize broadly cross-reactive and ultrapotent neutralizing MAbs that blocked post-attachment steps, and whose binding mapped to functionally-important E2 domains A or B, suggesting that MAbs inhibit virus-host membrane fusion by preventing exposure of the E1 fusion loop. Additional studies characterized MAbs that induce structural changes on E2 domains A and B. Other mapped MAbs included human E1-specific MAbs cross-reactive across the alphaviruses, isolated from survivors of equine encephalitis virus infections. MAbs binding patterns and epitope mapping identified differences in E1 reactivity based on exposure of epitope on E1 through pH-dependent mechanisms or presentation on the cell surface prior to virus egress. We also used CHIKV E2/E1 mutants to map the binding site on E2 A and B domains of cell adhesion molecule MxA, identified as enhancing attachment and internalization into cells of CHIKV and other alphaviruses, by infectivity screens of cells targeted by CRISPR/ Cas9 gene knockouts. We also isolated human MAbs against CHIKV E2/E1. Our most potent MAb, IM-CKVO63, was highly neutralizing (50% inhibitory concentration, 7.4 ng/ml), showed high-affinity binding (320 pM), and gave therapeutic and prophylactic protection in animal models up to 24 h post-exposure. Epitope mapping identified an inter-subunit conformational epitope on E2 domain A. Subsequent studies demonstrated that IM-CKVO63 blocks both virus entry and virus release. To provide critical reagents for analyses of MAbs or serum immune responses to CHIKV infection, we developed a pseudotyped lentiviral reporter virus system for CHIKV, using reporter virus particles (RVPs) displaying E2/E1. These replication-incompetent RVPs perform one round of infectivity and enable safe (BSL-2) and reproducible virus neutralization assays with luminescent or fluorescent readout.

COMPARISON OF TRADITIONAL PLAQUE ASSAY TO IMMUNOFOCUS ASSAY FOR QUANTIFICATION OF CLINICAL (WILD-TYPE) YELLOW FEVER VIRUSES

Courtney A. Micheletti, Felicity J. Coulter, Bettie W. Kareko, William B. Messer

Oregon Health & Science University, Portland, OR, United States

Clinical isolates (wild-type) of yellow fever virus produce variable cytopathic effects (CPE) in tissue culture and are often reported to form faint or undetectable plaques using traditional in vitro plaque assays. While flow cytometry-based in vitro neutralization assays have also been developed, they are equipment intensive and less amenable to higher throughput approaches. We compare traditional 6 well vero-cell based plaque assay quantification of wild-type YFV isolates to a 96 well immunofocus assay for quantification of representative strains of wild-type YFV, including West Africa 1, East Africa, South America I and South America II genotypes. The immunofocus method was optimized to quantitate wild-type yellow fever virus using the commercially available pan-flavivirus monoclonal antibody 4G2, a mouse-monoclonal antibody which binds a conserved epitope on the fusion loop of flavivirus structural E protein. We evaluate the sensitivity of detection of wild-type YFV by plaque assay compared immunofocus-forming assay and the performance of serum plaque reduction neutralization test (FRNT) to serum focus reduction neutralization test (FRNT) against select wild-type YFVs. Our results demonstrate that yellow fever virus isolates, when compared to traditional plaque assay, can be reliably quantified by 96-well immunofocus-forming assay in vitro assay using commercially available reagents.

A PANEL OF WILD-TYPE YELLOW FEVER VIRUSES REVEAL NEW INSIGHTS INTO THE POTENCY AND BREADTH OF 17D-ELIGITED NEUTRALIZING ANTIBODIES IN A VACCINATED COHORT

Felicity Jane Coulter

Oregon Health & Science University, Portland, OR, United States

Yellow fever (YF) is a severe disease caused by the prototypic flavivirus, yellow fever virus (YFV), transmitted by mosquitoes. Significant control of YFV has been achieved through vaccination with the 17D vaccine. In 2016 the historical vaccination regime of prime, followed by boost every 10-yr was changed to single vaccination by the CDC and WHO based on data that show long-term persistence of YFV neutralizing antibodies (NAbs). Yet, analyses of NAb titers (NTs) following single-dose vaccination estimate approximately 20% of vaccinees have NTs that fall below assay limits of detection (LoD) by 10-yrs post-vaccination. Furthermore, assays used to establish NTs typically only assess neutralization against the attenuated 17D vaccine strain virus, and serum neutralizing potency against clinically relevant wild-type (WT) YFVs remains largely unknown. Using a panel of 17D-immune sera from a non-endemic human cohort <10-years post vaccination (n = 50), we investigated breadth and potency of NAbs using focus reduction neutralization assays. Breadth and potency were assessed using a panel of WT YFVs representative of the 7 YFV genotypes found globally, including contemporary isolates of clinical significance. We found significantly reduced potency of NAbs against WT viruses compared to 17D, particularly to strains belonging to the South American genotype responsible for the recent outbreak in Brazil. Pairwise amino acid distances and antigenic cartography were used to characterize antigenic relatedness of WT YFV to one another. To further understand variables that may play a role in directing Ab responses <10-years post-vaccination, we employed multiple variable regression evaluating the effects of time since- and age at vaccination, and Zika and dengue virus infection history on 17D-immune sera potency and breadth. Importantly, for subject serum samples with NTs against 17D at or just above the LoD, we define potency against WT YFVs, a more authentic and stringent test of potential neutralization. These data suggest potentially elevated risk of vaccine breakthrough, which may better inform future vaccination recommendations.

CHARACTERIZATION OF MBC-DERIVED MABS FROM AN INDIVIDUAL WITH SEQUENTIAL DENGUE AND ZIKA VIRUS INFECTIONS

D. Ryan Bhowmik1, Benjamin D. McElvany2, Nancy R. Graham2, Aravinda M. de Silva1, Sean A. Diehl3, Alena Janda Markmann1

1University of North Carolina at Chapel Hill, Chapel Hill, NC, United States; 2University of Vermont, Burlington, VT, United States; 3Oregon Health & Science University, Portland, OR, United States

Zika virus (ZIKV) is a mosquito-borne flavivirus first isolated in 1947 in Uganda that has caused large outbreaks in the Americas in 2015 and 2016. ZIKV is transmitted by the same Aedes species mosquitoes that transmit dengue viruses (DENV). With tropical climates rising further north above the equator, there is an increased risk of ZIKV and DENV co-transmission in the southern United States as Aedes aegypti mosquitoes travel to these warmer climates. Therefore, with increased populations at risk of infection, there is a need to better understand how concurrent or prior flavivirus exposures can impact protective immunity to ZIKV in order to develop candidate vaccines. In this study, we aim to understand the human serum and memory B-cell (MBC) response in individuals exposed to ZIKV as their first known flavivirus exposure and in those who were exposed to ZIKV secondary to a prior DENV infection to understand whether DENV infection affects MBC responses to secondary ZIKV infection. Here, we assess the breadth...
and depth of binding of strongly neutralizing ZIKV monoclonal antibodies (mAbs) isolated from MBCs of three individuals - two with primary and one with secondary ZIKV infections. Furthermore, we have isolated a panel of seven mAbs from the MBCs of a secondary ZIKV infected individual; mAbs 2E9, 1D2, and 1C6 show type-specific binding and neutralizing response to ZIKV, while mAbs 2G7 and 3C9 show a DENV/ZIKV cross-reactive response but a highly specific DENV2 neutralizing response. These latter mAbs represent a previously unrecognized class of DENV-neutralizing mAbs, exhibiting broadly reactive binding capacity but serotype-specific functional activity.

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TARGETS OF DENGGUE CROSS-NEUTRALIZING POLYCLONAL SERA

D. Ryan Bhowmik, Matthew G. Hvasta, Devina J. Thiono, Brian Kuhlman, Aravinda M. de Silva, Alena J. Markmann
University of North Carolina at Chapel Hill, Chapel Hill, NC, United States

The four dengue (DENV) serotypes infect 100-400 million individuals yearly, causing a half a million cases of life-threatening hemorrhagic fever and shock syndrome. Each DENV serotype has unique (type-specific) as well as shared (cross-reactive) antibody epitopes on the viral envelope (E) protein. Typical primary DENV infections are mild yet stimulate durable protective immunity to the serotype of infection; importantly, primary DENV infections also generate antibodies that cross-react with other DENV serotypes as well as with Zika virus and other related flaviviruses. In contrast, secondary infection with a different DENV serotype leads to the development of a new population of polyclonal serum antibodies that cross-neutralize and protect against DENV 1-4 but surprisingly do not cross-neutralize or protect individuals from Zika virus and other flaviviruses. A major difference in the E proteins of DENV and Zika viruses is the presence of two as opposed to one glycosylation site on the DENV E protein, which may significantly affect antibody responses elicited to these two closely related flaviviruses. Furthermore, we currently do not understand the specific molecular properties of Zika virus neutralizing or DENV broadly cross-neutralizing antibodies secreted into plasma by long-lived plasma cells. Using specific engineered DENV antigens, viral chimeras, and novel imaging techniques, we are able to isolate serum antibodies from individuals exposed to multiple DENV serotypes and to identify protective E protein targets of dengue cross-neutralizing polyclonal serum.

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DETERMINANTS OF IMMUNIZATION IN POLIO SUPER HIGH-RISK UNION COUNCILS OF PAKISTAN

Sajid Bashir Soofi, Imtiaz Hussain, Muhammad Umer, Shabina Aniff
Aga Khan University, Karachi, Pakistan

The current polio epidemiology in Pakistan poses a unique challenge for global eradication as the country is affected by ongoing endemic poliovirus transmission. Across the country, union councils (UCs) that serve as core reservoirs for poliovirus with continuous incidences of polio cases are categorized as super-high-risk union councils (SHRUCs). A cross-sectional survey was conducted in 39 SHRUCs using a two-stage stratified cluster sampling technique. 6,976 children aged 12-23 months were covered. A structured questionnaire was used for data collection. Data were analyzed using STATA version 17. Based on both vaccination records and recall, 48.3% of children were fully-, 35.4% were partially-, and 16.3% were non-vaccinated in the SHRUC districts. Three-quarters or more of the children in SHRUC districts of Sindh and Khyber Pakhtunkhwa (KP) received a vaccination card, whereas only one to two-thirds of the children in the Balochistan SHRUCs did so. The dropout rate between vaccine visits was higher than the WHO-recommended cutoff point of 10% for all vaccine doses in the SHRUC districts. The of being fully vaccinated was higher among the children of educated parents. Full vaccination was found significant among the children of any SHRUC districts compared to district Killab Abdullah. In conclusion, context-specific strategies with more focus on community engagement and targeted mobilization, along with robust monitoring mechanisms, would help address the underlying challenges of under-immunization in the SHRUCs.

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IDENTIFYING SOURCES OF HEPATITIS E VIRUS TRANSMISSION DURING OUTBREAKS THROUGH MODEL-DRIVEN ESTIMATION

Sophia Tan1, Shahzor Rizvi1, Nila Cibu1, Benjamin J. Singer1, Andrew S. Azman2, Nathan C. Lo1
1University of California, San Francisco, San Francisco, CA, United States, 2Johns Hopkins Bloomberg School of Public Health and MSF Switzerland, Baltimore / Geneva, Switzerland

During a waterborne infectious disease outbreak, such as an outbreak of hepatitis E virus in a refugee camp, understanding the relative contribution of environmental sources of infection (e.g., contaminated water) versus person-to-person transmission is key to informing an effective public health and vaccine response. Given the long and variable incubation period for hepatitis E virus (2-9 weeks), distinguishing between epidemic curves driven by environmental sources and person-to-person transmission is challenging. Here, we used person-level case data (with household structure) from simulated outbreaks to develop a statistical method that identifies modes of infection. We simulated epidemic curves for hepatitis E outbreaks using individual-based dynamic mechanistic models under different assumptions on the mode of infection: (i) environmental source (independent of human infection); (ii) person-to-person transmission; and (iii) hybrid modes including both sources. To determine the contribution of person-to-person transmission in different outbreak scenarios, we generated most-likely infection networks and estimated the instantaneous reproduction number (Rt), accounting for temporal clustering of cases within households. We found the average Rt in the population in scenarios with an environmental source alone (100% environmental), 25% person-to-person transmission (75% environmental), and 75% person-to-person transmission (25% environmental) was similar (Rt=2.2); however, the contribution to Rt from within households (a representative proportion of person-to-person transmission) was estimated to be 0.4%, 6.4%, and 30%, respectively. Accounting for household structure in Rt provided a reliable measure for both the predominant mode of infection and the contribution of person-to-
person transmission even when the epidemic curves appeared similar. This method may be applied in waterborne infectious disease outbreaks to guide public health response and reactive vaccine interventions.

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ALTERNATIVE MODALITIES AMPLIFYING TRANSMISSION OF EASTERN EQUINE ENCEPHALITIS VIRUS IN AVIAN HOSTS, THE FACTS ARE IN THE FECES

Kristi M. Miley, Kelli Barr, Thomas Unnasch
University of South Florida, Tampa, FL, United States

Eastern Equine Encephalitis virus (EEEV) is considered the most pathogenic arbovirus in the United States with approximately 35% mortality in severe infections and long-term neurological sequelae among survivors. EEEV is transmitted through the bite of an infected mosquito. However, recent data suggests non-vector modalities could affect EEEV transmission. Experimental animal models using House Sparrows and Zebra Finches (Passerines) has shown that fecal-oral transmission potential exists that may amplify EEEV in its natural host environment, providing an alternative explanation for increased transmission in Florida during winter months when mosquito populations are low. Upon experimental infection of passerines with 105 PFU of virus (IV), EEEV was detectable in avian feces for a minimum of five days following infection. Further research revealed that birds exposed to infective feces through fecal-oral route displayed no symptoms of illness, yet demonstrated RT-PCR results indicative of EEEV infection in feces and various organs including the liver, heart, and brain (CT between 24.04 to 34.90 in feces, 24.06 to 32.50 in liver, 29.41 to 33.03 in heart and 26.84 to 33.92 in the brain). Continued research could determine whether such transmission is possible among avian hosts in their natural environment, which would impact EEEV risk potential. Passerine birds are an important enzootic host for EEEV transmission and confirmation of EEEV transmission through fecal-oral route in the avian host adds an alternative modality to the transmission cycle and the ecology of this virus. Furthermore, these findings can direct future research and public health efforts for EEEV surveillance and control with an alternative method of transmission confirmed.

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MUTATIONS OF THE FLAVIVIRUS CONSERVED RESIDUES IN THE ENVELOPE PROTEIN DOMAIN I-DOMAIN III LINKER ATTENUATE THE MOUSE NEUROINVASIVE PHENOTYPE OF WEST NILE VIRUS

Bailey E. Maloney1, Adrienne E. Pohl1, Natalia C. Ball1, So Lee Park1, Emily K. Mantlo1, Alexis D. Schlieper1, Claire Y. Huang2, Alan D. Barrett3, Stephen Higgs1, Dana L. Vanlandingham1, Yan-Jang Huang1
1Kansas State University, Manhattan, KS, United States, 2Centers for Disease Control and Prevention, Fort Collins, CO, United States, 3University of Texas Medical Branch, Galveston, TX, United States

West Nile virus (WNV) is an emerging flavivirus that has caused over 25,000 cases of neurological disease and 2,300 deaths in the United States since 1999. Development of WN vaccines is a public health priority, especially live-attenuated vaccines (LAVs) that can elicit protective immunity with a single immunization. In the 21st century, candidate WN LAVs are expected to be rationally designed and have a defined molecular basis of attenuation. The envelope (E) protein is a class I fusion protein that facilitates the cell entry of WNV, making it a major component for the rational design of candidate LAVs. Rearrangement of the three domains encoded by the discontinuous sequence of the E gene (EDI, EDII, EDIII) triggers the conformational change of the E protein from dimer in the virion to trimer in the host cell to induce the membrane fusion process during viral entry. Specifically, the relative movement between EDI and EDIII stabilizes the E protein trimer that exhibits the membrane fusion activity. Therefore, the EDI-EDIII linker region has been postulated to be a target for the engineering of attenuating mutations. In this project, we undertook site-directed mutagenesis analysis of eight flavivirus conserved residues in the EDI-EDIII linker region, using the cDNA infectious clone of WNV NY99 strain. The E-L295 residue was shown to be suited for the rational design of candidate LAVs. Alternative amino acid substitutions of the E-L295 residue exhibit a varying level of attenuating effect on the mouse neuroinvasive phenotype of WNV in outbred Swiss mice, including the fully attenuated E-A295S mutant that remains capable of multiplying to high infectivity in Vero cells, the acceptable substrate for the manufacturing of flavivirus LAVs. Significantly, the WNV E-L295 residue is conserved between different flaviviruses, warranting the investigation of the equivalent E residue as the target for the engineering of broadly effective attenuating mutations.

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INFLUENCE OF BREEDING SITES MICROBIOTA AND NUTRIMENT CONTENT ON Aedes aegypti MICROBIOTA AND VECTOR COMPETENCE FOR ARBOVIRUSES

Elodie Calvez, Lyzza Hery, Caitlin Gaete, Isaure Quetel, Aicha Loial, Christelle Dollin, Sébastien Breurec, Anubis Vega-Rúa
Institut Pasteur de la Guadeloupe, Les Abymes, Guadeloupe

Since several decades, arboviruses represent major public health problems in tropical and subtropical regions. Their transmission is complex and influenced by several factors associated to mosquito, virus, host and environment. Among these factors, nutrition and microbiota appeared to be crucial for the mosquito development. However, little is known about the influence of the nutriments and field breeding site bacterial communities on the mosquito’s ability to transmit arboviruses. Here, we aimed to i) evaluate the influence of breeding site microbiota on Aedes aegypti microbiota as well as on the transmission of dengue (DENV) and Zika (ZIKV) viruses, and ii) study if the addition of commercial diet in the breeding site water could influence Ae. aegypti fitness, lifespan, microbiota, and ability to transmit arboviruses. All these investigations were performed in both field and laboratory waters. In field vs laboratory condition, metagenomics data revealed a modification of Ae. aegypti microbiota at the larval and adult stages depending on the breeding site water microbial composition. Furthermore, even if DENV transmission efficiencies were homogenous, ZIKV transmission seemed to be modulated indirectly by the breeding site. The evaluation of the influence of diet on Ae. aegypti demonstrated that the nutriments contained on the diet could significantly modulate mosquito’s pupation, emergence and survival rates, body size and microbiota in both laboratory and field waters. Interestingly, the diet seemed to also modify the ability of Ae. aegypti to be infected by DENV. These results highlight the importance of the breeding site water and nutriments on microbiota establishment in Ae. aegypti, as well as on its fitness and ability to transmit arboviruses. Furthermore, they emphasize the need of standardization of vector competence evaluations at regional or international scales to obtain an accurate risk estimation and for an enhanced preparedness in view of arbovirus spread.

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THE EFFECT OF ENVIRONMENTAL TEMPERATURE ON TRANSMISSION DYNAMICS AND VIRAL GENETICS AMONG CULEX TARSALIS MOSQUITOES INFECTED WITH RIFT VALLEY FEVER VIRUS (RVFV)

Arielle W. Glass
Colorado State University, Fort Collins, CO, United States

Rift Valley Fever Virus (RVFV) is an emerging, zoocratic arbovirus endemic to sub-Saharan Africa with a potential to invade new geographic areas, including the United States. Common mosquito species in the US, including Culex tarsalis, have been shown to be efficient vectors of RVFV. However, the ability of insect vectors to become infected and transmit arboviruses is mediated by environmental temperature, which is increasingly important to understand in the context of a rapidly changing climate. Therefore, the objective of our study is to evaluate the impact of temperature on the transmission efficiency of RVFV as well as population genetics of the virus in different tissue compartments of the mosquito over the course of infection. Culex tarsalis mosquitoes were infected with RVFV through an artificial,
infectious blood meal and held at 18°C, 28°C and 32°C. Mosquitoes were dissected to obtain legs and wings, midguts, carcasses, and saliva at 7- and 14-days post infection to determine how temperature influences transmission in this competent vector species. RNA extracted from the samples will be used study genetic interactions between the mosquito and virus by performing transcriptomic sequencing and gene expression analyses. Data collection and analysis are ongoing, but preliminary results on dissemination and transmission rates at each temperature will be presented. These experiments will fill critical gaps in our knowledge on RVFV infection dynamics and maintenance in a competent mosquito vector to inform the development of effective control strategies.

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VECTOR COMPETENCE OF Aedes aegypti to Dengue Serotypes Two and Three in Ayawaso West District in Ghana

Deborah Pratt1, Michael Amoa-Bosompem2, Christian Obirikorang2, Euudosia E. Agbossou1, Gideon Aning Boateng3, Patience L. Adams1, Mufeez Abudu6, Kwaku A. Osei6, Christopher N.L.T. Mensah3, Joseph Osei6, Samuel Dadzie6, Joseph H. K. Bonney1

1Department of Virology, Noguchi Memorial Institute for Medical Research, Accra, Ghana, 2Department of Biomedical and Diagnostic Sciences, University of Tennessee, Knoxville, TN, United States, 3Department of Molecular Medicine, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, 4University of New South Wales, Kensington, Australia, 5School of Medicine and Dentistry, University of Ghana, Accra, Ghana, 6Department of Parasitology, Noguchi Memorial Institute for Medical Research, Accra, Ghana

Dengue fever is an emerging arboviral disease which is found in tropics and sub-tropical climates. This fast-growing infectious disease records about 100 to 400 million infections per year. In Africa, the prevalence of dengue disease is 14%. Aedes aegypti, the transmitting vector has become abundant in Africa due to urbanization and trans-Atlantic trade. This poses a risk to a potential outbreak of some arboviral diseases including dengue. Some studies have shown that Ae. aegypti is more efficient than Ae. aegypti formosus which is thought to be predominant in Africa. Recent studies have however shown that the domestic forms of Aedes species have taken over from the forest types. In Ghana, limited information is known of the subspecies of Aedes mosquitoes and the efficiency of them transmitting dengue virus. This study therefore investigates the vector competency of Ae. aegypti in an urban location in the country. Larvae and pupae were collected and reared in the insectary until they emerged. Adult female aedes mosquitoes were infected with dengue 2 and 3 serotypes and then midgut infection and virus dissemination were established by RT-qPCR. It was realized that the infectivity rate of both dengue serotypes was high with DENV-2 and DENV-3 having 71.4 % and 60.7%, respectively. The dissemination rate was significantly higher in dengue 2 compared to dengue 3 in infected mosquitoes with DENV-2 having a 100% dissemination rate in the colony while DENV-3 had a 50% dissemination rate. These findings indicate that the high susceptibility to dengue virus in the study site in the Ayawaso west district in Accra poses a potential risk for future outbreak of the disease. The country therefore should enhance vector control tools and strategies to prevent dengue fever in the absence of an effective treatment or vaccine

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CELLULAR MECHANISMS INVOLVED IN THE VIRAL INTERFERENCE OF INFECTION BETWEEN DENGUE AND YELLOW FEVER VIRUSES IN Aedes Mosquito Cells

Jeffersoner B. Guimarães, Vitor G. Floriano, Carlos F. Capato, Benedito Antonio Lopes da Fonseca

School of Medicine of Ribeirão Preto, Ribeirão Preto, Brazil

Yellow Fever (YFV) and dengue viruses (DENV) are arthropod-borne viruses transmitted by Aedes mosquitoes and, in Brazil, the main transmission vector is Aedes aegypti. DENV is prevalent throughout Brazil, causing outbreaks every year, but since 1942 Ae. aegypti-transmitted YFV has not been responsible for outbreaks of urban origin. Due to the absence of urban Yellow Fever outbreaks, we hypothesized that mosquito infection by one virus would interfere with each other infection, a process known as viral interference of infection. Then, we showed that C6/36 (Ae. albopictus) mosquito cells initially infected with the DENV would not allow infection of the YFV (wild-type and 17DD strain). In short, experiments were performed in C6/36 cells, initially infected with DENV-2 for 7 days and subsequently infected with YFV (DENV-2/YFV). After YFV infection, DENV-2-infected cells died more quickly than when they were infected only with the DENV-2 and, under light microscopy, the DENV-2/ YFV-infected mosquito cells showed morphological changes, such as, increased cell volume. Considering apoptosis as a possible mechanism for cell death, RT-qPCR-based experiments showed that DENV-2/YFV sequential infections of C6/36 cells resulted in activation of the apoptosis pathway, with upregulation of the DREDD gene (CASP8), an initiator of the cell death cascade on the apoptosis pathway. Additionally, upregulation of the Michelob_x gene, a positive regulatory gene for cell death, and downregulation of the IAP-2, a cell death regulator gene, were observed in DENV-2/YFV infections compared with DENV-2 alone. Furthermore, the IMP gene expression, a gene responsible for maintaining the integrity of cell membrane, was strongly downregulated in the context of the same experiment (DENV-2/17DD infections). Our data indicate that DENV-2/17DD infections of C6/36 cells reduced cell viability due to a lack of control of programmed cell death. In the nature, this impairment of mosquito cell membrane maintenance may reduce the Ae. aegypti vectorial capacity in the transmission of the YFV in the urban environment, when sequentially infected with DENV and YFV.

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Aedes aegypti Mosquito Bites Enhance Infection of Myeloid Cells with Dengue Virus in Human Skin

Priscila M. Da Silva Castanha1, Sasha Azar2, Jason Yeung3, Megan Wallace1, Gwendolien Kettenburg1, Simon Watkins1, Ernesto T A Marques Jr.1, Nikos Vasilakis4, Simon M. Barratt-Boyces1

1University of Pittsburgh, Pittsburgh, PA, United States, 2University of Texas Medical Branch and Houston Methodist Research Institute, Galveston and Houston, TX, United States, 3Department of Biochemistry and Molecular Biology, University of Texas Medical Branch, Galveston, TX, United States, 4Department of Pathology, Center for Vector-borne and Zoonotic Diseases, Center for Biodefense and Emerging Infectious Diseases, Center for Tropical Diseases, and Institute for Human Infection and Immunity, University of Texas Medical Branch, Galveston, TX, United States

Biting of humans by infected Aedes aegypti mosquitoes is the primary mode of transmission for several arborviruses of public health importance, including the dengue virus (DENV1-4). However, little is known about the immune response at the site of infection. We inoculated Aedes aegypti adult females with DENV-2 by intrathoracic injection before exposing full-thickness human skin explants to infected mosquitoes. Skin explants inoculated with 4 logs of DENV-2 by a bifurcated needle were used as a control to assess the effects of mosquito probing on cutaneous immunity. Skin explants were analyzed by confocal microscopy using antibodies to cell-specific markers and NS3 protein, and RNA sequencing of whole skin biopsies. NS3 expression was detected in keratinocytes in the epidermis and the extent of infection was similar between Aedes-probing or needle skin inoculation. In contrast, DENV inoculation by Aedes-infected mosquitoes resulted in earlier and increased replication of DENV in the dermis, reaching a 2-fold boost in infected cells at 24h when compared to needle inoculation. Within the dermis, increased replication of DENV by Aedes-infected mosquitoes was mediated by earlier and increased recruitment and infection of macrophages. Increased infection of Langerhans cells but not dermal dendritic cells also contributed to increased DENV replication observed in mosquito-bitten skin. In the absence of DENV, Aedes bites boosted the recruitment of macrophages to the bite site, but not other myeloid cells. Transcriptomic comparisons between mock or infected (needle or Aedes) groups were performed across astmh.org
three-time points (2, 8, and 24th post-infection). Pathway Analysis identified canonical pathways relevant to experimental conditions at different time points and revealed evolving transcriptomic signatures tied to virus delivery method. Our findings reveal that DENV delivery by Aedes-mosquitoes bites induces a cutaneous immune response that favors virus infection and dissemination in human skin.

**VECTOR COMPETENCE OF US BORDER AEDES AEGYPTI MOSQUITOES FOR DENGUE VIRUS SEROTYPE 1 ISOLATED FROM A MEXICO BORDER COMMUNITY**

**Pedro M. Palermo**, Jeanette Orboegozo, Douglas Watts

University of Texas at El Paso, El Paso, TX, United States

Dengue is the most important mosquito-borne viral human disease in the tropical and subtropical regions of the world. In the last decades, dengue outbreaks have been reported in urban communities located in northern Mexico and in the Southeast Texas of the United States (US). Aedes aegypti, the main vector of dengue viruses (DENV), is distributed along the US-Mexico border region. Several dengue cases have been reported from Ciudad Juarez, a Mexican border city neighboring El Paso-Texas during the past few years, including the isolation of DENV-1 from a febrile patient with no travel history outside Ciudad Juarez. In this study, our aim was to determine if strains of Ae. aegypti mosquitoes from El Paso and Rio Grande Valley were competent vectors of the DENV-1 Ciudad Juarez isolate. Ae. aegypti mosquitoes from both US border communities were orally exposed to two different DENV-1 doses (6 log 10 PFU/mL or 4 log 10 PFU/mL) and maintained from 7 to 21 days at 28 °C. Individual mosquito body, legs/wings, and saliva were tested by plaque assays and identified by an immunofluorescence assay. Infectivity rates ranged from 76% to 93% in both Ae. aegypti strains after the mosquitoes ingested 6 log PFU/mL of DENV-1 and significantly decreased to 16% to 63% after ingesting 4 log PFU/mL of the virus. Dissemination rates significantly increased (p<0.05) through time (7 to 14 days) in both Ae. aegypti strains, with no associations between dissemination rates and the viral dose. DENV-1 transmission rates in Rio Grande and El Paso Ae. aegypti mosquitoes were 3.23% and 5.71%, respectively. These results showed that the DENV-1 efficiently infected and disseminated in both US border Ae. aegypti strains, but the very low transmission rate suggested the presence of a salivary gland barrier in these U.S. border Ae. aegypti strains. This study highlights the epidemiological importance of evaluating the risk of DENV-1 introduction and transmission in the U.S. border communities. While further studies are needed, these preliminary findings could explain the very low prevalence of dengue cases in the Rio Grande Valley and the absence of cases in the El Paso community.

**EFFICACY AND SAFETY OF AN INACTIVATED WHOLE- VIRION SARS-COV-2 VACCINE (CORONAVAC) IN BRAZILIAN HEALTHCARE PROFESSIONALS: THE PROFISCOV STUDY**

**José Moreira**, Elizabeth Patrício, Patrícia Emília Braga, Ricardo Palacios, Mauro Teixeira, Fabiano Ramos, Gustavo Romero, Fabio E. Leal, Luiz Carlos Junior, Luis Ferreira Carneiro, Francisco Hideo Aoki, Eduardo Barbosa Coelho, André M. Siqueira, Sonia M. Raboni, Danise S. Oliveira, Paulo de TO Castro, Cor J. Fontes, Ana Lucía L. de Oliveira, Chris Gast, Mauricio L. Noqueira, Fernanda C. Boullos, Esper Kallás

1 Instituto Butantan, São Paulo, Brazil, 2 Universidade Federal de Minas Gerais, Belo Horizonte, Brazil, 3 Centro de Pesquisa Clinica do Hospital São Lucas da PUCRS, Porto Alegre, Brazil, 4 Universidade de Brasilia, Brasilia, Brazil, 5 Universidade Municipal de São Caetano do Sul, São Paulo, Brazil, 6 Instituto Emilio Ribas, São Paulo, Brazil, 7 Einstein, São Paulo, Brazil, 8 State University of Campinas, São Paulo, Brazil, 9 Faculdade de Medicina de Ribeirão Preto da Universidade de São Paulo, São Paulo, Brazil, 10 Instituto Nacional de Infectologia Evandro Chagas (IN/IFIOCruz), Rio de Janeiro, Brazil, 11 Universidade Federal do Paraná, Curitiba, Brazil, 12 Hospital Escola da Universidade Federal de Pelotas, Pelotas, Brazil, 13 Hospital de Câncer de Barretos, Barretos, Brazil, 14 Universidade Federal de Mato Grosso, Cuiabá, Brazil, 15 Universidade Federal de Mato Grosso do Sul (UFMS), Campo Grande, Brazil, 16 PATH, Seattle, WA, United States, 17 Faculdade de Medicina de Sao José do Rio Preto, São José do Rio Preto, Brazil, 18 Instituto Butantan, São Paulo, Brazil

The inactivated SARS-CoV-2 vaccine, Coronavac (Sinovac, China), offers several advantages. Frontline healthcare professionals (HCP) in Brazil were highly impacted early in the pandemic, putting them at high risk of infection from COVID-19. This study reports the final analysis of vaccine efficacy (VE) and safety in HCP. In this phase 3, randomized, placebo-controlled trial conducted at 16 Brazilian sites, we assigned adults (≥18 years) in a 1:1 ratio to receive two intramuscular doses of Coronavac or placebo 14 days apart. The primary efficacy was symptomatic COVID-19 cases confirmed >14 days post-second dose. Exploratory efficacy analyses were the VE against COVID-19 with fever≥1 day, World Health Organization (WHO) progression score ≥3 and ≥4. Safety was evaluated by the frequency of solicited and unsolicited adverse reactions (ARs) up to 7 days after vaccination (NCT04466596). Between July 2020 and February 2021, 12,688 participants underwent randomization, and 11,620 were included in the pre-protocol efficacy population. The mean age was 39.2 ±10.9, with 64% females and 57% having any comorbidity. An interim analysis conducted on December 16, 2020 (median follow-up time: 148 days) showed a VE of 50.4% (95% CI: 35.2-61.2), authorizing the vaccine for emergency use in January 2021. In the final analysis (median 371 days of follow-up), VE was 44.6% (95% CI: 34.9-52.8). The VE against COVID-19 with fever≥1 day, WHO≥3, and WHO≥4 was 64.3%, 82.1%, and 100%, respectively. The incidence of ARs was higher in the vaccine than in the placebo (80% grade 1). No difference in serious adverse events within 7 days after any dose between groups was found (0.8% vs. 1.0%). In conclusion, a two-dose regimen of Coronavac administered to adult frontline healthcare
professionals was well-tolerated and conferred modest protection against symptomatic COVID-19; however, protection against severe disease was high.

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PRIOR VACCINATION ALTERS THE DYNAMICS OF THE EVOLVING T CELL RESPONSE IN HUMANS UNDERGOING CONTROLLED CHALLENGE WITH DENGE VIRUS 1

Rekha R. Rapaka1, Heather Friberg2, Christopher D. Colbertson3, Joel V. Chua4, Jeffrey R. Currier2, Kirsten E. Lyke1
1Center for Vaccine Development and Global Health, University of Maryland School of Medicine, Baltimore, MD, United States, 2Viral Diseases Branch, Walter Reed Army Institute of Research, Silver Spring, MD, United States, 3Institute for Human Virology, University of Maryland School of Medicine, Baltimore, MD, United States

T cell correlates of protection from dengue infection are poorly defined in humans; however, memory T cell responses are implicated in both host protection and pathology. A clinical trial was performed to evaluate the efficacy of a dengue vaccine consisting of a priming dose of purified inactivated tetravalent dengue (TDEN) vaccine followed by booster vaccination with a live attenuated TDEN vaccine administered at an interval of 90 or 180 days after. Between 27 and 60 months after booster vaccination, six vaccinated volunteers (vaccinees) and four unvaccinated controls underwent live virus human challenge (LVHC) with 3.25 X 103 PFU Dengue Virus 1 (DENV-1) strain 45AZ5, administered subcutaneously. Five of six vaccines and all four controls developed RNAemia and clinical symptoms of dengue fever, with earlier and shorter RNAemia and symptom duration observed in vaccinees compared to controls. PBMC collected prior to challenge (baseline) and post-challenge were stimulated in vitro with six peptide pools spanning the full DENV-1 proteome and analyzed by mass cytometry (30 analytes), and traditional flow cytometry. We observed distinct patterns of CD4+ and CD8+ T cell responses that differed depending on the development of RNAemia and prior vaccination status. While the majority of RNAemic vaccinees produced CD8+ T cell responses by day 90 after LVHC, the control volunteers did not produce strong CD4+ or CD8+ T cell responses relative to vaccinees after LVHC. Additional effector function and multifunctional responses, co-stimulatory molecule expression, and memory subset data will be presented to compare the maturation of T cell responses in a dengue human infection model in subjects with and without history of dengue vaccination.

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CHARACTERIZING A NOVEL DENGUE VACCINE BY LEVERAGING CLINICAL TRIAL DATA WITH A MULTI-LEVEL MODEL

Manar Alkuzweny, Guido Esparza, T. Alex Perkins
University of Notre Dame, Notre Dame, IN, United States

A safe and effective vaccine that can be universally administered could be key to effectively controlling dengue. Takeda’s QDENGA, which recently completed phase-III clinical trials, is a potential candidate for such a vaccine. However, initial trial results suggest that QDENGA may have differential protection by outcome, infecting serotype, and baseline serostatus. To accurately account for this differential protection when projecting impact of QDENGA, it is necessary to obtain estimates of protection that accord with how impact projection models are parameterized. We did so by developing a multi-level model using a survival analysis framework that simultaneously considers trial-wide, country-level, age-specific, and serostatus-specific clinical trial data on reported cases and hospitalizations in both treatment and placebo arms to estimate vaccine and epidemiological parameters. We found that protection varied by both serotype and serostatus, with protection against disease ranging from 70.9% (95% CI: 62.0, 80.6) among seropositives infected by DENV-2 to -17.4% (95% CI: -87.4, 42.2) among seronegatives infected by DENV-4. Importantly, using a multi-level model to account for shared baseline epidemiological characteristics between arms allowed us to obtain efficacy estimates with reduced uncertainty relative to trial estimates. This is highlighted by our estimates for protection against hospitalization due to DENV-3 among seronegatives. While the trial estimated an efficacy of -87.9% (95% CI: -573.4, 47.6), indicating enhanced risk of disease, our model estimates a value closer to the null (-21.7%, 95% CI: -79.6, 33.1). We used these model-derived efficacy estimates to project public health impact, which indicates that QDENGA could avert 38% (95% CI: 96-42) of cases among baseline seropositives and 1% (95% CI: 0.4-17) of cases among baseline seronegatives, reflecting uneven protection by serostatus. These results suggest that while QDENGA may be an important tool in controlling dengue, the heterogenous protection it offers may hamper its impact.

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DURABLE B AND T CELL IMMUNITY TEN YEARS AFTER DENGUE VACCINATION OF FLAVI-NAIVE PEOPLE

Sarah George, June D’Angelo
Saint Louis University, Saint Louis, MO, United States

Limited data are available on durability of immunity after dengue vaccination of flavivirus-naive people living in non-endemic areas. However this data is critical as followup studies in dengue seronegatives receiving both the Sanofi and Takeda vaccines have shown different levels and serotype-specificity of protection after vaccination compared with dengue seropositives. Further, durability of immunity is a key question for any vaccine. We enrolled 13 people who had received an early version of Takeda’s dengue vaccine as part of an NIH-sponsored study and 13 people who had prior natural dengue infection, and characterized durability of neutralizing antibodies and T cell responses to DV1-4. T cell responses were measured by re-expanding memory CD4+ and CD8+ T cells with live DV1-4 for 7 days in vitro. The number of re-expanded dengue-specific memory CD4+ and CD8+ T cells was determined by flow cytometry, using IFNy and K6/17 as markers of memory immunity and proliferation, compared with mock-expanded cells. B cell responses were measured by neutralization assays in Vero cells and secretion of dengue-specific antibodies from restimulated memory B cells. We found flavivirus-naive recipients of an early version of the Takeda live attenuated tetravalent dengue vaccine had re-expandable memory T cells to DV2 and some people had re-expandable memory T cells to DV4 10 years post vaccination. Flavivirus-naive vaccine recipients also had neutralizing antibodies to DV2 with cross-reactive antibodies to DV1, 3, and 4. As volunteers reported no travel to dengue endemic areas after vaccination and no other flavivirus infections or vaccinations, we conclude these are persistent memory responses to vaccination. In contrast, while people who had natural infection had detectable neutralizing antibodies (predominantly to DV3), only volunteers who had 2 dengue infections had re-expandable memory T cells years after infection (to DV1 and 3). We conclude that a single dengue infection does not produce durable memory T cell immunity in previously flavivirus-naive living in non-endemic areas, while an early version of Takeda’s live attenuated dengue vaccine does.

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CONDUCTING AN EBOLA VACCINE TRIAL IN A REMOTE AREA OF THE DEMOCRATIC REPUBLIC OF THE CONGO: CHALLENGES, MITIGATION, AND LESSONS LEARNED

Ynke Lariviére1, Trésor M. Zola2, Gwen Lemeray3, Bernard Osangir2, Paul P. Vermeiren3, Solange Mililo2, Rachel Meta4, Emmanuel Esanga5, Junior Matangila5, Jean-Pierre Van geertruyden1, Pierre Van Damme2, Vivi Maketa5, Patrick Mitashiri, Hypolite Muhindo-Mavoko5
1University of Antwerp, Antwerp, Belgium, 2University of Kinshasa, Kinshasa, Congo, Democratic Republic of the

Conducting a vaccine trial in a low- and middle-income country (LMIC) can present unique challenges and lessons learned. This Ebola vaccine trial, enrolling 699 healthcare providers and frontliners and jointly set up by the University of Antwerp (Sponsor) and the University of Kinshasa (Principal Investigator), was conducted in Boende, a remote town in the Democratic Republic of the Congo (DRC), between December 2019 and October 2022 (ClinicalTrials.gov: NCT04186003). While being bound to strict international...
regulations, this trial, exemplary for being a public-private partnership, required collaboration between several international stakeholders (i.e., two universities, a pharmaceutical company, and a clinical research organization), local communities and government agencies. Throughout its 34-month duration, the Sponsor and Principal Investigator team had to address several logistical and administrative challenges, cultural differences, language barriers and regulatory, political and ethical considerations, while tailoring and adapting the study to the specific local context. Lessons learned include the importance of community engagement and clear communication with participants in all phases of the study, but also among different stakeholders. Challenges, mitigations and lessons learned are presented in categories, ranging from safety management, trial documentation and logistics, communication, and climate conditions to financial and administrative hurdles. Ultimately, to reach the successful end of the vaccine trial in this remote Ebola endemic area of DRC, careful planning, collaboration, and great flexibility and adaptability was often required from all involved parties. As the vaccine trial presented in this paper was able to obtain high retention rates (i.e., 92% of participants completed the study) despite the encountered challenges, we hope that other international teams aspiring to conduct similar trials in remote areas of LMICs, can learn from these challenges, mitigations and lessons learned.

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THE IMMUNOGENICITY OF A TETRAVALENT LIVE DENGUE VACCINE (DENVAXIA) ADMINISTERED TO CHILDREN IN THE PHILIPPINES WITH BASELINE IMMUNITY TO ONE DENGUE VIRUS SEROTYPE

Lindsay C. Dahora 1, Cameron Adams 1, Laura White 1, Emily Freeman 1, Lakshmanan Premkumar 1, Jedas V. Daag 2, Maria V. Crisostomo 2, Kristal-An Agrupis 2, Michelle Ylade 2, Camila D. Odio 3, Leah C. Katzelnick 3, Aravinda M. de Silva 1

1University of North Carolina at Chapel Hill, Chapel Hill, NC, United States, 2University of the Philippines- Manila, Manila, Philippines, 3National Institute of Allergy and Infectious Disease, National Institutes of Health, Rockville, MD, United States

Antibody-dependent enhancement (ADE) of dengue virus (DENV) infection occurs when an individual with non-neutralizing dengue antibodies from prior exposure to dengue experiences a second infection with a different serotype. Due to the increased risk of ADE and subsequent severe disease in individuals with only one previous infection, vaccination against dengue will likely be most beneficial for this at-risk population. We aimed to define the properties of antibodies in a cohort of 215 primary immune children aged 9-14 from the Philippines who did (60%) or did not (40%) receive a single dose of Dengvaxia. Paired blood samples collected before vaccination (baseline) and 12-18 months after vaccination (Year 1) were tested for antibodies stimulated by the vaccine or incident natural DENV infections. To detect and distinguish vaccine responses from natural infections, we developed a multiplexed, bead-based serological assay to simultaneously quantify serum antibodies to different structural and non-structural antigens from the four DENV serotypes, Zika, and Yellow fever viruses (vaccine backbone). At Year 1, 29% of children had no detectable antibody response to the vaccine, while the remaining 71% had specific antibody responses to one or more vaccine antigen. Children with baseline immunity to DENV4 responded poorly to the vaccine (relative response 0.498, p=0.01) compared to children with prior immunity to DENV1, 2, or 3. All clinically apparent and symptomatic DENV infections in Year 1 were detected by serology using immunodominant DENV antigens that were absent in the vaccine. In Year 1, 36% of unvaccinated and 28% of vaccinated children experienced a natural infection (relative risk = 0.79; 95% CI = 0.53 - 1.19). Studies to assess vaccine clinical efficacy and safety in years 1 through 5 post-vaccination are ongoing.

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CHARACTERISING THE IMMUNOGENICITY AND EFFICACY OF SECOND-GENERATION DENGUE VACCINE

Bethan Naomi Cracknell Daniels, Neil Ferguson, Ilaria Dorigatti Imperial College London, London, United Kingdom

A second-generation vaccine (TAK-003) developed by Takeda Pharmaceutical has recently completed its phase III trial and received licensure for use in multiple countries, including Indonesia and Brazil. While overall results indicate that the vaccine is efficacious against symptomatic and hospitalised dengue, efficacy has also decayed with time and may depend on several factors such as the vaccine recipient’s age and baseline serostatus, as well as the infecting serotype. Critically, efficacy estimates are subject to large uncertainty, due to the limited number of cases in subgroups. No efficacy estimates have been published by age, infecting serotype and baseline serostatus. A Bayesian survival model was developed to refine efficacy estimates of TAK-003. We calibrated the model to publicly available data on virologically confirmed dengue cases and hospitalisations, by trial arm, baseline serostatus, and either age-group or infecting serotype, up to 54-months. A second model was developed to infer the underlying neutralising antibody titres induced by TAK-003 and to link them with protection against clinical disease. Our survival model fits the observed trial attack rates well, including by baseline serostatus, infecting serotype and age-group. In agreement with published trial data, we found that vaccine efficacy is highest in baseline seropositive individuals and against DENV-2. By month 36, no statistically significant efficacy was estimated against DENV-1, DENV-3 and DENV-4 in the baseline seronegative group. Despite the limitations of fitting to aggregated publicly available data, we have developed a survival model which can reconstruct the observed attack rates and links antibody dynamics with protection. The model supports a mechanism of action whereby TAK-003 is efficacious against DENV-2 irrespective of baseline serostatus, whereas efficacy against DENV-1,-3 and -4 depends on an individual’s past dengue exposure. As vaccine rollout is imminent, refined characterisation of the vaccine’s complex efficacy profile can help policymakers determine the optimal use of TAK-003.

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SAFETY AND IMMUNOGENICITY OF A 40 MG ADJUVANT DOSE OF A CHIKUNGUNYA VIRUS VIRUS-LIKE PARTICLE (CHIKV VLP) VACCINE: RESULTS FROM THREE PHASE 2 CLINICAL TRIALS

James M. McCarty 1, Jason Richardson 2, Lisa Bedell 1, Patrick Ajiboye 1, Sufia Muhammed 1, Mila Micceta 1, Lauren Tindale 2, Debbie Anderson 2, Tobi Laureth 3, Jason Mundy 4, Melinda Hamer 1, David Saunders 1, Sarah Royalty-Tred 1, Kelly Warfield 1

1Stanford University, Stanford, CA, United States, “Emergent Biosolutions, Gaithersburg, MD, United States, “Walter Reed Army Institute of Research, Silver Spring, MD, United States, “Uniformed Services University (USU) F. Edward Hebert School of Medicine, Bethesda, MD, United States

PXVX0317 is an aluminum hydroxide adjuvanted form of the CHIKV VLP vaccine that has previously demonstrated a good safety profile and robust immunogenicity. Here, we report the safety and immunogenicity of the selected single adjuvanted 40μg dose of PXVX0317 in 145 subjects 18-64 years of age from three phase 2 trials. In close range study PXVX-CV-317-001, a >4-fold rise over baseline in anti-CHIKV serum neutralizing antibody (SNA), as determined by a luciferase-based virus neutralization assay (NT80), occurred in 100% of the 40μg vaccine recipients, with a GMT of 1713, by 28 days after dosing. The immune response was durable, with a >4-fold rise in 90% of vaccine recipients and a GMT of 280 at day 731. In study EBSI-CV-317-002, which compared prior recipients of heterologous alphavirus vaccines with alphavirus vaccine-naïve controls, a >4-fold SNA rise at day 8 than controls (66.7%, p=0.021). The GMTs peaked in both groups at day 22 and were similar at this and all subsequent
visits. In open-label study EBSI-CV-317-010, the rate of >4-fold rise in anti-CHIKV SNA at day 22 was 100%, when GMTs peaked at 2365. In each study, the 40μg dose was well tolerated and the majority of solicited adverse events (AEs) were of mild or moderate severity. The most common local solicited AE was injection site pain, while the most common systemic AEs were fatigue, headache, and myalgia. In study EBSI-CV-317-002, there were no significant differences in the incidence of solicited AEs between prior recipients of alphasulfone vaccines and alphasulfone vaccination-naive control subjects. Across the 3 studies, there were no vaccine-related study discontinuations or SAEs. A single 40μg dose of the adjuvanted CHIKV VLP vaccine, PXVX317, was well tolerated in three phase 2 clinical trials, including in prior recipients of a heterologous alphasulfone vaccine, and generated a rapid SNA response which persisted for 2 years. Phase 3 studies of a 40μg dose of PXVX317 are ongoing.

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IN VIVO EFFICACY OF SULFADOXINE PYRIMETHAMINE IN PREGNANT WOMEN INFECTED WITH PLASMODIUM FALCIPARUM IN MALI

Coulibaly Oumou
University of Sciences, Technics and Technology of Bamako, Mali

Pregnant women infected with Plasmodium falciparum and her newborn are relatively a high risk to develop low birth weight, maternal anemia, prematurity, and mother death. Sulfadoxine Pyrimethamine as Intermittent Preventive Treatment is recommended from week 13 to delivery to prevent P. falciparum infection during pregnancy. In some parts of Africa, P. falciparum resistance to Sulfadoxine Pyrimethamine has reached a high threshold warranting. This study aimed to assess the level of efficacy of Intermittent Preventive Treatment with Sulfadoxine Pyrimethamine. A prospective observational study was conducted from 2018 to 2019 during routine consultation to collect data on SP delivery and malaria. Pregnant women were included during the first administration of Sulfadoxine Pyrimethamine when gestational age was between 16 and 30 weeks. In vivo efficacy was determined after 42 days of follow up, according to the 2009 World Health Organization standard protocol. A descriptive analysis was done to determine the proportion of preventive failures, Kaplan Meier curve and Log rank test were used to determine the time of failure and confounding factors. A total of 254 women were enrolled with a mean age of 22 years. Efficacy with and without molecular correction was 99.2 percent and 82.3 percent respectively. The real efficacy was not influenced by site, residence, gestate, Insecticid Treated Net use and anemia according to the survival curve, P value is lower to 0.05, Log rank. The proportion of anemia decreased significantly during follow-up from 81.4 percent to 64.5 percent, P value is lower to 0.001. Sulfadoxine Pyrimethamine remains effective to prevent P. falciparum infection and anemia during pregnancy. However, it’s monitoring is essential in the framework of the surveillance of this molecule.

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TRANSCRIPTOMIC APPROACH TOWARDS UNDERSTANDING THE MOLECULAR MECHANISMS OF IMIDAZOLOPIPERAZINE (IPZ) IN THE MALARIA PARASITE PLASMODIUM FALCIPARUM

Mohamed MAIGA1, Antoine Dara1, Devendra Kumar Gupta2, Abdoulaye Djimde1, Laurent Dembele1
1Université des Sciences des Techniques et des Technologies de Bamako (USTTB), Bamako, Mali, 2Novartis Institute for Tropical Diseases, California, CA, United States

Imidazolopiperazine (IPZ), KAF156, a close analogue of GNF179 is a promising antimalarial candidate. IPZ is effective against Plasmodium falciparum and P. vivax clinical malaria in humans with transmission blocking property in animal models and effective against liver stage parasites. Despite these excellent drug efficacy properties, in vitro parasites have shown resistance to IPZ. However, the mechanism of action and resistance of IPZ remains poorly understood. To decipher the mechanism of action and resistance of IPZ, we performed a differential transcriptome study using bulk RNA sequencing on P. falciparum Dd2 strains wild type and its mutant resistant to IPZ (pfcarl, pfact and pfugt known to confer drug resistance to IPZ in vitro) in GNF179 exposed and unexposed conditions of their schizont stages. We report in wild type parasites GNF179 treatment down regulated putative lipase enzymes disrupting thus the hydrolysis of Phosphoinositols 4,5, 1-biphosphate (PIP2) and Triglyceride (TAG) which are essential for P. falciparum survival and proliferation as well as membrane permeability and protein trafficking. Furthermore, we found that in wild type parasites, GNF179 disrupted lipid metabolism, transport and synthesis by repressing expression of Acyl CoA Synthetase, export lipase 1 and esterase enzyme. Finally, our data report that genes involved lipid metabolism are associated to IPZs resistance. These transcriptomic data constitute a great advance to identify the mechanism of action and resistance of IPZs. It’s essential to perform qPCR to validate level of the differentially expressed genes in this study and perform antibody experiments to target key genes which have been validated by qPCR.

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INVESTIGATION OF MARKERS OF ARTEMISININ RESISTANCE AT SELECTED INTERVALS DURING THE 72-HOUR PERIOD AFTER ARTEMISININ BASED COMBINATION THERAPY DOSING IN KISUMU WESTERN KENYA

Apollo Asenath1, Dennis Juma1, Benjamin Opat1, Raphael Okoth1, Gladys Chemwori1, Redemptah Yeda1, Charles Okello1, Agnes Cheruuyiot1, Timothy E. Egbo2, Hosea M. Akala1
1Department of Emerging Infectious Diseases (DEID), United States Army Medical Research Directorate-Kenya (USAMRD-K), Kenya Medical Research Institute (KEMRI), 2United States Army Medical Research Directorate-Africa (USAMRA-D).

Post-treatment parasitemia occurs in at least 25% infections in sub-Saharan Africa despite continued efficacy of artemisinin-combination therapy (ACT) across Africa. Plasmodium falciparum mutation in chloroquine resistance transporter gene (pfcrt76), multi-drug resistance gene1 (pfmdr1), deubiquinating enzyme gene (pfcubp-1) as well as clathrin vesicle-associated adaptor 2, μ subunit encoding gene (pcap2mu) and multi-drug resistant protein 1 gene (pfrmr1) have been associated with recurrent parasitemia. As new markers of ACT resistance in Africa continue to be unveiled across different regions, surveillance of changes in these polymorphisms during the course of treatment is useful in establishing their role in ACT treatment outcome. Each of the 118 P. falciparum samples obtained from individuals enrolled in an ACTs clinical efficacy study comparing outcomes of artemisinin-lumefantrine versus either dihydroartemisinin-piperaquine or artemether-mefloquine between 2013 and 2015 were screened at three or four-time points; day zero before start of treatment then days 2 and 3 after initiation of treatment plus the day of subsequent parasitemia by microscopy. Sequence analysis was done to evaluate genotyped frequency of drug resistance polymorphisms, Pfmdr1, gene copy number and genetic diversity typing of the 12 microsatellite loci. The results indicated that the most polymorphic loci of pfap2mu and pfcut-1 genes were S145C at 18% and E1528D at 19%. Pfcrt1 86, 184 and 1246 had significant increase in wild type alleles at time-points 1 and 4 (p <0.05). Multiple copies of the pfmdr1 gene were observed in 4.5% of the samples analyzed. Microsatellite profile analysis showed that the mean number of alleles in all the loci across the 8 populations ranged from 9.250 to 1.000. The mean parasite clearance half-life was 2.63 hours (IOR). Low clearance rates attained in this study suggests that ACTs are still effective treatment in Kenya. However, increased wild-type pfmdr1 86,184 and 1246 as well as polymorphisms in pfap2mu and pfcut-1 in post day zero suggest that these genes could be responding to ACT dosing and therefore require continued monitoring.
Sulfadoxine-pyrimethamine (SP) plays a critical role in malaria chemoprevention across Africa. However, the increasing P. falciparum resistance to SP has impeded its protective efficacy. The genetic mechanism of SP resistance is well-established and is caused by mutations in the dihydropyrofolic reductase (dhfr) and dihydropiropterase synthase (dhps) genes. Recently, the 431V mutation in the dhps gene has emerged in West Africa, and studies suggest that it may decrease the effectiveness of SP. The principle objective of this study is to investigate the possibility that there is a selective sweep associated with the upstream intron of the dhps variants carrying the 431V mutation. Preliminary evidence suggests that a linked intron mutation located in a microsatellite region upstream of the dhps variants is associated with the 431V haplotypes. We utilize high-throughput Illumina sequencing of the intron variant and associated dhps polymorphism to explore two alternative explanations for the observed association in clinical samples. The first explanation is that the mutation has phenotypic significance for the SP resistance haplotype. The second hypothesis is that the intron variant may have spread by genetic hitchhiking to linked dhps variants with the 431V mutation as they emerged and spread under positive selective drug pressure arising through the continuing use of SP. Linked polymorphisms are a useful tool for investigating the dispersal of resistant haplotype lineages across the African continent.
PHENOTYPIC VALIDATION OF MOLECULAR MARKERS ASSOCIATED WITH SEASONAL MALARIA CHEMOPREVENTION AND ONGOING SELECTIVE SWEEPS IN SENEGAL

Yaye Dié Ndiaye1, Katelyn Brenneman2, Wesley Wong3, Stephen Schaffner4, Abdoulaye Tine1, Mouhammad Sy1, Tolla Ndiaye1, Amy Gaye1, Mame Fana Ndiaye1, Mariama Touré1, Ngoye Gadiaga1, Alfa Sene1, Awa Bineta Demé1, Baba Dieye1, Mamadou Samb Yade1, Khadim Diongue1, Fatou Ba Fall1, Doudou Sene5, Medoune Ndilö1, Ibrahima Diallo1, Mame Cheikh Seck1, Aïda Sadikhi Badiane1, Jules François Gomis3, Mouhammadou Ndiaye1, Mamadou Alpha Diallo1, Ibrahima Mbaye Ndiaye1, Bronwyn MacInnis3, Sarah Volkman1, DYNNIR Wirth1, Dacouda Ndiaye1
1CIGASS/Cheikh Anta Diop University, Dakar, Senegal, 2Harvard T.H. Chan School of Public Health, Boston, MA, United States, 3The Broad Institute, Cambridge, MA, USA, 4Centre for Tropical Medicine and Global Health, Nuffield Department of Medicine, University of Oxford, Oxford, United Kingdom, Bangkáok, Thailand, 5Centre for Tropical Medicine and Global Health, Mahidol University, Bangkok, Thailand

Drug resistance in Plasmodium falciparum is a major threat to malaria control efforts. Senegal is a malaria-endemic country that has implemented successive antimalarial drug-based strategies for two decades. Previously, we profiled several known drug resistance markers (pfcrt, pfmdr1, pfldhps, pfldhrs, and pfketich13) and their surrounding haplotypes from 3,284 samples (collected 2000 – 2020) collected from febrile patients with malaria at health facilities spread throughout low (no Seasonal Malaria Chemoprevention (SMC)), moderate (SMC started in 2019), and high transmission zones (SMC started in 2014) in Senegal. We observed rapid changes at Pfcrt K76T and Pfldhps A437G that coincide with the implementation of SMC in 2014, which we hypothesize reflects SMC-induced changes in resistance or parasite fitness. We also found evidence of a selective sweep at chromosome 7, 8, 9, and 11 based on identifying regions of the genome with excess identity-by-descent in genetically related parasites. To test this hypothesis and determine whether drug resistance was responsible for the selective sweeps, we grouped parasites into categories based on whether parasites were mutant at Pfcrt K76T, Pfldhps triple mutant (N51C, C59R, and S108N), and Pfldhps A437G, and part of the identified selective sweeps. Representative parasites from each grouping were then culture-adapted and phenotyped for drug susceptibility to several antimalarials (chloroquine, amodiaquine, lumefantrine, mefloquine, piperaquine, sulfadoxine, and pyrimethamine) and phenotyped for in vitro parasite fitness. This strategy allowed us to assess the individual contribution of each mutation to the assayed drug resistance and fitness phenotypes and test for potential epistatic interactions between markers. Phenotypic assessment and genetic validation of each of these mutations in a Senegalese background is necessary to assess the impact of SMC and determine if it is causing changes in drug resistance in the population. Ongoing molecular surveillance and genetic validation of these markers will be used to continue to monitor and guide drug interventions.

EVIDENCE OF CHLOROQUINE SUSCEPTIBLE PLASMODIUM FALCI PARUM MALARIA IN AN URBAN MEDIUM TRANSMISSION ZONE IN ZAMBIA

Bertha Kasonde1, Sydney Mwanza1, Victor Daka2, Michael Nambozi1, Phidellis Malungu1
1Tropical diseases research centre, 71769, Zambia, 2Copperbelt University, Ndola, Zambia

Plasmodium falciparum resistance to anti-malarial drugs remains a major hindrance to malaria control and elimination. The P. falciparum parasite has developed resistance to most antimalarial drugs introduced in countries endemic to malaria. Many countries have observed decreases in the prevalence of chloroquine resistance with the discontinuation of chloroquine use. In Zambia, chloroquine was used as the first-line treatment for uncomplicated malaria until treatment failures led the Ministry of Health to replace it with artesunate-lumefantrine in 2003. There are limited treatment alternatives and with the threat of the emergence of resistance to the available anti-malarial drugs, a reintroduction of chloroquine could be a viable option. This study was conducted to determine the prevalence of the chloroquine resistance-associated Pfcrt-76T and Pfmdr1-86Y mutations in blood samples collected from patients in Ndola, a medium malaria transmission zone, an urban setting in Zambia. A cross-sectional study was conducted at Chipuluwuku clinic in Ndola district between January and March 2020. Samples were collected from all malaria-positive individuals attending the clinic. Rapid Diagnostic Tests were used to screen for malaria-positive individuals. Parasite DNA was extracted from Dried Blood Spots and blood slides were collected by finger-prick from all malaria-positive individuals. Polymerase Chain Reaction-Restriction

DEV ELOPMENT AND EVALUATION OF A NOVEL PROTOCOL TO ASSESS THE EFFICACY OF SEASONAL MALARIA CHEMOPREVENTION (SMC) USING SULFADOXINE, PYRIMETHAMINE AND AMODIAQUINE IN AN AREA OF HIGH ANTIMALARIAL DRUG RESISTANCE IN NAMPULA, MOZAMBIQUE.

Craig Bonnington1, Sonia Enosse2, Mercia Sitoe2, Ivan Alejandro Pulido Tarquino3, Joel Tarning3, Mallika Imwong1, Francois H. Nosten2, Nicholas J. White3, Baltazar Candrinhoi
1Malaria Consortium, London, UK & Centre for Tropical Medicine and Global Health, Nuffield Department of Medicine, University of Oxford, Oxford, United Kingdom, 2Department of Molecular Tropical Medicine and Genetics, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand, 3Centre for Tropical Medicine and Global Health, Nuffield Department of Medicine, University of Oxford, Oxford, United Kingdom & Shoklo Malaria Research Unit, Faculty of Tropical Medicine, Mahidol University, Mae Sot, Thailand, Mae Sot, Thailand, 4National Malaria Control Programme, Ministry of Health, Maputo, Mozambique, Maputo, Mozambique

Seasonal Malaria Chemoprevention (SMC) requires that a 3 day course of sulfadoxine, pyrimethamine and amodiaquine (SPQ) provide 28 days protection from malaria infection among children 3-59 months living in areas with highly seasonal malaria. Chemoprevention efficacy is a function of a chemopreventive drug’s ability to clear existing sub-patent malaria infections and to prevent new infections over the four week period of protection. A novel and simply implemented protocol aims to determine if sub-optimal protection occurs with complete adherence to a 3 day regimen of SMC and, if it occurs, whether it results from drug resistance or low drug exposure. Directly observed therapy (DOT) of SPMQ was given to children of 3-59 months on days 0, 1 and 2 as the first round of SMC. Large volume dry blood spot (DBS) and thick smear microscopy slides were collected on days 0, 7 and 28. Pharmacometric analysis was done on DBS samples on days 7 and day 28. Parasitemia, detection, quantification and genotyping was conducted for identified antimalarial drug resistance genes; Pfmdr1, Pfldhps, Pfldhrs, Pfketich13. Small volume dry blood spots were taken one month prior to the implementation of the first cycle of SMC and over 28 days after the final cycle and genotyped for the drug resistance genes. Preliminary results indicate a high rate of day 28 slide positivity and low level parasitemia in Mozambique. Large volume dry blood spots indicate significant survival of low level drug resistant parasites in the presence of therapeutic drug levels. Genotyping of breakthrough parasitemia indicates the same genotypes are those causing disease taken from symptomatic children during the same period. Full results and interpretation will be presented. Preliminary results suggest that although SMC is impacting disease, SPQ is not clearing existing sub-patent infection despite the presence of therapeutic drug levels. This indicates that the effectiveness using this SMC drug regimen Mozambique may be short lived in this province. Full results will be presented. This serves as a novel evidence-based method to measure chemoprevention efficacy of SMC.
INSIGHTS INTO THE MODE OF ACTION AND A NOVEL MUTANT PFCRT-MEDIATED MECHANISM OF RESISTANCE TO THE ANTIMALARIAL CLINICAL CANDIDATE ZY-19489

John Okombo1, Laura Hagenah1, Jess Bridgford1, Tarrick Gaňaš2, Kurt Ward3, Eva Gil-Turbe1, Tomas Yeo1, Matthias Quick1, Sachel Mok1, Manuel Llinás2, David Fidock1
1Columbia University Irving Medical Center, New York, NY, United States, 2Pennsylvania State University, State College, PA, United States, 3Columbia University and University Hospital of Tübingen, Tübingen, Germany

There is an urgent need for new antimalarial candidate drugs. One such molecule is ZY-19489, a fast-killing triaminopyrimidine currently in Phase II trial in combination with ferroquine (FQ). The ZY-19489 + FQ combination can replace artemisinin-based combination therapies (ACTs) as a non-ACT treatment option in case of global emergence of artemisinin resistance hence insights into its mode of action and mechanism of resistance are imperative. This study aimed to leverage in vitro selection and whole genome analysis (IVSWGA), quantitative trait loci (QTL) analysis, stage-specificity experiments and transport studies using P. falciparum lines expressing wildtype (Dd2Dd2) or mutant P. falciparum chloroquine resistance transporter (pfcrt) alleles to characterize the mechanisms of resistance to ZY-19489. IVSWGA identified parasites with a PICTR N246H mutation that conferred a 9-fold shift in IC50 in Dd2Dd2-N246H lines compared to Dd2Dd2. QTL analysis of recombinant progeny of an NF54xPF7 cross mapped the activity of ZY-19489 to a chromosome 7 region harboring pfcrt, thus validating the influence of this locus on ZY-19489 activity. Data from purified PICTR reconstituted in liposomes also showed that ZY-19489 blocks PICTR-mediated efflux of CQ from the digestive vacuole (DV), implying competition of ZY-19489 with CQ for the PICTR binding cavity. Curiously, Dd2Dd2-N246H parasites were highly sensitive to known DV-acting drugs including CQ. No change in susceptibility was observed for lumefantrine or atovaquone. These mutants also had distended DVs reminiscent of PICTR variants earlier shown to have hemoglobin (Hb) processing defects. Heme fractionation assays provided evidence of ZY-19489 inhibiting early steps of Hb degradation and metabolism in ZY-19489-treated lines. Stage-specificity tests showed that ZY-19489 is most potent against schizonts and rings, the latter recently shown to be the initial assembly stage of the parasite’s Hb processing machinery. These data implicate PICTR N246H mutation in mediating ZY-19489 resistance.
and piperaquine. To gain insight into antimalarial drug resistance trends, we surveyed for key P. falciparum polymorphisms from 10-16 health facilities across Uganda from 2016-22. We further assessed for evidence of evolutionary selection of resistant isolates by evaluating diversity in genomic regions flanking resistance loci. Five mutations in the targets of sulfadoxine (PdHPS 437G, 540E) and pyrimethamine (PdHFR 51I, 59R, 108N) were very common (80-100%). The prevalence of PdHFR 164I, and PdHPS 581G mutations, which mediate higher level antifolate resistance, varied between sites and over time. The PdHFR 164I mutation was most common at 4 sites in southwestern and central Uganda (20-75%), with prevalences increasing from 2016-17 (14%) to 2022 (30%), and increases were also seen in other parts of the country. The PdHPS 581G mutation was also most common in the four sites in southwestern and central Uganda, although significant temporal changes in prevalence were not detected. Mutations in PCTRT and PmMDR1, associated with aminooquinoline resistance, were increasingly uncommon. The PCTRT 76T allele was detected in 5/16 sites in 2021 and 4/16 sites in 2022, and was consistently seen only in western Uganda bordering Democratic Republic of Congo. The PmMDR1 86V mutation, which was previously very common, was absent at all sites from 2018-2022. The 1246D mutation decreased, with 0% prevalence at 12 sites in 2022. In regard to evolutionary selection, isolated with antifolate and aminooquinoline resistance-associated alleles showed similar diversity, compared to wild type isolates, in genomic regions flanking the resistance loci. These results suggest limited evidence of recent selection, consistent with stable transmission of resistant isolates.

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**RISK FACTORS OF CHEMOPROPHYLAXIS FAILURE MALARIA AMONG BANGLADESHI UN PEACEKEEPERS IN AFRICA**

Syed Abul Hassan Md Abdullah1, Md Golam Dostogir Harun2, Md. Mahabub Ull Arwan3, Tania Sultana4

1South Asia Field Epidemiology and Technology Network (Safetynet), Bangladesh, Dhaka, Bangladesh, 2icddr,b, Dhaka, Bangladesh, 3Office of Health Affairs, West Virginia University, Morgantown, WV, United States, 4Radha Burnenn, bangladesh, Dhaka, Bangladesh

Mefloquine has been recognized as a highly effective malaria chemoprophylaxis for nonimmune travelers in endemic areas. Bangladesh arms forces participating in United Nation Peace Keeping Mission for a long, mainly for the African region. In spite of prophylaxis intake, the number of malaria cases among deployed troops is alarming. This study identified the risk factors associated with chemoprophyaxis failure in malaria. A longitudinal case-control study was conducted among 859 Bangladesh UN Peacekeepers deployed in DR Congo from Feb 2018 to Jan 2019. Troops used mefloquine (5mg/kg body weight) as weekly malaria chemoprophylaxis. Persons who suffered from Malaria during UN mission tenure were considered as case and location-matched (camps) 1:3 control has been taken. Face-to-face interviews were done using a semi-structured, questionnaire to gather information related to lifestyle, compliance with prophylaxis intake, and other malaria preventive measure. An unobtrusive observational assessment was also carried out and noted a document on preventive practices. Logistic regression analysis was performed to determine the best predictors. Among 859 troops, 80(2.3%) suffered from malaria during the last 12 months of study time, considered as a case while 258 respondents had been taken as control. Respondents having body weight index below normal (AOR: 0.18, CI: 0.04-0.79), doing lighter duty (AOR: 4.37, CI: 1.09-17.24), no or rarely physical exercise (AOR: 0.08, CI: 0.01-0.36), having sleep disturbance (AOR: 0.43, CI: 0.19-0.97), history of Malaria (AOR: 0.21, CI: 1.04-4.40), experienced side effects of mefloquine (AOR: 0.23, CI: 0.06-0.88), irregular intake of mefloquine (AOR: 0.17, CI: 0.04-0.67), chemoprophylaxis took at own arrangement (AOR: 5.15, CI: 2.14-12.39), not properly use mosquito repellent during night duty (AOR: 0.30, CI: 0.10-0.53), mosquito net were not properly hanged (AOR: 0.14, CI: 0.02-0.81) found significantly associated. Adopting a healthy lifestyle, strict compliance of chemoprophylaxis intake and malaria prevention drills with close monitoring can reduce the disease burden.

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**SUSCEPTIBILITY OF PLASMODIUM FALCI PARUM ISOLATES FROM EASTERN UGANDA TO GANAPLACIDE AND PHOSPHATIDYLINOSITOL 4-KINASE INHIBITORS**

Oriona Kreutzfeld1, Stephen Orena2, Martin Oktiwi3, Patrick Turmwebaze1, Oswald Byaruhanga4, Thomas Kataro5, Melissa D. Conrad6, Jennifer Legac2, Oszkan Aydemir7, David Giesbrecht8, Samuel L. Nsobya9, Melanie Rouiller10, Jeffrey A. Bailey11, Roland A. Cooper3, Philip J. Rosenthal12

1University of California San Francisco, San Francisco, CA, United States, 2Infectious Diseases Research Collaboration, Kampala, Uganda, 3UMass Chan Medical School, Worcester, MA, United States, 4Brown University, Providence, RI, United States, 5Medicines for Malaria Venture, Geneva, Switzerland, 6Dominican University of California, San Rafael, CA, United States

Novel antimalarials are urgently needed to combat rising resistance to available drugs. During drug discovery and development, compounds need to be tested for activity against parasites now circulating in the field. We assessed ex vivo drug susceptibilities to the imidazolopiperazine ganaplacide and inhibitors of phosphatidylinositol 4-kinase (PI4K4) of Ugandan Plasmodium falciparum isolates collected from 2016 to 2022. For ganaplacide, decreased susceptibility of laboratory strains has been linked to polymorphisms in P. falciparum cyclic amine resistance locus (PICARL), acetyl-CoA transporter (PIAC), and UDP-galactose transporter (PIUGT). For PI4K4 inhibitors belonging to the 2-amino pyridine and 2-amino pyrazine series, mutations in PI4K4 have been linked to resistance. We also tested related PAN-kinase inhibitors. Drug susceptibilities were assessed using the 72-hour SYBR Green growth inhibition assay. The median IC50 for ganaplacide was 13 nM, but individual isolates had up to 5-fold increased IC50s. Compounds targeting PI4K4 showed a wide range of activity (median IC50s 1-200 nM), with varied susceptibilities for individual isolates. Median IC50s for two lead PI4K4 inhibitors, MMV048 and UCT943, were 65 and 11 nM, respectively. To determine phenotype-genotype associations, we sequenced a large number of the studied isolates using molecular inversion probe and standard dideoxy sequencing. Both PICARL and PI4K4 were highly polymorphic, with 9 mutations in PICARL and 23 in PI4K4 present in >5% isolates. None of these mutations had previously been selected by in vitro drug pressure or was associated with altered ex vivo susceptibility of Ugandan strains to the compounds. No mutations were observed in PIAC or PIUGT. Overall, Ugandan P. falciparum isolates were highly susceptible to these compounds under development as next-generation antimalarials, consistent with a lack of pre-existing or novel resistance-conferring mutations in circulating Ugandan parasites.

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**QPCR ANALYSIS OF RING STAGE SURVIVAL ASSAYS FOR SURVEILLANCE OF ARTEMISININ PARTIAL RESISTANCE IN PLASMODIUM FALCIPARUM**

Martin Oktiwi1, Douglas A. Shoue2, Lisa A. Checkley2, Mackenzie A.C. Sievert2, Frida G. Ceja3, Patrick K. Turmwebaze4, Jeffrey A. Bailey5, Melissa D. Conrad6, Philip J. Rosenthal7, Michael T. Ferdig8, Roland A. Cooper3

1Infectious Disease Research Collaboration, Kampala, Uganda, 2University of Notre Dame, South Bend, IN, United States, 3Dominican University of California, San Rafael, CA, United States, 4Infectious Diseases Research Collaboration, Kampala, Uganda, 5Brown University, Providence, RI, United States, 6University of California, San Francisco, CA, United States, 7University of California, San Francisco, CA, United States

Recent reports from Africa of emergence and spread of kelch13 mutations and artemisinin-partial resistance (ART-R) highlight the urgent need for improved malaria surveillance methods. Current approaches to detect ART-R include the ex vivo ring stage survival assay (RSA). The original RSA (% survival, relative to controls, 66 h after a 6 h 700 nM pulse of DHA) is a technically burdensome assay, requiring extensive microscopy time to count parasites. We recently developed an extended recovery RSA (eRSA) that relies on longer culturing time after DHA exposure followed by qPCR
To determine whether this assay developed for in vitro assays of parasite cultures was valid for studies of fresh isolates, we applied eRiRSA to dried blood spots preserved on filter paper, collected from symptomatic Plasmodium falciparum malaria patients in 2022 from northern and eastern Uganda, an area of emerging ART-R and kelch13 mutations. Fresh parasite samples were exposed to a pulse of 700 nM DHA, and analyzed by standard RSA microscopy at 72 h, and collected on filter paper at 120 h for qPCR analysis. 72 h RSA survival ranged from 0-50% (median 3.1%); fold change in RSA Ct values (a measure of parasite survival) ranged from 1.5 - 500 (median 43). Results with the two assays demonstrated a strong inverse correlation (Spearman, r = -0.8; N = 88). Kelch13 mutations (C469Y and A675V) associated with ART-R in other studies were detected in 24% of samples and were moderately associated with higher RSA eRiRSA survival values (Fisher’s exact test p=0.033). Some samples classified as ART-R (greater than 10% survival) by both microscopy and qPCR had wild type kelch13 genotypes, indicating the importance of a phenotypic surveillance approach. Our results show that collecting filter paper samples for subsequent RSAs by qPCR is an attractive option for surveillance of ART-R in samples from remote field locations with limited resources.

Recent reports of artemisinin partial resistance in Africa suggest that complete resistance to artemisinin-based combination therapies (ACTs) might emerge soon. This study utilized samples collected during therapeutic efficacy studies to assess the trends of molecular markers associated with resistance or reduced susceptibility to the current drugs. Samples (n=2,015) were collected at eight sites in Tanzania, in Kigoma, Mbeya Mtwaras, Mwanza, Morogoro, Pwani, Tanga, Mbeja, Mtwaras, Mwanza and Tabora: at five-time points between 2016 and 2021. Capillary sequencing was used to detect drug resistance markers in pfmdr1 gene, encoding for multi-drug resistance protein and kelch 13 gene (k-13) associated with artemisinin resistances. Sequencing success was ≥80.0% across all the years and 1,733/1,778 (97.5%) samples had k-13 wild-type parasites, while 24 (2.1%) had synonymous mutations (at codons P417P, C469C, R471R, V487Y, F505F, G535G, R539R, and S624S). Only eight samples (0.4%) had non-synonymous where seven mutations are not validated by WHO (416V, E433D, R471S, P475S, A578S with one sample except G613E which had two samples) and one sample from Morogoro region in 2020 with R622 (validated by WHO). For pfmdr1, N86 (wildtype) was 100% while Y184F mutant samples increased from 30% in 2016 to about 60% in 2021. D1246Y mutations occurred in four samples (0.23%); two from Morogoro and Tanga in 2016 and other two from Kigoma and Morogoro in 2020. pfmdr1 haplotypes (N89Y, Y184F and D1246Y) were constructed with 1,708 samples and 985(57.66%) had NVD while 719 (42.09%) had NFD. Minor haplotypes included NYY (in three samples, 0.17%), YFD (two samples 0.12%) and (NFD (one sample, 0.05%). NVD haplotype decreased from 60% in 2018 to 45% in 2021 while NFD increased from 38% in 2016 to 55% in 2021. These findings show that validated k-13 mutations were only detected in one sample (with R622) from Morogoro in 2020. Remarkable changes were observed in pfmdr1 markers with an increase in Y184F mutations and NFD haplotype. Intensified surveillance is urgently needed to monitor the trends of these mutations and their potential impact on the performance of ACTs.
The emergence and spread of resistance to all first-line antimalarials threatens our ability to treat and contain malaria. Therefore, new antimalarial therapies that exploit novel targets and pathways essential for multiple life-cycle stages are highly sought after for primary prophylaxis and transmission blocking, in addition to acute treatment. We have previously identified the molecular target of halofuginone (HFG), one of the most potent known antimalarials and a synthetic derivative of the natural product febribugline, as the cytosolic prolyl-tRNA synthetase (PfcProRS). PfcProRS a member of the aminoacyl-tRNA synthetase (aaRS) family, whose canonical function is to charge tRNA with cognate amino acids for downstream use by the ribosome for protein biosynthesis. Although HFG is highly effective against malaria, parasites quickly develop resistance (~5 generations) through increased intracellular proline, which competes with HFG in the PfcProRS active site. Furthermore, HFG inhibits human ProRS with comparable potency, limiting the therapeutic window of this inhibitor class. To identify and optimize novel ProRS inhibitors that are insensitive to elevated proline levels and selective for PfcProRS over the human homolog, we have developed a novel time-resolved Förster resonance energy transfer (TR-FRET) assay for human and Plasmodium ProRS ligand characterization. The single-step assay is HTS-compatible and enables the sensitive and quantitative profiling of small molecule inhibitors in substrate-dependent (proline, ATP) fashion. Supported by this platform we have developed a new series of proline-uncompetitive PfcProRS inhibitors that are insensitive to HFG-resistance mechanisms. To enable the development of selective PfcProRS inhibitors, we have established a high-throughput synthetic strategy to rapidly access focused libraries for SAR exploration. Preliminary studies have yielded mid-nanomolar inhibitors with ~3-fold selectivity for the parasite enzyme. In parallel, we have successfully screened over 60,000 compounds to identify novel chemotypes for ProRS inhibitor development.

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The WHO recommends rational antimarial medicines use to avert antimicrobial resistance (AMR). In Kenya, malaria treatment guidelines recommend the right dose of Artemether Lumarfantrine (AL) as the first-line treatment for test-positive uncomplicated malaria. Cases are recorded in registers, summarized monthly, then uploaded to Kenya Health Information System (KHIS). The KHIS data indicate a mismatch between patients tested positive and AL doses dispensed. We assessed the impact of biannual malaria commodities review meetings on commodity stewardship. A standard template was shared with the 47 counties to collect aggregated data on the number of patients tested, confirmed positive, and treated, as well as commodities used per county. Key information was downloaded from KHIS for the County Referral Hospitals (CRHs), for April and October 2022 in an excel spreadsheet. This included number of patients tested positive, number of patients by weight categories and doses of AL dispensed per CRH. Comparison of total patients tested positive to total number of patients treated was done. Conversion of doses dispensed into tablets was done to compare with number of tablets that should have been dispensed based on the reported patient numbers by weight category. We compared the performance in April and October 2022. The median months of stock (MOS) for AL in the counties was 13 (IQR 6.2 – 26.3) while for mRDTs was 3.4 (IQR 2.3 - 7.3). Generally, for AL, mRDT, Artemesine injection, Sulfadoxine-Pyrimethamine, and Insecticide-Treated Nets (ITNs), under-stocked counties increased from 21% to 24.3%, adequately stocked increased from 32% to 36%, overstocked reduced from 48% to 40%. The TNs were best stocked (61%) while AL 12s was worst (8%). Treatment for only patients who tested positive improved from 28% to 57%. For CRHs, concordance between number of positive patients and treated improved from (26% to 57%). Number of facilities having concordance improved from 16% to 38%. The review meetings helped improve malaria commodity stocking and rational use in 2022. The Ministry should improve inclusivity in meetings to strengthen antimicrobial stewardship.

INVESTIGATING THE ACCURACY OF MALARIA DIAGNOSTIC TESTS: A BAYESIAN META-ANALYSIS COMPARING CONVENTIONAL AND ULTRASENSITIVE RAPID DIAGNOSTIC TOOLS

Muhammed Elfaituri, Taha Khaled
University of Tripoli, Tripoli, Libyan Arab Jamahiriya

Malaria remains a significant public health challenge, affecting millions of people worldwide. Rapid, accurate, and sensitive diagnosis of malaria is crucial for effective disease management and control. Conventional rapid diagnostic tests (cRDTs) have been widely utilized for malaria diagnosis; however, recent advancements in diagnostic technology have led to the development of ultrasensitive rapid diagnostic tests (uRDTs). This study aims...
to evaluate and compare the diagnostic performance of cRDTs and uRDTs for malaria diagnosis through a Bayesian meta-analysis of diagnostic test accuracy in the available literature. A comprehensive search of electronic databases, including PubMed, Embase, and Web of Science, was conducted to identify relevant studies published up to September 2022. Studies comparing the diagnostic performance of cRDTs and uRDTs for malaria diagnosis were included. The primary outcomes were sensitivity, specificity, and false positive rate. A Bayesian random-effects model was employed to pool the results and calculate summary statistics. In this meta-analysis, 15 studies comprising 18,602 samples were included. The pooled sensitivity of uRDTs was substantially higher than that of cRDTs (62.6% vs. 51.5%) in the first year of the study (CI 95% for uRDTs: 47.4% to 75.6% vs. 47.3% to 67.4%). The sensitivity demonstrated a slight difference between uRDTs and cRDTs (97.8% vs. 99.1%) in the first year of the study (CI 95% for uRDTs: 94.7% to 99.1% vs. 98.4% to 99.3%). The false positive rate (1 - specificity) of uRDTs was 2.2% (CI 95% for uRDTs: 0.9% to 5.3%) compared to 1.6% (CI 95% for cRDTs: 0.7% to 4.4%) for cRDTs. Subgroup analyses revealed consistent performance across diverse transmission settings and asymptomatic populations. This study highlights the superior diagnostic performance of ultrasensitive rapid diagnostic tests (uRDTs) over conventional rapid diagnostic tests (cRDTs) for malaria diagnosis. Implementing uRDTs in endemic regions could significantly enhance case detection and improve disease management, warranting further research on cost-effectiveness and feasibility across diverse settings.

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PRECLINICAL PERFORMANCE AND USABILITY EVALUATION OF A NEW POINT-OF-CARE TEST FOR GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY

Rebecca K. Green1, Gornpan Gornsawun2, Paw Khu Moo2, Chanikan Thipwong2, Stephanie Zobrist1, Laypaw Archasukan2, Huyen Nguyen3, Huong Nguyen3, Cindy S. Chu4, Emily Gerth-Gonzalo J. Domingo1, Germana Bancone4

1PATH, Diagnostics, Seattle, WA, United States, 2Shoklo Malaria Research Unit, Mahidol-Oxford Tropical Medicine Research Unit, Mahidol University, Mae Sot, Thailand, 3Centre for Tropical Medicine and Global Health, University of Oxford, Oxford, United Kingdom, 4Mahidol-Oxford Tropical Medicine Research Unit, Mahidol University, Bangkok, Thailand

Current treatment guidelines for radical cure of Plasmodium vivax malaria recommend the use of 8-aminoquinolines, which can result in potentially life-threatening complications if administered to people deficient in the glucose-6-phosphate dehydrogenase (G6PD) enzyme. Accordingly, treatment guidelines recommend testing for G6PD deficiency (G6PDd) prior to administration of such drugs. The Wondfo G6PD/Hb Test is a new point-of-care (POC) G6PD test that can help decentralize testing and expand access to safe treatment methods. A retrospective diagnostic accuracy study was performed on 264 frozen blood specimens (34 deficient, 23 intermediate, 207 normal) previously collected in Mae Sot, Thailand. Index testing was performed in duplicate under typical lab and field conditions (22.8°C - 27.9°C; 28%-52% humidity; 28.8°C -31.5°C, 58%-71% humidity, respectively). Reference testing by spectrophotometer (G6PD) and HemoCue 301+ (hemoglobin) were performed on site. The Wondfo G6PD/Hb Test demonstrated sensitivity of 1 (95% CI: 0.9-1) and specificity of 0.94 (95% CI: 0.9-0.97) among G6PDd males and females in lab conditions with similar performance in field conditions (sensitivity 1, 95% CI: 0.89-1; specificity 0.94, 95% CI: 0.9-0.96). Among intermediate females (G6PD activity <70%), the test demonstrated sensitivity of 1 (95% CI: 0.85-1) and specificity of 0.37 (95% CI: 0.3-0.45) in lab conditions and similar performance in field conditions (sensitivity 1, 95% CI: 0.85-1; specificity 0.34, 95% CI: 0.26-0.42). Optimization of thresholds on the Wondfo Test achieved sensitivity of 1 (95% CI: 0.85-1) and specificity of 0.94 (95% CI: 0.89-0.79) for intermediate females. A usability evaluation was performed, and data will be presented. A prospective market equivalency study is underway in Memphis, TN, US, to evaluate test performance on fresh venous and capillary specimens (planned completion May 2023).

Performance will be reported. These data provide key insights into product performance for a promising new POC G6PD test to inform further product development efforts and evidence generation for regulatory approval.

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PRACTICAL FACILITY-LEVEL APPROACHES TO REDUCE MALARIA TEST POSITIVITY RATES IN OYO STATE, NIGERIA

Motunrayo Fagbola1, Esther Ayandipo1, Tosin Orhorhambru1, Abiodun Ojo2, Abimbola Olayemi2, Olatayo Abikoyer2, Uchenna Nwokonna2, Foluke Adeyemo2, Olutujin Muideen2, Bolaji Olufemi2, Anja Huestis5, Allan Were6, Thomas Hall6, Erkwagh Dagba7, Veronica Momoh7, Jules Mihigo7

1U.S. President’s Malaria Initiative for States, Management Sciences for Health, Oyo, Nigeria, 2U.S. President’s Malaria Initiative for States, Management Sciences for Health, Abuja, Nigeria, 3Oyo State Malaria Elimination Program, Oyo, Nigeria, 4Oyo State Primary Healthcare Board, Oyo, Nigeria, 5Oyo State Hospitals Management Board, Oyo, Nigeria, 6Management Sciences for Health, Arlington, VA, United States, 7U.S. President’s Malaria Initiative, United States Agency for International Development, Abuja, Nigeria

Malaria Test Positivity Rate (TPR) is used to assess the effectiveness of malaria interventions. However, over the years, this rate has remained high in routine data across Nigeria, including Oyo state. The high TPR has been inconsistent with other data triangulated from therapeutic efficacy studies and fever monitoring exercises reporting national TPR average of 49-52%; suggesting that the high TPR may reflect poor quality data and conduct of malaria diagnosis. This study reports on the effect of the two measures introduced to improve the accuracy of TPR data using secondary quantitative data from the National District Health Information System (DHIS) for both primary healthcare centers (PHCs) and secondary health facilities (SHFs). The two measures were facility-level audits of archived used RDT cassettes at 733 PHCs introduced in September 2021, and a 12-day basic malaria microscopy training (BMMT) at 17 SHFs which was completed in June, 2021. There was a sustained decline in state malaria RDT test positivity rate from 69% in October 2021 to 55% in October 2022 at PHCs. Furthermore, a period review from Jan - Sept, 2022 showed TPR decline from 61% - 54% when compared to January to September 2021 with TPR range from 72% - 76%. An independent T-test was done to compare the mean TPR for each year with a statistically significant decline (= 14.857, p<0.01). At SHF, following the BMMT in June 2021, the microscopy based TPR declined from 60% in July 2021 to 37% in July 2022. Period review done from July 2020 - June 2021 shows TPR of 62% -52% respectively compared to TPR 60%-39% in July 2021 - June 2022 respectively. An independent T-test was done to compare the mean TPR for each year in the SHF's with a statistically significant decline (= 3.622, p 0.02). This study concludes that supervised archiving and auditing of cassettes is a model that can be considered for future scale-up to continue the positive trend at PHCs, while BMMT should be further encouraged for accurate microscopy-based diagnosis. The findings reinforce the critical role of capacity building of human resources and the influence of audits on increasing the accuracy of malaria diagnosis and data reporting.

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THE ROLE OF PSYCHOSOCIAL FACTORS IN PROMPT AND APPROPRIATE CARE SEEKING FOR FEVER IN CHILDREN UNDER 5: FINDINGS FROM THE KENYA MALARIA BEHAVIOR SURVEY

Zoe M. Hendrickson1, Elvis Oyugi2, Jacinta Opondo2, Mildred Shieshia2, Joseph Millward1, James Andath1, Jayme Hughes1, Jeremiah Ochieng3, Grace Milhose4, Jennifer Boyle1, Anna McCartney-Melstad1, Carol Underwood5

1Breakthrough ACTION Project, Johns Hopkins Center for Communication Programs, Baltimore, MD, United States, 2Division of National Malaria
Psychosocial factors can influence caregivers’ care-seeking for fever for children under 5 (US), yet there remains a paucity of evidence examining these factors in Kenya. To assess correlates of care-seeking for fever among children, Kenya’s Division of National Malaria Program (DNMP) and Breakthrough ACTION Kenya conducted the Malaria Behavior Survey in 2022 in 8 malaria-endemic counties in Western Kenya. The study population included 920 female caregivers of US from 1,456 households. 33% reported that a child they cared for had a fever in the last 2 weeks, and 80% of those caregivers reported seeking care for fever. Bivariate and multivariable logistic regression models examined the socio-demographic and psychosocial factors associated with prompt and appropriate care-seeking, defined as care sought same or next day from a health facility or community health volunteer (CHV) by caregivers of US with a fever in the 2 weeks before the study. 61% of caregivers sought care same or next day, and 64% sought care from a health facility or CHV. Only 50% sought prompt and appropriate care. In the final model, respondents discussing malaria with their partner in the past 6 months had 2 times increased odds (95% CI: 1.15-3.64) of seeking prompt and appropriate care compared to those who had not. Those who perceived that CHVs always had malaria rapid diagnostic tests had 1.9 higher odds of seeking prompt and appropriate care (95% CI: 1.1-3.4). While perceiving that CHVs always had treatment for malaria trended in the same direction, associations were not statistically significant. Household wealth and perceived distance to a facility were also not significantly associated with prompt and appropriate care-seeking. Less than half of respondents (46%) reported that they spoke with their partner about malaria in the last six months, and only 50% reported that CHVs always have rapid diagnostic tests. These results suggest that programs should encourage partner communication about malaria and improve caregivers’ perceptions of CHVs. There is also a need to explore the influence of other health system and structural factors on care-seeking.

THE EXPERIENCE OF TESTING AND TREATMENT FOR MALARIA IN THE RETAIL SECTOR: COMPARING THE PROVIDER AND ATTENDANT REPORTED OUTCOMES

George Ambani1, Jeremiah Laktaba2, Joseph Kipkoche3, Emmah Kimachas4, Lucy Abel5, Tabitha Chepkwony5, Emily Robie6, Mark Amunga7, Aggrey Wekesa7, Diana Menya8, Wendy Prudhomme-O’Meara9

1Academic Model Providing Access to Healthcare, Moi University, Eldoret, Kenya, 2College of Health Sciences, Moi University School of Medicine, Eldoret, Kenya, 3Duke Global Health Institute, Duke University, Durham, NC, United States, 4College of Health Sciences, Moi University School of Public Health, Eldoret, Kenya

Improving access and adherence to parasitological diagnosis is critical for malaria control. Over 70% of ACTs consumed in Kenya are sold in the private retail sector, yet they rarely offer malaria diagnostics. We conducted a cluster-randomized trial in 39 registered medicine retail outlets in Western Kenya designed to improve uptake of testing and adherence to results. Outlet attendants of study outlets were trained to perform mRDTs, capture photos of cassettes and enter information about clients on a mobile App. Simultaneously, outlet clients with or history of fever in the last 48 hours were randomly selected for exit interviews. In this study, we compare the experiences of testing and treatment as reported by the outlet through the mobile app compared to reports by clients at exit. 25,454 (47.2%) malaria diagnoses were reported by outlets through the mobile app. 2,462 (42.8%) clients who consented to an exit interview also reported having an mRDT. Both data sources showed similar demographics for those who tested. We noted important differences in the experiences of testing and adherence reported by outlets compared to clients; 11.0% of clients had positive mRDT reported in the app by the outlets compared to 35.3% from exit interviews. 97% of test positive patients received a first-line ACT as reported by the outlet but only 77% by client report. For test-negative clients, 35% received an ACT based on outlet reports compared to 25% by client report. Among 109 clients randomly selected for re-test at exit, nearly two thirds of those who reported a positive test from the shop had a negative mRDT at exit. Contrasting outcomes reported by the provider and the client highlight barriers to improving testing and adherence for malaria as well as challenges for monitoring care management in the retail sector, including accurate communication of results to the client, poor confidence in a negative result and reluctance to withhold antimalarials from test-negative clients.

COMPARING MALARIA RAPID DIAGNOSTIC TEST AND PCR FOR DETECTION OF PLASMODIUM FALCIPARUM INFECTIONS IN SCHOOL MALARIA PARASITAEMIA SURVEY IN TANZANIA

Sylvia F. Mkalla1, Frank Chaky2, Fabrizio Molteni3, Billy Ngasala4

1COSTECH, Dar es Salaam, Tanzania, United Republic of, 2National Malaria Control Programme, Ministry of Health, Community Development, Gender, Elderly and Children, Dodoma, Tanzania, United Republic of, 3Swiss Tropical and Public Health Institute, Dar-es-salaam, United Republic of Tanzania, Dar es Salaam, Tanzania, United Republic of, 4Muhimbili University of Health and Allied Sciences, Dar es Salaam, Tanzania, United Republic of

Malaria rapid diagnostic tests (mRDTs) are increasingly utilized in school malaria parasite surveys as part of national monitoring and evaluation efforts. However, mRDTs based on histidine-rich protein2 (HRP2) detection may yield false positives due to persistent antigenemia after effective antimalarial treatment, and false negatives due to low parasitemia or HRP2/3 gene deletion. In 2017, we evaluated HRP-Il’s diagnostic performance against polymerase chain reaction (PCR) for detecting Plasmodium falciparum infection in 17,051 primary schoolchildren across eight regions in Mainland Tanzania. Based on PCR the prevalence of Plasmodium falciparum malaria infection was 19.2% (95% CI 18.6-19.8). Compared to PCR, mRDT had a sensitivity of 76.2% (95% CI = 74.7-77.7) and specificity of 93.9% (95% CI = 93.5-94.3). At the conference, we will present the contribution of HRP2/3 to false positive results. Thus, PCR and other molecular methods should be considered as additional diagnostic tools for use in schools and other epidemiological surveys.

COMPARISON OF TWO METHODS FOR DETECTION OF GAMETOCYTES IN BURKINA FASO YOUNG CHILDREN WHO RECEIVED THE MALARIA VACCINE CANDIDATE BK-SE36

Edith Christiane C. Bougouma1, Palacpac Nirianne2, Sophie Hourd3, Issiaka Soulama4, Samuel Serme1, Emilie S. Badoum1, Issa Njie5, Alfred B. Tiono5, Sam A. Coulbaly1, Alphonse Ouedraogo1, Hori Toshirho2, Sodionmon B. Sinima2

1gras, Ouagadougou, Burkina Faso, 2Department Of Molecular Protocology, Research Institute For Microbial Diseases, Osaka University, Suita, Osaka, Japan, Osaka, Japan, 3Institut De Recherche En Sciences De La Santé (Inser), Ouagadougou, Burkina Faso, 4centre National De Recherche Et De Formation Sur Le Paludisme, Ouagadougou, Burkina Faso, 5Ouagadougou, Burkina Faso

Plasmodium falciparum gametocyte is the only stage in the malaria parasite life cycle that is transmissible from the human host to the mosquito vector. As their density in peripheral blood is typically low, gametocytes are often undetected by conventional light microscopy of thick blood smears. RNA-based molecular detection method, described as more sensitive, remains however a challenge in remote field settings. In this study, we compared two detection methods of gametocyte, namely Light Microscopy (LM) and Reverse Transcription Polymerase Chain Reaction (RT-PCR) using blood samples of participants of a clinical phase Ib trial assessing the BK-SE36 malaria blood-stage vaccine (registry PACTR201411000034120). Peripheral blood samples from healthy participants were obtained from two age cohorts: 25-60-month-old (n=54) and 12-24-month-old (n=54) children. Gametocyte detection using LM and RT-PCR was conducted on a total of 756 samples collected from the 108 subjects attending 7 clinical
trial scheduled visits. RT-PCR was sensitive, specific, and superior to LM. Overall, 6.62% of the samples from all visits were gametocyte positive by LM, whereas gametocyte positivity by RT-PCR was more than threefold higher (6.62 vs 22.19 %, p = 0.0164). When collected prior to the first vaccination, 12.9% of the samples were positive by LM, vs. 19.4% by RT-PCR. Both methods showed a decrease in gametocyte prevalence after two vaccinations, still with higher positivity when assessed by RT-PCR: 2.77% and 17.3% (p = 0.0002) for LM and RT-PCR, respectively. The difference in detection method results was also observed when the analysis was done per age cohort (cohort 1: 3.85 and 5.77 % [p = 0.032] and cohort 2: 1.92 and 28.85 % [p < 0.001] for LM and RT-PCR, respectively).

The present study compares standard LM and RT-PCR for gametocyte detection using samples collected during a clinical trial. The study confirms the utility of RT-PCR in remote field settings.

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**PERFORMANCE EVALUATION OF CONVENTIONAL RDT, HIGHLY SENSITIVE RDT, AND POLYMERASE CHAIN REACTION TO IDENTIFY MALARIA INFECTION AMONG PREGNANT WOMEN ATTENDING FIRST ANTENATAL CARE VISITS IN CHADIZA DISTRICT, ZAMBIA**

Conceptor Mulube1, Victoria Seffren2, Mulenga Mwenda1, Bupe M. Kabamba3, Chabu C. Kangale3, Marie-Reine I. Rutagwera3, Travis Porter5, Paul Psychas6, Busiku Hamainza4, Julie I. Thwing2, Maximillian Musunse3, Moonga Hawela4, Caroline Phiri-Chibawe3, Atlanta, GA, United States, 3PATH PAMO Plus, Lusaka, Zambia, 4Zambia Ministry of Health National Malaria Elimination Centre, Lusaka, Zambia, 5PATH Malaria Control and Elimination Partnership in Africa (MACEPA), Lusaka, Zambia, 6Malaria, Global Health Center, Centers for Disease Control and Prevention, Atlanta, GA, United States, 7PATH PMO Plus, Lusaka, Zambia, 8Zambia Ministry of Health National Malaria Elimination Centre, Lusaka, Zambia, 9PATH Malaria Control and Elimination Partnership in Africa (MACEPA), Seattle, WA, United States, 10US President’s Malaria Initiative, Centers for Disease Control and Prevention, Lusaka, Zambia

Antenatal Plasmodium falciparum infection can cause placental malaria and adverse pregnancy outcomes. In high transmission settings, pregnant women may harbor low density infections that are undetectable with conventional rapid diagnostic tests (cRDTs), but which can still lead to adverse pregnancy and birth outcomes. Understanding the degree to which a more sensitive RDT can detect asymptomatic infections in pregnancy has implications for earlier treatment to improve birth outcomes. We assessed the sensitivity of a high-sensitivity rapid diagnostic test (hsRDT) and cRDT versus polymerase chain reaction (PCR) during routine surveillance of pregnant women at their first antenatal care (ANC) visit in Chadiza District. From September to October 2020 and December 2021 to March 2022, consenting women attending their first ANC at 21 facilities were tested with both the NxTek Eliminate RDT (hsRDT, Abbott 05FK140) and either the SD Bioline or First Response cRDT (similar sensitivities). A dried blood spot sample was collected for the detection of malaria by PCR and was used as the gold standard for all outcomes. A total of 1,396 women were assessed by all three tests. Malaria prevalence was 13.5% (95% CI: 11.7 - 15.4) by cRDT, 14.5% (95% CI: 12.7 - 16.4) by hsRDT, and 16.8% (95% CI: 14.8 - 18.8) by PCR, while cRDT sensitivity and specificity was 64.1% (95% CI: 57.6 - 70.3) and 96.7% (95% CI: 95.5 - 97.7) respectively, and 65.0% (95% CI: 58.5 - 71.1) and 97.7% (95% CI: 94.4 - 96.8) for hsRDT. Prevalence by PCR was markedly different when stratified by gravidity, with 21.0% (95% CI: 17.3 - 25.2), 19.3% (95% CI: 15.0 - 24.2), and 12.8% (95% CI: 10.4 - 16.5) for primigravida, secundigravida, and multigravida (3+ prior pregnancies), respectively. Similar results were demonstrated with conventional RDT and HSRDT. Smaller than desired sample size precluded finding a significant difference between cRDT and hsRDT. Considering the potential life-long impact of poor birth outcomes, larger studies to better define the sensitivity difference, as well as a cost-benefit analysis, are warranted to assess the potential impact/utility of hsRDT in pregnancy.

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**REINFORCING ADHERENCE TO NATIONAL GUIDELINES ON MALARIA CASE MANAGEMENT IN PRIVATELY-OWNED HEALTH FACILITIES: A CASE STUDY FROM OYO, NIGERIA**

Chinwe Nweze1, Abimbola Olayemii, Augustine Firima1, Arja Huestis1, Olusesan Ishola-Gbeni1, Thomas Hall1, Allan Were3, Esther Ayandipo1, Tosin Orhorhammeru1, Jay Okiokpolomi1, InaBasi Inglass1, Olatayo Akabye1, Uchenna Nwokenna1, Foluke Adeyemo1, TitiIayo Famade1, Erkwagh Dagba1, Veronica Mornoh1, Jules Mihigo1

1United States President’s Malaria Initiative for States, Management Sciences for Health, Cross River, Nigeria, 2United States President’s Malaria Initiative for States, Management Sciences for Health, Abuja, Nigeria, 3United States President’s Malaria Initiative for States, Management Sciences for Health, Lagos, Nigeria, 4United States President’s Malaria Initiative for States, Management Sciences for Health, Oyo, Nigeria, 5State Malaria Elimination Program, Ministry of Health, Oyo, Nigeria, 6United States President’s Malaria Initiative for States, Bayan Global, Oyo, Nigeria, 7United States President’s Malaria Initiative, United States Agency for International Development, Abuja, Nigeria

High-quality health services necessitate delivering the right care and correct treatment, at the right time to the needs and preferences of service users, while minimizing harm and resource waste. In Nigeria, private health facilities (PHF) account for at least a third of the country’s 34,000 health institutions, and 50% of patients with a fever seek care in highly unregulated privately owned health establishments (NMCP, 2018). Given these large proportions, this study aims to examine the effect of capacity-building efforts on the quality of malaria care at 128 PHF reporting into the National Health Management Information System that were selected based on high patient load and geographical spread in three senatorial districts of Oyo state. Two clinicians at each PHF (n = 256) were trained on malaria case management using the updated guidelines and on the prevention of malaria in pregnant women, with monthly supportive supervision for one year post training. A pre (Jan-Dec 2020)-and post (Jan-Dec 2021) (twelve months before and after the intervention) Wilcoxon signed rank test at a 95% confidence level with an alpha value of 0.05 was undertaken to assess malaria quality-of-care indicators. The facility-specific findings reveal that the rate of fever testing and the proportion of confirmed uncomplicated malaria treated in accordance with national guidelines increased from 85% to 98% (p<0.05) and 96% to 100% (p<0.05) respectively, while clinically diagnosed malaria decreased from 15% to 2% (p<0.05). The proportion of pregnant women receiving intermittent preventive treatment in pregnancy (second and third dose (IPTp2 & IPTp3)) increased from 44% to 63% (p<0.05) and 12% to 35% (p<0.05) respectively. The outcomes of this analysis suggest the necessity of capacity building and on-the-job supportive supervision of private sector providers to improve the quality of malaria care. There is also a need for the government to reinforce regulatory oversight towards ensuring adherence to national guidelines on malaria case management to build sustainable quality malaria services in the private sector.

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**MULTIPLEXED DROPLET DIGITAL PCR-AMPLICON SEQUENCING TO UNDERSTAND PLASMODIUM VIVAX TRANSMISSION IN THE ETHIOPIAN HIGHLANDS**

Gustavo Da Silva1, Yalemwerk Evnetu2, Lise Carlier3, Wossenseng Lemma4, Nege Berhan5, Cristian Koepfli1

1University of Notre Dame, Notre Dame, IN, United States, 2Gondar University, Gondar, Ethiopia, 3Noul Inc, Yongin, Korea, Republic of Korea, 4State Malaria Elimination Program, Ministry of Health, Oyo, Nigeria, 5State Malaria Elimination Program, Ministry of Health, Oyo, Nigeria

Genomic epidemiology has become a key methodology to understand malaria transmission, but few protocols are available for species other than Plasmodium falciaparum. We developed a novel genotyping protocol for Plasmodium vivax targeting 44 microhaplotypes selected by screening publicly available genomes to find highly variable regions. Multiplexed amplification was done by droplet digital PCR (ddPCR) and followed by amplicon sequencing (AmpSeq). We applied our assay to 43 samples collected in Gondar (Ethiopian highlands) between November 2019 and
October 2020, with further samples currently being sequenced. Ethiopia accounts for 12% of the global cases of P. vivax, and the highlands are known for heavy seasonal migration of laborers from the lowlands in June-November (~400,000 laborers each year). Their return is associated with an increase in malaria cases. We aimed to understand the proportion of imported and locally transmitted cases, and genetic distinctions among parasite populations. 37/44 markers were observed in all 43 samples, regardless of parasite density (from 1 to ~100 parasites/μL). We observed 474 high quality SNPs, and each sample had a unique haplotype (h=1). 35/43 samples carried multiple clones. 25 samples had genotypic similarity higher than 85%, clustering into one group. One of these individuals reported travel to the lowlands before symptoms occurred. This could indicate importation of an infection from the highly malarious lowlands. 18 samples did not show high similarity to any other sample. Multiple samples carried SNPs possibly conferring drug resistance. 27/43 samples carried the Y976F mutation in pvmdr1, 13/43 the A383G mutation in pvdhps, and 2/43 the S58R mutations in pvdhps, indicating potential resistance against chloroquine (Y976F), sulfadoxine (A383G), and antifolates (S58R). In conclusion, our new assay is suitable to elucidate P. vivax transmission and potential resistance against the current first-line treatment.

Severe malaria is associated with multiple syndromes, including cerebral malaria (CM), which is associated with a high risk of death (~16%). Currently, there are no validated biomarkers that can help in the diagnosis or predict the risk of developing severe or CM. Most of the proposed biomarkers to date are molecules secreted by endothelial or immune cells in response to inflammatory stimuli. While inflammation is an important biomarker to date are molecules secreted by endothelial or immune cells in response to inflammatory stimuli. The Behavioral Economics Prototypes (BEP) are decision-making-centered delivery approaches to improve health provider adherence to testing, differential diagnosis, and treatment of confirmed malaria cases in line with national guidelines. A study aimed to analyze health providers’ adherence to parasitological diagnosis of uncomplicated malaria cases in selected Primary Health Centers (PHCs) implementing BEP in Akwa Ibom state. A study of 355 health providers from 275 PHCs on the application of BEP for fever case management using a stepwise cascade approach with on-site post-training and 1 year and 6 months follow-up supportive supervision. Fifty BEP facilities were randomly selected from those trained using inclusion criteria (data availability, no stock outs for malaria rapid diagnostic test kits (RDT) during the study period. Quantitative data (2020 pre-BEP and 2022 during BEP implementation) from the National Health Management Information System were analyzed for adherence to parasitological diagnosis with RDTs using Wilcoxon rank sum test with an alpha value of 0.05. Results showed a statistically significant increase in testing rates (W=771, p-value = 9.9 x 10^-4), 94% before to 99% after 2 years of intervention, and decrease in test positivity from 73% to 62%, which was also significant (W=1637, p-value = 3.0x10^-4). The findings reinforce that scaling up BEP could improve testing of fever cases for malaria, leading to correct identification of cases which could improve treatment services. However, further research could be conducted to effects of possible confounders and determine causality.

BRAIN ENDOTHELIAL SECRETORY BIOMARKERS FOR SEVERE AND CEREBRAL MALARIA

Claudia Gomes1, Rosaura Varo2, Miquel Duran-Frigola3, Alfredo Mayor4, Quique Bassat5, Ana Rodriguez6

1New York University School of Medicine, New York, NY, United States, 2Barcelona Institute for Global Health, Barcelona, Spain, 3Ersilia Open Source Initiative, Cambridge, United Kingdom

Severe malaria is associated with multiple syndromes, including cerebral malaria (CM), which is associated with a high risk of death (~16%). Currently, there are no validated biomarkers that can help in the diagnosis or predict the risk of developing severe or CM. Most of the proposed biomarkers to date are molecules secreted by endothelial or immune cells in response to inflammatory stimuli. While inflammation is an important contributor to severe and CM, the role of Plasmodium falciparum-infected erythrocytes interactions with the endothelium has not been explored as a trigger for endothelial secretion of potential biomarkers. To identify biomarkers specifically secreted by endothelial cells in response to P. falciparum, we have performed the transcriptomic analysis (RNAseq) of human brain microvascular endothelial cells (HBMEC) incubated with P. falciparum infected red blood cells (RBC) lysates compared to control RBC. Selection of genes upregulated in response to the parasite coding for proteins that are predicted to be secreted from endothelial cells, yielded 14 candidate genes. Each candidate was tested at the protein level, finding that 8 of the candidate proteins were secreted by HBMEC in response to P. falciparum infected RBC lysates (ADAMTS18, Angiopoietin-like 4, BDNF, Brevican, Erythroferrone, Inhibin-β E, KNG1, Reelin). We then determined the level of the 8 selected candidate biomarkers in the plasma of a cohort of Mozambican children with non-severe (n=128) and severe (n=136) malaria, including CM cases (n=23). This analysis identified 2 candidate biomarkers that significantly differentiate the groups of non-severe and severe malaria: ADAMTS18 (AUROC 0.77, p<0.0001) and Angiopoietin-like 4 (AUROC 0.67, p<0.0001); and 2 candidate biomarkers that differentiate the groups of severe malaria (caused by any other complication but CM) and CM: Angiopoietin-like 4 (AUROC 0.68, p=0.005) and Inhibin-β E (AUROC 0.70, p=0.0024). A biomarker signature that could accurately predict or identify the development of severe and/or CM would facilitate rapid and accurate diagnosis of patients resulting in improved care.

COMPARATIVE ANALYSIS OF PRIMARY HEALTH CARE PROVIDERS’ ADHERENCE TO PARASITOLOGICAL DIAGNOSIS OF UNCOMPPLICATED MALARIA USING BEHAVIORAL ECONOMICS PROTOTYPES IN AKWA IBOM, NIGERIA

Methodius Okouzi1, Ubong Umoren1, Ime Akpan1, Chinwe Nwese2, Abimbola Oluyemi3, Uchenna Nwokenna2, IniAbasi Inglass1, Erasette Ekong1, John Oro3, Arja Huéstis1, Thomas Hall2, Olugbenga Mikuolu1, Veronica Momoh1, Erkwagh Dagba2, Jules Mhirigo1

1United States President’s Malaria Initiative for States, Management Sciences for Health, Akwa Ibom, Nigeria, 2United States President’s Malaria Initiative for States, Management Sciences for Health, Cross River, Nigeria, 3United States President’s Malaria Initiative for States, Management Sciences for Health, Abuja, Nigeria, 4State Malaria Elimination Program, Ministry of Health, Akwa Ibom, Nigeria, 5United States President’s Malaria Initiative for States, Management Sciences for Health, Arlington, VA, United States, 6United States President’s Malaria Initiative, United States Agency for International Development, Abuja, Nigeria

Malaria is a major global public health problem with an estimated 232 million annual cases. Nigeria accounts for 27% of the 2022 global burden. The United States Presidents’ Malaria Initiative for States (PMI-S) project partners with State Malaria Elimination Programmes (SMEPs) to provide comprehensive malaria case management in Nigeria, consisting of parasitological confirmation of suspected cases and appropriate treatment of confirmed cases using quality-assured antimalarials. The Behavioral Economics Prototypes (BEP) are decision-making-centered delivery approaches to improve health provider adherence to testing, differential diagnosis, and treatment of confirmed malaria cases in line with national guidelines. A study aimed to analyze health providers’ adherence to parasitological diagnosis of uncomplicated malaria cases in selected Primary Health Centers (PHCs) implementing BEP in Akwa Ibom state. PMI-S collaborated with the SMEP to train 355 health providers from 275 PHCs on the application of BEP for fever case management using a stepwise cascade approach with on-site post-training and 1 year and 6 months follow-up supportive supervision. Fifty BEP facilities were randomly selected from those trained using inclusion criteria (availability, no stock outs for malaria rapid diagnostic test kits (RDT) during the study period. Quantitative data (2020 pre-BEP and 2022 during BEP implementation) from the National Health Management Information System were analyzed for adherence to parasitological diagnosis with RDTs using Wilcoxon rank sum test with an alpha value of 0.05. Results showed a statistically significant increase in testing rates (W=771, p-value = 9.9 x 10^-4), 94% before to 99% after 2 years of intervention, and decrease in test positivity from 73% to 62%, which was also significant (W=1637, p-value = 3.0x10^-4). The findings reinforce that scaling up BEP could improve testing of fever cases for malaria, leading to correct identification of cases which could improve treatment services. However, further research could be conducted to effects of possible confounders and determine causality.

EVALUATION OF THE STANDARD 6GPD RAPID TEST FOR THE DETERMINATION OF THE ENZYMATIC ACTIVITY OF 6GPD

Yassamine Lazrek1, Stephane Moutereau1, Manon Discours1, Ayman Ztouli1, Denis Blanchet1, Emilie Mosnier1, Magalie Demar1, Lise Musset1

1Institut Pasteur de la Guyane, Cayenne, French Guiana, 2Département de Biochimie-Biologie Moléculaire-Pharmacologie-Génétique Médicale, LBMFR Hémoglobinoopathies et biochimie du globule rouge, Hôpitaux Universitaires Henri Mondor. Université Paris-Est Créteil, IMRB Equipe PRÈMÉRIT, Laboratoire d’excellence LABEX, Paris-Est Créteil, France, 3Laboratoire Hospitalo-Universitaire de Parasitologie-Mycologie, Centre Hospitalier André-Rosemon, Cayenne, French Guiana, 4Aix

astmh.org
The 8-aminoquinoline drugs, primaquine and tafenoquine, are effective against the liver-stage of Plasmodium vivax, specifically the hypnozoites. However, they can cause severe hemolytic anemia in people with Glucose-6-Phosphate-Dehydrogenase (G6PD) deficiency. Thus, the WHO recommends a quantitative assay to assess the enzyme activity before prescription. The objective of this study was to evaluate the performance of the “STANDARD G6PD” from DBIOSensor, a rapid quantitative test for the determination of G6PD activity, compared to theracentic method used as gold standard. A panel of 166 individuals has been selected based on previous G6PD results in order to represent all levels of G6PD activities and obtain three groups of individuals (36 severe deficient, 18 intermediate and 112 normal). In addition, a validation of methods according to the ISO 15189 standard for medical biology has been conducted. The sensitivity/ specificity values [95% confidence intervals] of the rapid test to detect deficient males/females was 100% [87-100]/ 100% [75-100] and 88% [79-97]/ 89% [82-96], respectively. Regarding intermediate females, it was of 61% [39-84]/ 82% [73-91]. The semiquantitative interpretation of the results would also be presented. A next-generation sequencing of the entire G6PD gene has been done to document the misclassified results. On deficient samples, the repeatability and reproducibility of the method determine a coefficient of variation (CV) at 9.4 and 9.7%, respectively. A R² of 0.87 has been obtained when comparing the results obtained in the field from venous or capillary blood. A four days storage between -2°C did not impact sample results beyond the CV, whatever the level of G6PD activity. According to the robustness of the test, the incubation time of the sample in the buffer should stay under 5 minutes. The deployment of such a rapid, easy-to-use and implement method would be a major step forward in the elimination process of P. vivax nowadays conducted in some countries of the Guiana Shield.

APPLICATION OF MACHINE LEARNING IN A RODENT MODEL FOR RAPID AND ACCURATE PARASITE COUNTS

Sean Yanik, Hang Yu, Nattawat Chaiyawong, Prakash Srinivasan, Stefan Jaeger

1 Johns Hopkins, Baltimore, MD, United States, 2 National Library of Medicine, Rockville, MD, United States

Rodent malaria models (e.g., Plasmodium yoelii and P. berghei) serve as important preclinical antimalarial and vaccine testing tools. Efficacy measurements of these models often require manually counting parasite-infected red blood cells (RBCs), a time-consuming and repetitive process. We have developed machine learning (ML) software to expedite these studies by automating the counting of Plasmodium-infected RBCs in rodents. Previous ML methods created by our group, designed to count P. falciparum-infected RBCs in humans, accurately measure parasitemia in humans but need to be optimized to measure parasitemia in rodent models. We retrained our ML model to target P. yoelii, instead of P. falciparum, in mouse RBCs, which are much smaller than human RBCs. Our improved algorithm reliably measured P. yoelii-infected RBCs at a wide parasitemia range (0.13-74.12%). Automated parasitemia measurements strongly correlated with manual results (r = 0.996). The program was highly accurate for parasitemia >1%, with a median error rate of 2.06% (mean error rate= 6.74%). Low parasitemia (<1%) affected count accuracy (up to 2-fold). However, our new software was designed to allow optional user verification of infected RBCs and to make corrections, an especially quick process at parasitemia <1%. The software is being developed as a stand-alone desktop application for Windows and Mac OS. The dataset is currently being trained to differentiate between parasite stages and between reticulocytes and mature RBCs. This approach can be applied to other rodent malaria-infected RBCs, and we are currently verifying the program’s accuracy counting P. berghei-infected RBCs. Automation by ML for quick and accurate quantification of blood-stage parasitemia will help in the rapid evaluation of novel vaccines and antimalarials in an easily accessible in vivo malaria model.
malaria DNA. pfhrp2 gene genotyping was done using the one-step PCR method (CDC). Among the 763 total RDT negative samples, 29 (3.8%) were positive by PET-PCR. Of the 29 PCR-positive samples, microscopy identified 7 P. falciparum infections (parasite densities ranging 40 to 8700 parasites per microliter), 1 P. malariae and 1 P. ovale, while 20 were negative by microscopy. Of the 7 P. falciparum, only 2 (3.2%) were identified as potentially pfhrp2 deleted parasites. Validation of these findings using Oxford Nanopore Technology sequencing methods to confirm and map any deletion is ongoing. These findings suggest a very low prevalence of pfhrp2 deleted parasites in Senegal. Continued surveillance as part of the process of quality control and assurance of malaria diagnosis in the country is ongoing both detect and monitor changes in the frequency of any detected pfhrp2 or pfhrp3 gene deletion that may undermine efforts to control and eliminate malaria in Senegal. Regular surveillance is recommended to ensure appropriate use of malaria RDTs.

DETECTION AND QUANTIFICATION OF PLASMODIUM VIVAX DNA: CONCORDANCE BETWEEN PCR RESULTS ON PLASMA AND BLOOD PELLET SAMPLES FROM PATIENTS IN SENEGAL

Babacar Souleymane Sambe1, Aissatou Diagne1, Hélène Ataune Mawoungue Diatta1, Folley Mawulolo Gaba1, Ibrahim Sarr1, Arona Sabènè Diatta1, Serigne Ousmane Mbacké Diaw1, Rokhaya Sané1, Babacar Diouf1, Intès Vigan-WOMas1, Babacar Mbengue1, Makhatar Niang1

1Institut Pasteur de Dakar, Dakar, Senegal, 2Faculté Médecine Pharmacie et Odonto-stomatologie - Université Cheikh Anta Diop, Dakar, Senegal

Current malaria diagnosis relies mainly on microscopy and rapid diagnostic tests, both having shown several limitations in the detection of non-falciparum species, though the global agenda for malaria elimination and eradication does not preclude these species. In Senegal, the use of molecular diagnostic on long-term archived sera samples has been key to the discovery of Plasmodium vivax. To ensure the reliability of the diagnosis of P. vivax from plasma or serum samples as an alternative to the preferred red blood cells, our study evaluated the detectability and quantification of P. vivax gDNA in blood pellets and plasma samples from febrile individuals. Blood samples obtained from 616 febrile patients living in Kolda, Tambacounda, and Kédougou regions in Senegal, were first screened for Plasmodium species composition by 18S ssrRNA-based nested PCR. Paired blood pellets and plasma samples were selected from a subset of 50 P. vivax-positive patients matched by age and sex with 50 P. vivax-negative patients, and subjected to a cytochrome b-based qPCR to compare the detection and quantification of P. vivax genomic DNA between the two specimen types. We report 1.8% and 14.77% of single and mixed P. vivax infections in the study population, and a high concordance (84%) between the qPCR detection of P. vivax genomic DNA from paired blood pellets and plasma samples. All P. vivax negative samples from the blood pellets were also confirmed plasma-negative, and parasitaemia in blood pellets was higher compared to plasma samples. The results support investigations of P. vivax infections in archived plasma collections with a high degree of confidence to generate additional data on the neglected P. vivax malaria, and ultimately guide strategies to control the disease.
EXPANDING COMMUNITY CASE MANAGEMENT OF MALARIA TO ALL AGES CAN CONTRIBUTE TO UNIVERSAL ACCESS TO MALARIA DIAGNOSIS AND TREATMENT: RESULTS FROM A CLUSTER RANDOMIZED TRIAL

Andres Garchitorena1, Aina Harimanana1, Judickaelle Irinantenaina1, Hobisca Razanandraivo1, Tsinoj Rasaneaivo1, Chiarella Mattern1, Emilia Brazy1, Hoby Rabesondratora1, Dean Sayre2, Julie Gutman2, Laurens Lewis2, Rezily Mangahasimbola1, Celestin Razafinjato1, Aimee Ravocarinoys3, Voahangy Razanakotomalala3, Nicolas Ralemy1, Mahafa Andrianaisorolamanana1, Julie Pontarollo1, Aline Mukerabiror1, Anna Bowen2, Jocelyn Razafindrakoto2, Catherine Dentinger2, Laurent Kapesa2, Lauren Lewis2, Reziky Mangahasimbola1, Celestin Razafinjato1, Catherine Dentinger2, Laurent Kapesa2, Laura Steinhardt2

1Institut Pasteur Madagascar, Antananarivo, Madagascar, 2CDC, Atlanta, GA, United States, 3Ministry of Health, Antananarivo, Madagascar, *Inter Aide, Farafangana, Madagascar, 1PMI IMPACT, Antananarivo, Madagascar, 2U.S. President’s Malaria Initiative (PMI), USAID, Antananarivo, Madagascar

Global progress on malaria control has stalled in recent years, in part due to challenges in universal access to malaria diagnosis and treatment. Community health workers (CHWs) can play a key role in improving access to malaria care for children < 5 years (CU5), but national community health policies rarely permit them to treat older individuals. We conducted a two-arm cluster randomized trial in rural Madagascar to assess effects of expanding malaria community case management (mCCM) to all ages on health care access and use. Thirty health centers and their associated CHWs participated. The intervention involved additional CHW training on malaria case management, community sensitization on free malaria care, monthly CHW supervision, and reinforcement of malaria supply chains. Cross-sectional household surveys in ~1600 households were conducted at baseline (Nov-Dec 2019) and endline (Nov-Dec 2021). In addition, data were collected from health center and CHW registers for 36 months (2019-2021). Intervention impact was assessed via difference-in-differences analyses for survey data and interrupted time-series analyses for health system data. Rates of care seeking for fever and malaria diagnosis more than doubled in both arms (from 25% to over 50%), driven mostly by increases in CHW care. mCCM expansion yielded additional improvements for those over 5 years in the intervention arm (Rate ratio for RDTs done in 6-13-year-olds (RRR6-13yrs=1.65; 95% CI 1.45-1.87; RR for RDTs done in those 14+ years (RRR14+yrs=1.46; 95% CI 1.30-1.63), but increases were statistically significant only in health system analyses. mCCM expansion was associated with larger increases for populations living further from health centers (RRRDT6-13yrs=1.21 per km; 95% CI 1.19-1.23). Expanding mCCM to all ages can contribute to universal access to malaria diagnosis and treatment. In addition, strengthening community health and supply chain systems can achieve significant improvements even absent mCCM expansion.

DIGITALLY-ENHANCED RAPID MALARIA TESTING USING ARTIFICIAL INTELLIGENCE (AI) TO SUPPORT QUALITY CONTROL WITH COMMUNITY HEALTH WORKERS IN RWANDA

Noella Umulisa1, Eliau Mwiseneza1, Shawna Cooper2, Natalie Maricich3, Arnababte Mbitumyuremyi3, Sasha Frede2, Sam Smedinghoff2, David Hattery2, Yongshao Ruan2, Paul Isabelli2, Aline Uwimanila1, Jean Niyonzima2, Anastase Muhashyi1, Jean M. Hererimanana1, Marcel Manariyo1, Nadia Iriza1, Celestin Ntirandeka1, Angelique Mugirente1, Marie Rose Kayirangwa1, Gladys Tetteh1

1Jhpiego, Kigali, Rwanda, 2Audere, Seattle, WA, United States, 3Malaria and Other Parasitic Diseases Division/Rwanda Biomedical Center, Kigali, Rwanda, *Jhpiego, Baltimore, MD, United States

Following the WHO’s guidance for parasitological diagnosis of all suspected malaria cases, from 2010 to 2020, the use of malaria rapid diagnostic tests (mRDTs) increased 10-fold across sub-Saharan Africa. While this scale-up was an important step towards improved malaria case management and surveillance, RDT misadministration and misinterpretation errors remain a concern amongst community healthcare workers (CHWs). Traditional data quality assurance (QA) methods rely heavily on visits to health facilities and communities, and assume patient register data is accurate, despite reports of over-treatment and a lack of provider confidence in negative RDT results. In Rwanda, CHWs contribute to the case management of more than 60% of uncomplicated malaria cases exclusively using nationally recommended RDTs for diagnostic testing and artemisinin-based combination treatments (ACTs). However, there is paucity of evidence around this statistic and weak QA systems. This study investigates whether a digital application (app) supporting CHWs in Rwanda could augment QA/QC efforts, providing a way to facilitate high quality community mRDT administration and real-time data surveillance to assess the accuracy of malaria test positivity rate (TPR). To date, 200 CHWs have completed 4,388 tests using the app with positivity rates of 15.2% P.f positive, 2.1% Pan positive and 9.5% P/P+ Pan positive. Baseline CHW survey results indicate over 90% of CHWs are supportive of using an app in their malaria testing flow and are excited about its potential to provide proof for their work. Final results available in July 2023 will assess the accuracy of CHW RDT interpretations against a trained group of RDT readers (Panel Read ground truth) and artificial intelligence algorithms (AI), indicating AI’s potential role in supporting timely overall TPR reporting accuracy for CHWs. This work has broader impact across disease areas (including other neglected tropical diseases), supporting decision-making and accurate reporting, and augmenting investment in mRDT AI technology that can be a multiplier for overall community health and febrile epidemic surveillance.


Calvino Tah Fomboh1, Awa B. Deme2, Akindeh Mbuhab Nji1, Jude Bigoga1, Peter Thelma Ngwa Niba1, Dorothy F. Achu2, Ateba Joel1, Neuly Ngande2, Irene Cavros3, Johan Ahmed4, Amin Munkt3, Jean Yves Mukambe1, Fritz Mbuha Tata1, Jean Paul Kenge Chedjou1, Souleymanou Souleymanou1, Judith Hedje5, Mamanou A. Diallo1, Mouhamad Sy2, Bassirou Ngom2, Amy Gaye2, Tollou Ndiaye2, Djiby Sow2, Akta Sene1, Ibrahimou M. Ndiaye2, Daouda Ndiaye2, Wilfred F. Mbacham2

1Biotechnology Center, University of Yaoundé I, Yaoundé, Cameroon, 2International Center for Research and Training in Applied Genomics and Health Surveillance, Université Cheikh Anta Diop, Dakar, Senegal, 3National Malaria Control Program, Cameroon Ministry of Public Health, Yaoundé, Cameroon, 4U.S. President’s Malaria Initiative, Malaria Branch, U.S. Centers for Disease Control and Prevention, Atlanta, GA, United States

Efforts focused on the treatment of uncomplicated Plasmodium falciparum malaria among children in the Center and North regions of Cameroon have been ongoing since 2011, with the most recent coverage being presented in 2021. These efforts have focused on the efficacy of different antimalarial drugs, including artemether-lumefantrine, artesunate-amodiaquine, and dihydroartesinin-piperaquine. The study aimed to assess the effectiveness of these drugs in treating uncomplicated P. falciparum malaria among children in the Center and North regions of Cameroon over the period 2021-2022. The study used a randomized controlled trial design and involved a large number of children enrolled in the study. The main outcomes measured were the efficacy of the drugs in terms of parasite clearance and clinical improvement. The results indicated that all three drugs were effective in treating uncomplicated P. falciparum malaria, with artemether-lumefantrine and artesunate-amodiaquine showing similar efficacy and dihydroartesinin-piperaquine showing slightly lower efficacy. However, all drugs demonstrated a high level of efficacy, with parasite clearance rates exceeding 90% in both arms. The study also highlighted the importance of improving malaria treatment outcomes in the region, as well as the need for continued monitoring and evaluation of antimalarial drug efficacies.
Since 2019, treatment for uncomplicated falciparum malaria in Cameroon has been one of three first-line therapies: artesunate-amodiaquine (ASAQ), artemether-lumefantrine (AL), and dihydroartemisinin-piperaquine (DP). In Côte d’Ivoire, artesunate-amodiaquine (ASAQ) and artemether-lumefantrine (AL) were implemented annually in the region since 2016.

Efficacy and safety of ACTs. In accordance with WHO’s guidance and standard protocol to routinely test antimalarial drug efficacy, we evaluated all three treatments in children 6 to 120 months in Cameroon’s Center and North regions from April 2021 to September 2022. In the Center, 178 patients were randomly assigned either ASAQ (n=89) or AL (n=89); in the North, where seasonal malaria chemoprevention (SMC) is implemented, 203 patients randomly received either AL (n=102) or DP (n=101). Clinical and parasitological responses were monitored for 28 (AL and ASAQ) or 42 (DP) days. Molecular correlation was performed on 52 late treatment failure samples using PCR analysis of msp1 and msp2 genes to differentiate recrudescence from reinfection. For AL, Kaplan–Meier PCR-corrected efficacy was 96.6% (95% CI 93.0-100%) in the Center and 93.8% (89.2-98.7%) in the North. PCR-corrected efficacy of ASAQ in the Center was 100%. PCR-corrected efficacy for DP in the North was 90.5% (84.8-96.6%). Amiplocine sequencing of 104 (52 day zero and 52 day of failure) samples was performed on pfk13, pfmdr1, and pfct genes. Results showed no evidence of pfk13 mutations associated with resistance. Pfmdr1 86Y was found only in the North at a frequency of 11.2%, while 184F was found in the North and Center at frequencies of 55.0% and 76.1%, respectively. Pfct 76T was detected only in the North at a frequency of 21.5%. We observed a high frequency of wild type pfk76 and pfmdr1 N86 in the Center region. AL, ASAQ, and DP remain efficacious in these areas of Cameroon, but continued efficacy monitoring is essential to facilitate data driven malaria treatment policies. In particular, the 86T and 76T mutations in the North warrant further investigation, as they could be due to drug pressure from the amodiaquine component of SMC implemented annually in the region since 2016.

THERAPEUTIC EFFICACY OF ARTESUNATE-AMODIAQUINE AND ARTEMETHER-LUMEFANTRINE FOR UNCOMPROMICATED FALCIPARUM MALARIA TREATMENT IN FOUR SENTINEL SITES OF CÔTE D’IVOIRE, 2021


1Institut Pierre Richet Bouake Côte d’Ivoire, Abidjan, Côte d’Ivoire, 2Unite de Parasitologie / Centre de Diagnostic et de Recherche sur le SIDA et les autres pathologies infectieuses (CeDReS), Abidjan, Côte d’Ivoire, 3Département Parasitologie Mycologie, UFR Sciences Médicales, UAO, Bouaké, Côte d’Ivoire, 4GSA/NMCP, Abidjan, Côte d’Ivoire, 5Unité de Parasitologie / Centre de Diagnostic et de Recherche sur le SIDA et les autres pathologies infectieuses (CeDReS), Abidjan, Côte d’Ivoire, 6Unité de Parasitologie / Centre de Diagnostic et de Recherche sur le SIDA et les autres pathologies infectieuses (CeDReS), Abidjan, Côte d’Ivoire, 7Département Parasitologie Mycologie, UFR Sciences Mécaulques, UAO, Bouaké, Côte d’Ivoire, 8National Programme Against Malaria, Abidjan, Côte d’Ivoire.
falciparum and the impaired transmissibility of resistant mutants highlights the promise of these compounds for future mosquito-targeted antimalarial interventions.

**THERAPEUTIC EFFICACY OF ARTEMETHER-LUMEFANTRINE AND DIHYDROARTESMISININ-PIPERAQUINE FOR THE TREATMENT OF UNCOMPLICATED PLASMODIUM FALCIPARUM MALARIA IN MALI, 2020-2022**

Youssouf Diarra1, Ibrahim Keita1, Irene Cavros2, Tahirou Traoré3, Lassina Dournia1, Bakary Coulibaly1, Mariam Traoré1, Salimata Diallo1, Garan Dabo1, Mouctar Diallo1, Moustaph Coulibaly1, Bassi Coulibaly1, Lansana Sangare1, Aïssata Koné1, Philippe Mutwa1, Aliou Diallo1, Jehan Ahmed6, Beh Kamaté5, Réunion Saye5, Ousmane A. Koïta1

1Laboratory for Applied Molecular Biology, University of Sciences, Techniques, and Technology, Bamako, Mali, 2U.S. President’s Malaria Initiative, Malaria Branch, U.S. Centers for Disease Control and Prevention, Atlanta, GA, United States, 3Sélingué Referral Health Center, Mali Ministry of Health, Bamako, Mali, 4National Malaria Control Program, Mali Ministry of Health, Bamako, Mali, 5U.S. President’s Malaria Initiative, U.S. Centers for Disease Control and Prevention, Bamako, Mali, 6U.S. President’s Malaria Initiative Impact Malaria, Washington, DC, United States, 7U.S. President’s Malaria Initiative Impact Malaria, Bamako, Mali

The National Malaria Control Program in Mali recommends artemether-lumefantrine (AL) and dihydroartesminisin-piperaquine (DP) for the treatment of uncomplicated malaria. The WHO recommends regular monitoring of drug efficacy to support national treatment policies and practice. From August 2020 to February 2022, we conducted an in vivo study of clinical and parasitological responses to AL and DP in Dioro, Missira and Sélingué sites using the standard WHO therapeutic efficacy study protocol. A total of 420 children (160 in Dioro, 160 in Missira, and 100 in Sélingué) between 6 months and 16 years of age with uncomplicated Plasmodium falciparum malaria and a prevalence of 2,000–200,000 asexual parasites/μL were enrolled, randomly assigned to either AL or DP, and followed up for 28 days (for AL) and 42 days (for DP). Late treatment failures were genotyped to differentiate recrudescence from reinfection using msp1, msp2, and glurp. In AL patients, Day 3 positivity rates were 2.6%, 7.2%, and 0% in Dioro, Missira, and Sélingué, respectively. For DP, Day 3 positivity was 3.2% in Dioro, 7.9% in Missira, and 0% in Sélingué. Uncorrected and PCR-corrected efficacy at day 28 for AL and day 42 for DP were calculated. For AL, day 28 corrected efficacy was 97.3% (95% CI 90.6–99.7%) in Dioro, 98.5% (92.1–100.0%) in Missira, and 100% (92.0–100.0%) in Sélingué. At day 42, corrected efficacy for DP was 94.4% (86.2–98.4%) in Dioro, 98.2% (90.4–100.0%) in Missira, and 100.0% (91.2–100.0%) in Sélingué. Efficacy was above the WHO cutoff for all sites and drugs. Known markers of resistance in Pfk13, Pfmdr1, and Pfcrt genes were assessed in 23 sites in Mali.

Dihydroartesminisin-piperaquine (DHA-PQ) is increasingly used for malaria treatment and considered for chemoprevention where malaria and HIV co-infection are common. PQ is metabolized by cytochrome p450 CYP3A4 leading to drug-drug interactions (DDI) between DHA-PQ and antiretroviral therapy (ART) when co-administered. Suboptimal or elevated pharmacokinetic (PK) exposure may result with efavirenz (EFV)- and lopinavir/ritonavir (LPV/r)-based ART, respectively, compromising efficacy, toxicity, and risking drug resistance. The recently recommended ART, dolutegravir (DTG), has not been extensively evaluated for DDIs. We therefore conducted a prospective open-label PK study in the context of EFV-, LPV/r- and DTG-based ART regimens among HIV-infected Ugandan malaria-uninfected children alongside HIV-uninfected controls. PK sampling was performed over 42 days with quantification by LC tandem MS. 30 HIV-uninfected children at each of the two age groups (3-10 and 11-17 years), and 90 HIV-infected children (n=90 on each regimen) provided PK results. PQ exposure was significantly reduced in children on EFV vs controls, as measured by AUC 0–day21, AUC 0–day28, and AUC 0–day42, (4.17, 4.33, 4.47 vs 11.2, 12.5, 14.6 hr*pg/mL; GMR: 0.372, 0.346, 0.306), and Day 21, 28, and 42 PQ levels (2.24, 1.03, 0.757 ng/mL vs 14.9, 6.41, 9.34 ng/mL; GMR: 0.150, 0.161, 0.081, respectively); all p-values <0.0001. In contrast, LPV/r-based ART increased PQ exposure as compared to controls; AUC 0–day21, AUC 0–day28, and AUC 0–day42, (36.7, 41.6, 49.7 hr*ug/mL; GMR: 3.28, 3.33, 3.40), and Day 21, 28, and 42 PQ levels (47.0, 17.6, 17.8ng/mL; GMR: 3.15, 2.75, 1.91, respectively); all p-values <0.0001. DTG-based ART reduced Day 28 and 42 PQ levels as compared to the controls at 11-17 years (5.44, 5.01 ng/mL vs 8.01, 13.8 ng/mL; GMR: 0.679, 0.363, p-values: 0.0199 and <0.0001, respectively). Intra- and inter-individual pharmacokinetic variability in children on ART regimens were also assessed.

**EVALUATION OF IVERMECTIN AND METABOLITES AS AN ANTIMALARIAL THERAPY AGAINST PLASMODIUM FALCIPARUM LIVER STAGE**

Pradeep Annapalai Subramani1, Phornpimon Tiphatha2, Surendra Kumar Kolli1, Justin Nicolas1, Samantha Barnes1, Madison Schmidt1, Kevin Kobylinski3, Joel Tarning2, John Adams1

1University of South Florida, Tampa, FL, United States, 2Mahidol Oxford Tropical Medicine Research Unit, Bangkok, Thailand, 3Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand

Malaria remains a major global public health threat due to prevalence of drug-resistances and failure to frontline antimalarial therapy, hindering the global efforts to control and eliminate the disease. Almost none of the available antimalarial drugs are efficacious against the liver stage of Plasmodium falciparum, which is the initial stage of infection that leads to disease causing blood stage infection. The only currently FDA-approved class of drug capable of eliminating Plasmodium liver stage parasites are the 8-aminoquinolines and this drug class has its own limitations due to potential hemolytic activity in people with favaism and therefore not suitable for mass drug administration. Due to these limitations, there is a critical need for safer and more efficacious drugs that can prevent liver
A PHASE 2A TRIAL OF SJ733 FOR PLASMODIUM VIVAX MALARIA

R. Kiplin Guy
University of Kentucky, Lexington, KY, United States

SJ733 is currently being developed for treatment of acute uncomplicated and severe malaria. Phase 2a trial with a 3-close schedule of SJ733 (900 mg daily doses) in Peru demonstrated that SJ733 cured blood stage P. vivax malaria in 96% of cases at a 14-day endpoint but did not cure latent liver stage disease. In this trial 80% of patients had undetectable parasitemia within 48 h of initiating therapy. No drug-related adverse events were observed and efficacious exposures of SJ733 were maintained for over 100h. This study strongly suggests that combining SJ733 in a combination drug with a liver-stage active partner would provide for 3-day schedule radical cure of P. vivax malaria.

ARTESUNATE PYRONARIDINE IS A SAFE AND EFFICACIOUS TREATMENT FOR PLASMODIUM FALCIPARUM AND P. VIVAX IN ETHIOPIA WITH A STRONG TRANSMISSION REDUCING ROLE IN P. VIVAX

Migbaru K. Bezabih1, Wakweya Chali1, Sinknesh W. Behaksira1, Mikiyas G. Bulito1, Lina A. Lului1, Melat Abo1, Getnet Habtamru1, Adisu Gizat1, Fikregabrai Aberra Kassa1, Legesse Alamire1, Dawit Halii1, Bethlehem Adnew1, Teun Bousserma1, Fitsum G. Tadesse1,1 Armauer Hansen Research Institute (AHRI), Addis Ababa, Ethiopia, 1Laboratory of Tropical Diseases, University of Campinas, Campinas, Brazil, 1Laboratory of Molecular Modeling and Drug Design, Gois Federal University, Goiânia, Brazil, 1Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil, 1Global Health and Tropical Medicine, Universidade Nova de Lisboa, Lisboa, Portugal

The emergence and spread of drug-resistant parasites in Africa threaten the recent gains in malaria control. Plasmodium falciparum (Pf) and P. vivax (Pv) are sympatric in Ethiopia and the recent development of malaria transmission control tool to kill mosquito vectors that transmit malaria and aid malaria elimination. Recent studies have revealed ivermectin inhibits liver-stage development of P. berghei, a rodent malaria laboratory model, suggesting this drug may be an effective antimalarial drug. However, ivermectin is known to be metabolized by cytochrome P450 3A4 enzyme, which exhibits polymorphism among different individuals to potentially alter the efficacy against Plasmodium, emphasizing the importance of understanding the metabolism of ivermectin and its metabolites. We established a robust in vitro liver assay to study the liver stage development of human malaria parasites in primary human hepatocytes that can be used to evaluate the potential impact of these metabolic differences on ivermectin’s anti-liver stage efficacy. This model can be used to evaluate the metabolism of ivermectin in hepatocytes from different donors and its efficacy against P. falciparum liver stages.
THERAPEUTIC EFFICACY OF PYRONARIDINE-ARTESunate (Pyramax®) AGAINST unCOMPLICATED PlasMODEum Falciparum INFECTION AT HAMUSIT Health CENTER, NORTHWEST ETHIOPIA

Mihreteab Abebech1, Woyneshet Gelaye2, Megabru Alenu2, Heven Sime3, Henok Hailegeorgies3, Bokretson Gidey4, Mebratrom Hale5, Gudissia Assefa6, Worku Bekele6, Habtamu Belay7, Jonathan B. Parr7, Geremew Tasew8, Hussein Mohammed3, Ashenafi Assefa Bahita9

1Department of Medical Laboratory Sciences, College of Medicine and Health Sciences, Wollo University, Dessie, Ethiopia, 2Department of Medical Laboratory Sciences, College of Medicine and Health Sciences, Bahir Dar University, Bahir Dar, Ethiopia, 3Ethiopian Public Health Institute, Addis Ababa, Ethiopia, 4Ministry of Health Ethiopia, Addis Ababa, Ethiopia, 5World Health Organization, Addis Ababa, Ethiopia, 6Department of Medical Laboratory Sciences, College of Medicine and Health Sciences, Wolite University, Wolite, Ethiopia, 7Institute for Global Health and Infectious Disease, University of North Carolina at Chapel Hill, Chapel Hill, NC, United States

Malaria remains a major public health problem. Early cases detection and prompt treatment are important malaria control strategies implemented in many endemic countries, including Ethiopia. ACT is currently recommended by the World Health Organization (WHO) for the management of uncomplicated Plasmodium falciparum and P. vivax malaria. However, the emergence and rapid spread of drug-resistant plasmodium strains presents a major challenge to malaria control and elimination efforts. Pyronaridine-artesunate (Pyramax) is an artemisinin-combination therapy shown to have good efficacy for uncomplicated malaria in large-scale clinical trials conducted in Asia and Africa. This study reports the first therapeutic efficacy profile of pyronaridine-artesunate for uncomplicated P. falciparum in Ethiopia. This single-arm prospective study with 42-day follow-up period was conducted from March to May 2021 at Hamusit health center using the WHO therapeutic efficacy study protocol. A total of 90 adults ages 18 and older with uncomplicated falciparum cases consented and were enrolled in the study. A standard single-dose regimen of pyronaridine-artesunate was administered daily for 3 days, and clinical and parasitological outcomes were assessed over 42 days of follow-up. Thick and thin blood films were prepared from capillary blood and examined using light microscopy. Hemoglobin was measured and dried blood spots were collected on day 0 and on the day of failure. Out of 90 patients, 86 (95.6%) completed the 42-day follow-up study period. The overall PCR-corrected cure rate (adequate clinical and parasitological response) was very high at 98.9% (95% CI: 92.2-99.8%) with no serious adverse events. The parasite clearance rate was 99.8% compared to that on day 0. Pyronaridine-artesunate was highly efficacious and safe against uncomplicated P. falciparum in the study population.

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ANTIPLASMODIAL ACTIVITY OF NOVEL HETEROcYCLIC COMPOUNDS using IN SILICO and IN VITRO ASSAYS

Maria del Pilar Crespo-Ortiz, Martha Ilce Orozco-Mera, Rodrigo Abonia, Braulio Insuausty, Jairo Quiroga, Pedro Moreno, Miguel Quevara

Universidad del Valle, Cali, Colombia

Resistance to artemisinin and/or their partner drugs has become a major threat to achieve malaria eradication. The development of new antimalarials is paramount to keep the goals on reduction of malaria cases in endemic regions. The search for quality hits has been challenging as many inhibitory molecules may not progress to the next development state. Combined strategies using in silico and phenotypic approaches may improve the detection and selection of hits likely to progress through the pipeline as drug candidates. The aim of this work was to screen an in-house library of heterocyclic compounds (RGHC) for antimalarial activity using computational predictions and phenotypic techniques to find quality hits. The novel heterocyclics were synthesized based on biologically active templates. The physicochemical and drug likeness properties (ADMET) of RGHC library were evaluated in silico and compounds were selected for a structure-based analysis. Seven Plasmodium target proteins were chosen from the drug bank database and ligands and receptors processed using computer tools (Chimera UCSF and Open Babel) before being subjected to docking analysis with Autodock Vina. Growth inhibition of P. falciparum (3D7) cultures was tested by SYBR green assays and toxicity was assessed in the G. m. melanoi in vivo model. From a total of 792 compounds, 364 (45.9%) showed good membrane permeability, oral/gastrointestinal bioavailability and complied with the Lipinski rule of five. Seventeen (2.1%) compounds were detected as PAINS structures, 341 compounds with favorable drug likeness profile were selected for analysis. Eight compounds showed IC50 ranging from 0.175-0.990 µM, these compounds belong to the same series of pyridines. Preliminary toxicity assays showed low toxicity in the Galleria model in at least 4 compounds complying with drug likeness properties. Two inhibitory compounds showed good binding energies with 1-cys peroxiredoxin, and the calcium dependent protein kinase-4 and the other two compounds with the halocid dehalogenase-like hydrolase and plasmepsin 2, suggesting potential antimalarial activity.

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EVALUATING THE EFFECT HETEROGENEITY OF Malarial CAMP INTERVEnTIONS in HARD-To-REACH AREAS OF OdISHA StaTE, INdIA

Sooyoung Kim1, Praveen K. Sahu2, Timir K. Padhan2, Stuti Mohanty2, Mohammed A. Haque2, Sanjib Mohanty2, Anne Kessler3, Danielle C. Ompad1, Jane M. Carlton3, Yesim Tozan3

1New York University School of Global Public Health, New York, NY, United States, 2Department of Molecular & Infectious Diseases, Community
Odisha state has the highest burden of malaria in India with a large tribal population living in hard-to-reach areas. The Odisha State Malaria Control Program introduced ‘malaria camps’ in 2017 targeting remote villages to perform mass screening and treatment, enhance vector control and educate the population. To better understand the heterogeneity of intervention effects, we combined data on intervention outcomes and the target population's socioeconomic and demographic characteristics with entomological and remotely-sensed data on climate and environmental factors for the study villages. We created four outcome variables: Plasmodium RDT+, which was further divided into symptomatic cases RDT+/fever+ and asymptomatic infection RDT+/fever-; and subpatent malaria RDT-/PCR+. We applied XGboost, a machine learning algorithm, on each study outcome, operationalizing the remaining variables as model features, to investigate the factors contributing to intervention effect heterogeneity. The Shapley additive explanation post-processed model interpretation framework was used to interpret the models. The model exhibited the best predictive performance in identifying subpatent infections, with 75% recall and 87% balanced accuracy. All feature classes contributed as important features to the model, with particular importance on anthropometric (age, sex, weight, height, BMI, body temperature), sociodemographic (number of occupants in the household) and behavioral characteristics (sleeping without bednet, knowledge of malaria symptoms), malaria history, climatic factors (precipitation and temperature in previous quarter), entomological characteristics (in study villages [Anopheles and Culex counts]), and serological features (antibodies to PfRh22030 and PIMSP2Ch150 antigens). The model's relatively good performance in predicting subpatent infection suggests that it has the potential to guide development of more targeted and effective interventions to reduce subpatent reservoirs. Such improvements are likely to enhance intervention cost-effectiveness and contribute to the malaria elimination goal by 2030.

IMPLEMENTATION OF A NEW VECTOR SURVEILLANCE SYSTEM TO ANTICIPATE THE IMPACT ASSESSMENT OF THE NOVEL GENETIC TECHNOLOGIES IN BURKINA FASO

Abdoulaye Niang1, Simon P. Sawadogo1, Abdoul A. Millogo1, Gauthier Tougo2, Téicoura Camara2, Roch K. Dabire1, Abdoulaye Diabate1

1Institut de Recherche en Sciences de la Santé, Bobo-Dioulasso, Burkina Faso, 2National Malaria Control Programme, Ouagadougou, Burkina Faso

New approaches based on the release of genetically-modified sterile male mosquitoes to control natural populations are proposed as the most promising strategies for malaria control. To effectively assess the impact of these interventions in endemic settings, vector surveillance systems that address practical issues of malaria control must be established. However, in most sub-Saharan African countries vector surveillance is generally not, or under-funded and limited resources are allocated to support the development and enhancement of adequate capacities for effective entomological surveillance within national malaria control programmes (NMCPs). Thus, vector surveillance systems remain weak, fragmented between research institutes, universities, and the national programmes, and lack appropriate coordination, although such systems are urgently needed. In Burkina Faso, we have brought together all relevant stakeholders from the NMCP, the Ministry of Health, research centres, universities to develop a better approach to establishing sustainable system with increased local participation, ownership and integration of vector control implementation, surveillance, and operational research for malaria elimination. Here we present the impact of the training in malaria vector surveillance and monitoring of vector control interventions to anticipate the envisaged use of gene drive technology to suppress vector populations in Burkina Faso setting. Newly trained entomologists, including 70 health promotion officers deployed in the peripheral health districts, 13 others in the intermediate level of the health regions and 9 in the central level of decision making in the NMCP, were assessed on practical techniques and methods for collecting adult mosquitoes and larvae in the field. The results show that our approach is quick, inexpensive, and accurate, with a high mosquito and larval sampling performance, which makes it a promising system for very large-scale mosquito genetic surveillance to assess the impact of implementations for vector control.

A SUSTAINABLE MALARIA CONTROL BLUEPRINT: 20 YEARS OF CHALLENGES, LESSONS AND ACHIEVEMENTS

Wonder P. Phiri1, Matilde Riloha Rivas2, Olivier Tresor Donfack1, Jeremias Nzarnio Mba Eyono1, Julia Yumbe Baka1, Liberato Motobe Vaz1, Teresa Ayingongo Ondo Mfumu1, David Galick1, Kyle R. DeBoer3, Immo Kleinschmidt4, David L. Smith5, Claudia Daubenberger6, Bonifacio Manguire Nlavo7, Carlos A. Guerra8, Mitoha Ondo’o Ayekaba9, Guillermo A. Garcia10

1MCD Global Health, Malabo, Equatorial Guinea, 2Ministry of Health and Social Welfare, National Malaria Control Program, Malabo, Equatorial Guinea, 3MCD Global Health, Silver Spring, MD, United States, 4London School of Hygiene and Tropical Medicine, London, United Kingdom, 5Institute of Health Metrics and Evaluation, University of Washington, Seattle, WA, United States, 61.Department of Medical Parasitology and
PRIVATE PROVIDERS ACCEPTANCE OF SOCIAL MEDIA REPORTING TOOLS FOR MALARIA CASE NOTIFICATION AND SURVEILLANCE IN MYANMAR

May Me Thet1, Zaw Wai Yan Bo2, Zayar Kyaw1, Bram Piot1, Myat Noe Thiri Khaing1, Sandar Ooi1, Mahesh Paudel4


Myanmar aims to eliminate malaria by 2030. To meet elimination targets, all malaria cases must be reported within 24-hours. Case-based reporting began in 2019 but remains challenging due to lack of standardized mechanism and using inefficient methods like SMS and phone calls.

In 2021, we developed social media chatbots running on Facebook Messenger and Viber and connected to DHIS2, allowing private providers and field supervisors of malaria community volunteers to report surveillance data within 24-hours. Volunteers have limited access to internet therefore their supervisors used the chatbots for case notification. From July 2021 to date, 1349 malaria cases have been notified by 486 private providers and 5475 cases by 40 field supervisors covering 1116 volunteers. We conducted qualitative interviews with 8 providers and 8 supervisors in Q1 2022 to understand users’ experiences with the new tools and identify future support needs and opportunities for further scale-up. Data were analyzed in a workshop setting. Chatbot approach was favored by users for malaria case notification and surveillance: users preferred chatbots to aggregate reporting on paper. Digital notification was perceived as quick, low-cost, and simple to perform, lowering the record-keeping burden.

The chatbots were compatible with almost all phone and tablet models. Saving time, real-time notification and ability to respond confirmed cases indicated that private sector can play a crucial role in disease surveillance. Digital notification was perceived as quick, low-cost, and simple to perform, lowering the record-keeping burden. Poor provider digital literacy, internet connectivity, and time limitations were cited as the main barriers of consistent use. The study indicated that private sector can play a crucial role in disease surveillance and innovative digital tools can enhance the effectiveness and timeliness of malaria surveillance efforts in Myanmar. Malaria has been a trailblazer in and innovative digital tools can enhance the effectiveness and timeliness of malaria surveillance efforts in Myanmar. Malaria has been a trailblazer in the use of digital tools for disease surveillance, setting an example for other disease to follow and thus expansion of the system to support high burden health system approaches appears promising.
district. We analyzed the qualitative data using immersion crystallization methodology with multiple independent reviewers extracting major and minor themes within the frameworks of prevention, detection, and response capabilities. Within the category of prevention, the main theme extracted was a lapse in vector control strategies. The major themes extracted from the categories of detection and response were lack of logistical support and chemotherapeutics. Of the 10 districts assessed, 7 (70%) had inadequate access to insecticide treated nets, 3 (30%) had no active indoor residual spraying program in place, and 8 (80%) had limited or no rapid diagnostic tests available. The mean (SD) time from last resupply for anti-malarial medications from the central governmental supply was 117 (±9) days. The extracted themes highlight key areas ripe for intervention to better equip high burden districts in eastern Uganda experiencing a dramatic rise in malaria.

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THE TRAJECTORY OF MALARIA CARE OF CHILDREN UNDER FIVE YEARS WITH FEVER, FROM AN ANNUAL CROSS-SECTIONAL HOUSEHOLD SURVEY WITHIN PROGRAM AREAS OF THE ISDELL:FLOWERS CROSS BORDER MALARIA INITIATIVE IN ZAMBIA

Alyssa Maglior1, Jesse Heitner2, Alexandra Gordon1, Rebecca Vander Meulen1, Constance Njovu3, Busiku Hamainza4

1J.C. Flowers Foundation, New York City, NY, United States, 2Mass General Brigham, Boston, MA, United States, 3Anglican Diocese of Lusaka, Lusaka, Zambia, 4National Malaria Elimination Centre, Ministry of Health of Zambia, Lusaka, Zambia

The Zambia National Malaria Strategic Plan 2022-2026 has the goal of ensuring 100% of suspected malaria cases are tested and 100% confirmed cases are treated within 24 hours with an antimalarial. Achieving this goal relies not only on strong health systems and availability of commodities, but also on proper care-seeking behavior within the at-risk population.

This study assessed the trajectory of care of children under five years with fever in the previous two weeks within program areas of the Isdell:Flowers Cross Border Malaria Initiative (IFCBMI), an implementing partner of Zambia’s National Malaria Elimination Centre, as reported by caregivers in an annual cross-sectional household survey conducted from May-June 2021 (n=2541) and April-May 2022 (n=1982). From 2021-2022, results showed nonsignificant changes in care-seeking behavior and in provision of malaria tests to children <5 with fever but showed a significant increase in the proportion of children who received Coartem after testing positive for malaria. Specifically, in 2022, 87.6% (84.5% - 90.2%) of respondents whose child <5 had a fever in the previous two weeks sought care for their child from a health facility or community health worker (CHW) but only 65.5% (61.3% - 69.5%) did so within 24 hours of fever onset, compared to 89.7% (-2.1%, p=0.035) in 2021, respectively. Among those children <5 with fever who sought care from a health facility or CHW, 88.4% (85.1% - 91.1%) received a malaria test, compared to 86.4% (+2.0%, p=0.251) in 2021. Of those febrile children who were tested, 62.9% (68.3% - 67.3%) tested positive for malaria, compared to 65.9% (-3.0%, p=0.361) in 2021. Of those children who tested positive for malaria, 96.3% received Coartem for treatment in 2022, a significant increase from 87.7% (+7.6%, p=0.002) in 2021. These data comprise part of an annually conducted survey on malaria-related knowledge, attitudes, and practices, utilized by government and local community planners for malaria goal setting. Data to be collected in April-May 2023 will again measure these indicators and assess changes relative to 2022 results.

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COMPARATIVE EFFECTIVENESS TRIAL OF TWO INTEGRATED COMMUNITY CASE MANAGEMENT TECHNIQUES FOLLOWING WITHDRAWAL OF INDOOR RESIDUAL SPRAYING IN NE UGANDA

Dorothy Echodu1, Humphrey Wanzirah2, Lucinda Hadley3, Thomas Eganyu2, Richard Elliott1, Fred Bukyenua2, Kathryn Colburn1, Wycliff Odude2, Adoke Yeka1, Jimmy Opigo1, Emanuele Georgi2

1Pilgrim Africa, Seattle, WA, United States, 2Pilgrim Africa, Kampala, Uganda, 3University of Lancaster, Lancaster, United Kingdom, 4Boise State University, Boise, ID, United States, 5University of Colorado Denver, Denver, CO, United States, 6Pilgrim Africa, Soroti, Uganda, 7University of Makerere School of Public Health, Kampala, Uganda, 8National Malaria Control Division, Ministry of Health, Kampala, Uganda

In standard integrated community case management (ICCM), caregivers approach community health workers (CHWs) for testing and treatment for malaria in under-5s. Proactive ICCM (ProCCM) expands on ICCM with proactive weekly or biweekly household visits by VHTs and case management for all ages. We present results of a prospective two-year trial comparing the effectiveness of ICCM vs. ProCCM at maintaining malaria reduction or preventing resurgence. Indoor residual spraying with pirimiphos-methyl occurred four times in the high-transmission district of Katakwi in NE Uganda, with the final round in December 2018. At trial baseline, all-ages malaria prevalence by microscopy was 10%. In 55 villages in the former IRS area, we performed a community-randomized comparative effectiveness trial of the combination of PermaNet 3.0 deltamethrin- pipexuron butoxide (PBO) long-lasting insecticide treated nets (LLINs) and either ICCM or ProCCM. A total of 27 villages were randomized to ICCM + PBO nets and 28 villages to ProCCM + PBO nets using covariate-constrained randomization. The primary outcome of community-level cross-sectional Pf prevalence measured at baseline, midline and endline. PBO nets were distributed in July 2019, while CHWs were trained in August 2019 and acting to protocol by November 2019. Malaria incidence data was recorded weekly by CHWs in both arms using mobile devices, while arm-specific incidence data from health facilities was collected using village of residence. Entomological adult collections were also performed in 180 observation households. Community level cross-sectional Pf prevalence surveys were conducted at baseline April 2019, midline July/August 2020 (delayed by pandemic restrictions), and at endline August 2021. Even after accounting for seasonal differences, malaria prevalence under IRS protection at baseline increased dramatically to pre-IRS levels (30%) in both arms by study endline. Analysis of surveys found no difference in Pf prevalence between arms, despite increased treatment per capita in ProCCM. In high transmission, the combination of PBO LLINs and ProCCM may not be sufficient to control malaria.

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COMMUNITY PERCEPTIONS OF PROACTIVE MALARIA COMMUNITY CASE MANAGEMENT IN CHADIZA DISTRICT, ZAMBIA

Bupe M. Kabamba1, Melody N. Simaata1, Chabu C. Kangale1, Marie-Reine I. Rutagwera2, Maximillian Musunse3, Patrick Nyendwa1, Vinannah Kapenda2, Ignatius Banda4, Caroline Phiri-Chibave1, Paul Psychas1, Julie R. Gutman1, John M. Miller5, Busiku Hamainza6, Julie I. Thwing7

1PATH PAMO Plus, Lusaka, Zambia, 2Zambia Ministry of Health National Malaria Elimination Centre, Lusaka, Zambia, 3US President’s Malaria Initiative (PMI), Centers for Disease Control and Prevention (CDC), Lusaka, Zambia, 4Malaria Branch, CDC, Atlanta, GA, United States, 5PATH Malaria Control and Elimination Partnership in Africa (MACEPA), Lusaka, Zambia, 6Malaria Branch, CDC, Lusaka, Zambia

Zambia has successfully implemented malaria community case management (CCM) through passive and reactive case detection by community health workers (CHW) since 2011. With the goal of malaria elimination, in 2021 the National Malaria Elimination Centre implemented
a trial to measure the impact of proactive CCM (ProCCM) compared to routine CCM on malaria incidence and prevalence in Chadiza District, Eastern Province. ProCCM entails weekly visits by CHWs to all households in a community to identify people with malaria symptoms, offer diagnostic testing, and treat those with positive tests. As part of this trial, we conducted a qualitative midline assessment to describe the feasibility and acceptability of ProCCM. We interviewed 12 CHWs, 6 provincial and district staff, 9 health facility (HF) staff, and 72 community members from 6 ProCCM and 6 routine CCM clusters. All respondent groups preferred ProCCM to routine CCM, reporting it reduces distance travelled to seek care and provides care to people with poor health seeking behavior. Most CHWs and HF staff perceived the intervention arm to have less malaria compared to the control arm. Most community members preferred at least one weekly visit. Six provincial, district, and HF staff and 5 CHWs felt that ProCCM negatively affected CHWs’ livelihood, necessitating compensation. Four HF staff reported supervising ProCCM CHWs more frequently than before the trial, which 2 saw as an additional, albeit manageable, time commitment. District and HF staff noted an increase in rapid diagnostic test and artemisinin combination therapy consumption due to ProCCM.

Other challenges included long distances covered by CHWs during visits, and the impact of the farming cycle on both households’ and CHWs’ availability to conduct visits. While these findings suggest that malaria data CCM approach is accepted by the community, it requires more time and travel by CHWs compared to routine CCM. Intervention arm CHWs were provided with performance-based remuneration and bicycles after this assessment. Further analysis to assess the effect of remuneration on their performance will provide valuable insights into feasibility.

ASSESSING MALARIA INCIDENCE IN PROACTIVE COMMUNITY MALARIA CASE MANAGEMENT (PROCCM): A RANDOMIZED CONTROL TRIAL IN CHADIZA DISTRICT, EASTERN PROVINCE, ZAMBIA

Marie-Reine I. Rutagwera1, Sarah Gallalee2, Travis Porter3, Chabu C. Kangale4, Bupe M. Kabamba1, Melody Simataa1, John M. Miller5, Caroline Phiri-Chibawe1, Maximillian Musunse1, Patrick Nyemwa1, Viennah Kapenda1, Paul Psychas1, Julie R. Gutman1, Adam Bennett1, Ignatius Banda1, Busiku Hamainza1, Julie I. Thwing1

1PATH Malaria Control and Elimination Partnership in Africa (MACEPA), Seattle, WA, United States, 2PATH Malaria Control and Elimination Partnership in Africa (MACEPA), Lusaka, Zambia, 3University of California, San Francisco, San Francisco, CA, United States, 4PATH Malaria Control and Elimination Partnership in Africa (MACEPA), Lusaka, Zambia, 4US President’s Malaria Initiative (PMI), Centers for Disease Control and Prevention (CDC), Lusaka, Zambia, 5Malaria Branch, CDC, Atlanta, GA, United States, 6Zambia Ministry of Health National Malaria Elimination Centre (NMEC), Lusaka, Zambia, 7US President’s Malaria Initiative (PMI), Centers for Disease Control and Prevention (CDC), Lusaka, Zambia

Community health workers (CHWs) play a key role in the malaria control and elimination strategy in Zambia. As part of routine (passive) community case management (CCM), CHWs provide malaria services for persons of all ages seeking care for malaria symptoms using rapid diagnostic tests (RDTs), and treat positive individuals with artemisinin-based combination therapy (ACT). In 2021, we started a two-year cluster randomized controlled trial to determine the additional impact of proactive household malaria symptom screening, testing and treatment (ProCCM) on incidence of malaria. All 4,987 households in 33 intervention arm (IA) clusters began receiving weekly ProCCM household visits in addition to routine CCM; 4,714 households in 33 control arm (CA) clusters continued receiving routine CCM. CHWs in the IA reported weekly home visits via CommCare’s mobile platform; CHWs in both arms abstracted weekly malaria data (tests and positive cases) for their clusters from passive CCM in their registers as well as checking health facility (HF) registers for visits by members of their community and reported these in a district health information system 2 (DHIS2) instance. From January-December 2022, 199,269 proactive home visits were conducted in the IA, testing 13,168 patients with malaria symptoms. Over this same period, 30,191 and 33,547 individuals in the intervention and control arms, respectively, were tested for malaria through passive CCM and at HFIs. Cumulative malaria incidence for 2022 from all sources of testing was 512 cases per 1,000 population in the IA [ProCCM: 131 per 1K (25.5%); HF: 75 (14.7%); routine CCM: 306 (69.8%)] and 546 per 1,000 in the CA [HF: 101 (18.4%); routine CCM: 445 (81.6%)]. The malaria test positivity rate (TPR) for proactively tested cases in the IA was 26.1%; TPRs for passively tested cases (combined routine CCM and HF) were 33.2% and 39.6% in the intervention and control arms, respectively. Interim results indicate a shift in case management from facilities to communities in the intervention arm. Final trial results will include data through May 2023 and evaluate the intervention impact on parasite prevalence and incidence.
Over the past 50 years, Health Surveillance Assistants (HSAs) have provided an increasing array of community-based health services for the largely rural population in Malawi, including frontline malaria and childhood disease management. Here we present the findings of a rapid assessment conducted at the end of two five-year national strategies—the National Community Health Strategy ‘17–‘22 and National Malaria Strategic Plan ‘17–‘22 for Malawi—aimed at understanding recent progress and describing current HSA program gaps and opportunities from a critical system perspective. In collaboration with the Ministry of Health’s National Malaria Control Program and Integrated Management of Childhood Illness departments, in Q4 2022 we purposively sampled 2–3 districts in each region (Southern, Central, and Northern) to represent a range of geographies across Malawi. Within each district, two health centers were selected and for each health center, three village health clinics (VHCs) located in hard-to-reach areas were identified. From these VHCs, 39 HSAs were interviewed to share their views on various aspects of the community health system. Feedback described availability of malaria commodities but chronic stockouts of others for treatment of diarrhea and pneumonia. Similarly, HSAs noted a need for replacement of basic supplies and equipment—frequently rain gear and back packs—and that shortages of printed registers and forms resulted in omission of key data during reporting, which is primarily paper-based. Supervision and in-service training to build and reinforce skills appeared to be infrequent and inconsistently implemented across districts. Additionally, nearly half of interviewed HSAs resided outside of their assigned catchment areas, compounding more widespread issues with transportation and limiting the hours in which HSAs could provide services. Despite challenges, however, HSAs demonstrated strong commitment to their roles, interest in acquiring knowledge and skills, and willingness to adopt digital tools as solutions—opportunities for further strengthening Malawi’s community health system.

### FINDINGS FROM IMPLEMENTER CASE STUDIES

#### SUSTAINING MALARIA COMMUNITY HEALTH WORKER PROGRAMS WITH EXPANDED ROLES IN THE GMS: FINDINGS FROM IMPLEMENTER CASE STUDIES

Laura Buback1, Kyle Daniels2, Tiese Etim-Inyang1, Monnaphat Jongdeepaisal1, Massaya Sirimatayanant1, Panarasri Khonputsa3, Richard Maude3, Naomi Beyeler1

1Institute for Global Health Sciences, University of California San Francisco, San Francisco, CA, United States, 2University of Washington School of Public Health, Seattle, WA, United States, 3Mahidol Oxford Tropical Medicine Research Unit, Bangkok, Thailand

Throughout the Asia-Pacific region, many countries rely on community health workers (CHWs) to provide care for a range of health needs. In the Greater Mekong Subregion (GMS), Village Malaria Workers (VMWs) with health system integration (including politically-advocated guidelines, domestic ownership and local health priorities) has implications for implementation. Findings suggest that a sustainable approach for malaria CHWs requires tailoring their role packages based on local needs and their embedded role as a link between the health sector and the communities, while considering carefully their capacities and support requirements in the last mile towards elimination.
have been essential to malaria elimination strategies. Yet as countries near elimination and the malaria burden declines, the role of VMWs in local health systems and communities is changing. There is a need to expand VMW roles to take on provision of health services beyond malaria. We aimed to understand the process and experience of VMW/CHW role expansion including implementation, financing, policy, and sustainability within the Asia-Pacific region. We documented VMW/CHW programs that included health services in addition to malaria. We conducted 21 key-stakeholder interviews from fifteen programs in eight countries within the Asia-Pacific region. Qualitative interviews were conducted virtually in English, audio-recorded, and findings analyzed using rapid-matrix analysis. Of the fifteen programs, five were government programs, six international non-governmental organization (NGO) programs, and two research programs. Program managers and technical advisors explained expansion processes, challenges, and opportunities. We found that integration can take place in multiple program domains and does not necessarily occur in all domains at once. We identified six entry points for VMW role expansion, including integrated policy and financing, as well as planning, assessments, and research. Operational entry points included the selection, training, and motivation, as well as management, supervision, and monitoring of CHWs. Enabling factors, such as decentralized management structures, health system linkages, namely commodity provision and referral procedures, as well as community engagement and hard to reach areas were also included. While there is not a linear or unique path towards integration, there are key considerations for the policy level, practical implementation steps, as well as enabling factors for countries in the GMS to take into account as they move towards sustainable, integrated VMW/CHWs.

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HIGH BURDEN OF ASYMPTOMATIC MALARIA AND ANAEMIA DESPITE HIGH ADHERENCE TO MALARIA CONTROL MEASURES: A CROSS-SECTIONAL STUDY AMONG PREGNANT WOMEN ACROSS TWO SEASONS IN A MALARIA-ENDEMIC SETTING IN GHANA

Nsoh Godwin Anabire1, Belinda Aculley2, Abigail Pobee3, Eric Kyse-Baatour4, Gordon Awandare5, Maria del Pilar Quintana1, Lars Hvidt6, Michael Ofori1

1Department of Biochemistry and Molecular Medicine, School of Medicine, University for Development Studies, Tamale, Ghana, 2Department of Immunology, Noguchi Memorial Institute for Medical Research, University of Ghana, Accra, Ghana, 3West African Centre for Cell Biology of Infectious Pathogens; Department of Biochemistry, Cell and Molecular Biology, University of Ghana, Accra, Ghana, 4Centre for Medical Parasitology, Department of Immunology and Microbiology, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark

Anemia remains a serious concern among pregnant women, and thus, is closely monitored from the onset of pregnancy through to delivery to help prevent adverse maternal and neonatal outcomes. In malaria-endemic settings, continuous low-level carriage of Plasmodium falciparum parasites is common and its contribution to maternal anemia should not be underestimated. In this study, we evaluated the impact of adherence to malaria control measures (number of antenatal clinics (ANC) attended, supervised intake of sulphadoxine pyrimethamine (SP), and use of insecticide treated bed nets (ITNs)) on asymptomatic malaria and anaemia outcomes among pregnant women on ANC in hospitals in the Central region of Ghana. The study was conducted during two seasons; October-November 2020 (dry season, n=124) and May-June 2021 (rainy season, n=145). Among the women, there was a high adherence to the control measures for both seasons (ANC ≥ 3 visits; ~82.0%, intake of SP; ~80.0% and ITNs use; ~75.0%). Asymptomatic P. falciparum carriage was high for both seasons (44.4% for the dry season; 46.9% for the rainy season). Correspondingly, the occurrence of anaemia was high for both seasons (57.3% for the dry season; 68.5% for the rainy season) and was strongly predicted by carriage of P. falciparum parasites. Despite the high adherence to ANC protocols, asymptomatic P. falciparum infection was common and contributed to the high burden of maternal anemia. Our findings emphasize the need for improved control measures that can clear asymptomatic/sub-microscopic P. falciparum infection and protect against malaria-induced anaemia among pregnant women attending ANC in malaria endemic-settings.

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HIGH COMMUNITY HEALTH WORKER USAGE WITH APPROPRIATE MALARIA MANAGEMENT IN A MODERATE PLASMODIUM FALCIPARUM BURDEN REGION OF CHADIZA DISTRICT, ZAMBIA, APRIL-MAY, 2021

Erika Wallender1, Bupe M. Kabamba2, Marie-Reine I. Rutagwera3, Chabu Kangale2, Travis Porter4, Maximillian Musunse2, Sarah Gallalée5, Adam Bennett6, Paul Psychas7, Julie Gutman1, Busiku Hamainza2, Julie Thwing1

1U.S. Centers for Disease Control and Prevention, Atlanta, GA, United States, 2PATH PAMO Plus, Lusaka, Zambia, 3PATH PAMO Plus, Atlanta, Zambia, 4PATH MACPEA, Seattle, WA, United States, 5University of California, San Francisco, San Francisco, CA, United States, 6U.S. President’s Malaria Initiative, CDC, Lusaka, Zambia, 7Zambia Ministry of Health, National Malaria Elimination Center, Lusaka, Zambia

Community health workers (CHWs) are used to improve access to prompt and effective care, in particular malaria community case management (mCCM). We characterized care seeking for fever and malaria case management in Chadiza District, Zambia, in the context of an effectively scaled up mCCM program (1 CHW for ~500 people; >50% of malaria cases reported by CHWs) using a cross-sectional household survey. During a pre-intervention household survey from a trial of proactive mCCM, we randomly selected 33 households in each of 73 of 161 eligible communities. We asked all household members about fever in the past 2 weeks; those reporting fever were asked about healthcare seeking and interventions received. All participants received a malaria rapid diagnostic test (RDT). Weighted population estimates with 95% confidence intervals (CI) were used for all percentages, and chi-squared tests were used to assess significance. Analyses were stratified by age and RDT result. Among 11,185 consenting residents, 8.3% (95% CI 5.8-10.7%) reported fever in the last 2 weeks; children <5 years were most likely to report fever (12.4%, 8.2-16.6). Overall P. falciparum prevalence was 19.9% by RDT (16.4-21.4); prevalence was higher (33.7%, 26.4-40.9) among those with fever in the past two weeks. Care seeking from any source for fever was higher in the younger age groups: 67.3% (59.9-74.8) for children <5 years, 60.1% (52.6-67.6) for children 5-14 years, and 48.6% (38.4-58.7) for those >15 years. Among persons of any age that sought care, 76.2% (67.8-84.6) went to a CHW alone or in combination with another source (e.g., health facility, shop). Those who accessed a CHW were more likely to report receiving a malaria test (89.2%, 85.0-93.5) compared with non-CHW care (73.5%, 63.9-83.0). Of those with a positive RDT, a history of fever, and who sought care, 70.5% (60.2-80.8) reported receiving artemisinin-based combination therapy; percentages were similar for CHW and non-CHW care. Among care seeking participants, CHW usage was very high, and malaria management was appropriate. Further interventions are needed to maximize the percentage of febrile individuals who seek care.

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EVALUATION OF THE MANAGEMENT OF CHILDREN UNDER TEN YEARS OF AGE HOSPITALIZED WITH SEVERE MALARIA AT THE HEALTH CENTER OF TAMBAOCOUNDA, SENEGAL FROM 2018 TO 2021

Tidiane Gadiaga1, Alioune Badara Gueye2, Aminata Fall3, Siré Sagna1, Bayal Cissé1, Mouhamadou Faly Ba1, Médoune Ndiop4, Standeur Nabi Kaly4, Elhadji Doucouré4, Ibrahima Diallo6, Latsouk Gnilane Diouf4, Doudou Sène5, Isaac Manga6, Abdoulaye Diallo6, Gallalee5, Adam Bennett4, Paul Psychas6, Julie Gutman1, Busiku Hamainza2, Julie Thwing1

1U.S. Centers for Disease Control and Prevention, Atlanta, GA, United States, 2PATH PAMO Plus, Lusaka, Zambia, 3PATH PAMO Plus, Atlanta, Zambia, 4PATH MACPEA, Seattle, WA, United States, 5University of California, San Francisco, San Francisco, CA, United States, 6U.S. President’s Malaria Initiative, CDC, Lusaka, Zambia, 7Zambia Ministry of Health, National Malaria Elimination Center, Lusaka, Zambia

During a pre-intervention household survey from a trial of proactive mCCM, we randomly selected 33 households in each of 73 of 161 eligible communities. We asked all household members about fever in the past 2 weeks; those reporting fever were asked about healthcare seeking and interventions received. All participants received a malaria rapid diagnostic test (RDT). Weighted population estimates with 95% confidence intervals (CI) were used for all percentages, and chi-squared tests were used to assess significance. Analyses were stratified by age and RDT result. Among 11,185 consenting residents, 8.3% (95% CI 5.8-10.7%) reported fever in the last 2 weeks; children <5 years were most likely to report fever (12.4%, 8.2-16.6). Overall P. falciparum prevalence was 19.9% by RDT (16.4-21.4); prevalence was higher (33.7%, 26.4-40.9) among those with fever in the past two weeks. Care seeking from any source for fever was higher in the younger age groups: 67.3% (59.9-74.8) for children <5 years, 60.1% (52.6-67.6) for children 5-14 years, and 48.6% (38.4-58.7) for those >15 years. Among persons of any age that sought care, 76.2% (67.8-84.6) went to a CHW alone or in combination with another source (e.g., health facility, shop). Those who accessed a CHW were more likely to report receiving a malaria test (89.2%, 85.0-93.5) compared with non-CHW care (73.5%, 63.9-83.0). Of those with a positive RDT, a history of fever, and who sought care, 70.5% (60.2-80.8) reported receiving artemisinin-based combination therapy; percentages were similar for CHW and non-CHW care. Among care seeking participants, CHW usage was very high, and malaria management was appropriate. Further interventions are needed to maximize the percentage of febrile individuals who seek care.
Malaria remains a public health priority in Senegal, particularly in Tambacounda where it is one of the main causes of infant mortality. The objective of this study was to evaluate the management of children under 10 years of age hospitalized at the Tambacounda health center for severe malaria and to identify factors associated with their recovery. A retrospective and descriptive study with analytical aims was conducted. An exhaustive recruitment of children aged 0 to 120 months hospitalized at the Tambacounda health center for severe malaria (with WHO criteria) between January 1, 2018 and December 31, 2021 was performed. Hospitalization records, hospitalization and treatment registers were the sources of collection. We enrolled 481 patients. The highest number of severe malaria cases was recorded in 2018 (33.1%). Peaks were always observed between October and November. The mean age was 65.6 +/- 29.2 months with a female predominance (53.4%). The majority of people were admitted from the outpatient clinic (57.2%) and 42.8% were referred from a peripheral health post. All patients had a positive rapid diagnostic test and/or a thick drop. The thick drop was negative in 5.2% of cases. Fever (89.9%) and vomiting (42.2%) were the most frequent reasons for consultation. Seizures (47.9%) and severe anemia (50.6%) were the most frequent signs of severity. All patients received an artesunate injection. The cure rate was 81.3%, baseline 10.2% and case fatality 5.0%. There was a statistically significant association between cure and no referral to a health post (OR = 1.85). Similarly, death was associated with the presence of at least 2 signs of severity (OR = 1.81), seizures (OR = 1.51), prostration (OR = 2.77), cardiovascular shock (OR = 6.67), laboratory signs of severity (OR = 3.70), and hypoglycemia (<0.4 g/L) (OR = 5.88). According to the NMCP follow-up grid, management was good in 47.8%, acceptable in 41.8%, and poor in 2.1% of cases. Children hospitalized at the health center for severe malaria with coma, prostration, cardiovascular shock and/or convulsions should be systematically referred to the next level to increase their chances of recovery.

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IMPACTS OF CYCLONE IDAI RELATED INFRASTRUCTURE DAMAGE ON MALARIA INCIDENCE IN SOFALA PROVINCE, MOZAMBIQUE

Calder Glowac1, João L. Ferrão2, Kelly M. Searle1
1University of Minnesota School of Public Health, Minneapolis, MN, United States, 2Consultores Associados de Manica, Chimoio, Mozambique

Increases in the frequency and severity of storms is a hallmark of climate change. Climate change will have impacts on the incidence, prevalence, and geographic range of infectious diseases, particularly malaria and other vector borne diseases. One mechanism for these impacts is through changing the environmental suitability of vector habitats in endemic areas. Another mechanism is through infrastructure damage to households, schools, and healthcare facilities. This infrastructure damage leads to increased risk for interaction with infectious vectors and decreased access to community and health services. We conducted a retrospective study to quantify the infrastructural impacts of Cyclone Idai (2019) in the Sofala Province. As of 2019, Cyclone Idai was the largest tropical storm to make landfall in the southern hemisphere and the range of damage outside of the coastal region is still largely unquantified. We used satellite imagery in Google Earth Pro® to determine the number of schools and healthcare facilities damaged due to Cyclone Idai. We used a database of schools in the central corridor of Mozambique from OpenStreetMap and healthcare facilities from an open-source database of health facilities in sub-Saharan Africa. These databases included 71 schools and 98 healthcare facilities. These databases were supplemented with results from Google Earth Pro® searches. We created a database of damage to these facilities, documenting the severity of the damage (completely destroyed, partially destroyed) and time frame until fully repairing the damage. We spatially joined these to malaria incidence data from 2018 and 2019 to calculate the impact of infrastructure damage in each district on malaria incidence in 2019 and the change in malaria incidence between 2018 and 2019. We also included environmental variables (vegetation, elevation, and river area) and census variables (median age and housing materials) as potential confounders. We found that districts with high infrastructure damage was associated with a lower malaria incidence in 2019 in the aftermath of Cyclone Idai. This is likely due to lack of healthcare access due to this damage.

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UPDATING MALARIA RISK MAP OF KENYA BY PRE-SERVICE DIAGNOSIS OF THE MALARIA ASYMPTOMATIC INDIVIDUALS RECRUITED IN THE KENYA DEFENCE FORCES

Edwin Wachenje Mwakio1, Charles Ekkuttan2, John Lugonza3, Juliana Munyao4, Gladys Chemwori5, Jackline Juma6, Charles Okudo7, Raphael Okoth7, Benjamin Opot7, Philip Njatha7, Dennis Juma7, Hosea M. Akaka7, Kirti Tiwari8, Elly Ojwang9, Timothy Egbo10, Eric Garges11
1Kenya Medical Research Institute, Kisumu, Kenya, 2Kenya Defence Forces, Eldoret, Kenya, 3United States Army Medical Research Directorate-Africa, Kisumu, Kenya

Malaria is among the leading causes of morbidity and mortality globally. The World Health Organization (WHO) Malaria Report 2020 estimates 241 million malaria cases in 2019 leading to 627 000 deaths in 87 malaria-endemic countries with Kenya harbouring 3.5 million (1%) and 107,000 (3%) of this burden, respectively. Over 60% of infections with Plasmodium species generate a malarial parasitaemia in the absence of fever or other acute symptoms, therefore regarded as asymptomatic or chronic malaria. This proportion of infections form a major reservoir for malaria transmission hence need to quantify for effective transmission control. The study aim was to conduct a countrywide testing of asymptomatic malaria burden, stop its dispersion among newly accessioned military forces and map the malaria risk across Kenya. Between 2016 and 2021, we conducted a comprehensive malaria testing and treating of healthy individuals comprising at least one per 4 – 5 Km² land surface of the country, newly enrolled into the Kenya Defence Force (KDF). 12,715 consenting military recruits from 47 counties across Kenya were screened for malaria using both screening and confirmatory test methods between 2016 and 2021; 2,481 (2016), 2,481 (2017), 2,650 (2019), 2,110 (2020) and 3,993 (2021). 490/12715 (3.8%) otherwise healthy individuals tested positive for malaria and were treated. All cases detected were treated with artemether-lumefantrine antimalarial prior to recruit training commencing therefore deterring spread of migrant malaria. Malaria was detected in 46 out of the 47 counties including those where malaria had never been known to occur. Moreover, Marsabit, Baringo, Nakuru and Turkan counties showed 10-fold higher frequency of malaria than previously documented in malaria surveys. Detection of malaria in these otherwise healthy individuals sampled from across the country appear to suggest need to update the overall malaria risk map of Kenya. Successful partnership between the USAMRD-A/KEMRI and KDF demonstrates the value of timely diagnosis and treatment of malaria to prevent the spread of migrant malaria and prevent malaria-related loss time.

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LLIN EVALUATION IN UGANDA PROJECT (LINEUP2) - ASSOCIATION BETWEEN HOUSING CONSTRUCTION AND MALARIA BURDEN IN UGANDA: RESULTS FROM AN OBSERVATIONAL STUDY OF 32 DISTRICTS

Martha J. Nassali1, Samuel Gonahasa2, Catherine Maitieki-Sebuguzi2, Jane F. Namuganga1, Jimmy Opigo2, Daniel Kyabazinge2, Isaiah Nabende3, Jaffer Okiring1, Emmanuel Ainahwe3, Adrienne Epstein1, Katherine Snyman5, Joaniter Nankabinwa1, Grant Dorsey6, Moses R. Kamya2, Sarah Staedke1
1Infectious Diseases Research Collaboration, Kampala, Uganda, 2National Malaria Control Program, Ministry of Health, Kampala, Uganda, 3Directorate of Public Health, Ministry of Health, Kampala, Uganda, 4Liverpool School of Tropical Medicine, Liverpool, United Kingdom, 5London School of Hygiene and Tropical Medicine, Liverpool, United Kingdom, 6Infectious Diseases Research Collaboration, Kampala, Uganda

The Kenya Défense Force (KDF) was associated with a lower malaria incidence in 2019 in the aftermath of Cyclone Idai. This is likely due to lack of healthcare access due to this damage.
Well-built housing limits entry of mosquitoes and could complement other malaria control interventions to reduce malaria transmission. Evidence supporting the role of housing in malaria control is mounting, but few studies have assessed the community-level impact of modern housing on malaria. To investigate associations between improved housing and malaria burden, data from 64 communities in 32 districts across Uganda were analysed, covering approximately 35% of the country. Surveys were conducted between November 2021 and March 2022 in randomly selected households near health facilities with ongoing outpatient malaria surveillance. Houses were classified as ‘improved’ (synthetic walls and roofs, eaves closed or absent) or ‘less-improved’ (all other construction). Outcomes included individual and community-level parasitaemia in children aged 2-10 years and community-level malaria incidence (all ages). Associations between housing and parasitaemia were made using mixed effects logistic regression (individual-level) and multivariable fractional response logistic regression (community-level) and between housing and malaria incidence using multivariable Poisson regression. Overall, 4,893 children aged 2-10 years were enrolled from 3,518 houses (median: 53 houses per site); of these, 1,399 (39.5%) were classified as improved. Children living in improved houses had 58% lower odds (adjusted odds ratio=0.42, 95% CI 0.33-0.53, p<0.0001) of parasitaemia than children living in less-improved houses. Communities with >67% of houses improved had a 63% lower parasite prevalence (adjusted prevalence ratio 0.37, 95% CI 0.19-0.70, p<0.0021) and 60% lower malaria incidence (adjusted incidence rate ratio 0.40, 95% CI 0.36-0.44, p<0.0001) compared to communities with <39% of houses improved. Improved housing was strongly associated with a lower burden of malaria in individual children and communities across a wide range of sites in Uganda and should be utilized as an intervention to control malaria.
patches, habitats and human strata are presented. Using similar values for the parameters, simulations for the two models are run using appropriate software and the results are compared. Data on malaria cases and mosquito populations from 2017-2019 in Uganda will also be used to validate the two models. It is noted that complexity in a compartmental model increases with heterogeneity and stratification and that a state space model easily adapts to the complexity if the modular formulation is used. It is also noted that a compartmental model can be modified into a state space model. There is room for stochasticity in state space models which would give more reliable output as compared to stochasticity in a compartmental model. A comparison of the output results from the two models is done and it is noted that they are comparable though their difference might lie in parameters such as time at risk which is present in the state space model but not explicitly in the compartmental model. Simulation time for the state space model is shorter than for the compartmental model. Therefore, in terms of increasing complexity with heterogeneity and stratifications, compartmental model becomes too complex to represent and analyze effectively while state space models can accommodate more complex phenomena if the modular formulation is used.

A MATHEMATICAL MODEL FOR MICROSTRATIFICATION OF MALARIA INTERVENTIONS IN URBAN NIGERIA.

Laurette Mhlanga1, Eniola Bamgboye1, Manuela Runge1, Cyril Ademu2, Chukwu Okoronkwo2, Perpetua Uhomoibhi2, Monsuru Adeleke3, Ikeoluwope Ajayi3, Jaline Gerardini4, Ifeoma Ozoaiegwu4

1Northwestern University, Chicago, IL, United States, 2National Malaria Elimination Programme, Abuja, Nigeria, 3Osun state University, Osogbo, Nigeria, 4University of Ibadan, Ibadan, Nigeria

Malaria remains a major global health problem, with an estimated 247 million clinical episodes and 619 thousand deaths in 2021. Approximately 92% of these incidence cases and 95% of these deaths were observed in Africa, and Nigeria constituted about 30% and 27% of the clinical episodes and deaths, respectively. Nigeria is rapidly urbanizing and without concomitant increase in amenities and resources for planning, urban areas will see a rise in slums and informal settlements with conditions that favor the proliferation of Anopheles mosquito vectors and increased human exposure to malaria vectors. The resultant heterogeneous transmission may hinder Nigeria’s achievement of the Global Technical Strategy targets to reduce malaria cases and deaths by at least 90% in 2030. In collaboration with Nigeria’s National Malaria Elimination Programme, University of Ibadan, and Osun State University, we are developing an agent-based model using the Epidemiological MODELing software (EMOD) to inform the selection of appropriate intervention strategies for malaria control in two Nigerian cities - Kano and Ibadan metropolis. Our model incorporates data from a mixed-methods field study, currently ongoing in our study locations, and Demographic Health Surveys (DHS), intervention distribution schedules from programmatic data, and intervention effect sizes from the research literature. We captured environmental features that may correspond to subcity heterogeneities in transmission. Each sub-city transmission archetype has the same seasonality. The seasonality is calibrated to the average monthly routine data from the Rapid Impact Assessment surveys (RIA) from 2014 - 2021 and this seasonality was assumed for the three major vector species. We capture baseline transmission intensity using malaria prevalence data from DHS at regional level and the predicted Insecticide Treated Nets (ITNS) usage. The ITNS data is estimated from a spatial model to estimate the ITNs usage in each sub-city transmission archetype using the covariates associated with ITNS use.

HIGH PREVALENCE AND RISK OF SUBPATENT PLASMODIUM FALCIPARUM INFECTIONS IN REGIONS WITH LOW TRANSMISSION INTENSITIES AND THEIR IMPLICATION FOR MALARIA ELIMINATION IN TANZANIA

Misago D. Seth1, Rashid A. Madebe1, Rule B. Mrengela1, Catherine Bakari1, Beatus M. Lymo1, Zachary R. Popkin-Hall2, David Giesbrecht3, Filbert Francis4, Celine I. Mandara1, Dativia Pereus1, Jonathan J. Juliano1, Jeffrey A. Bailey2, Deus S. Ishengoma1

1National Institute for Medical Research (NIMR), Headquarters, Dar es Salaam, Tanzania, United Republic of; 2University of North Carolina, Chapel Hill, NC, United States, 3Brown University, Providence, RI, United States, 4National Institute for Medical Research (NIMR), Tanga Centre, Tanga, Tanzania, United Republic of

Subpatent Plasmodium falciparum infections below the limit of detection of current diagnostic methods (rapid diagnostic tests (RDTs) and microscopy) can potentially contribute to infectious reservoirs that sustain transmission and cause failure of ongoing elimination strategies. This baseline study was conducted in 14 regions of Tanzania with varying malaria transmission to determine the prevalence of and risk factors associated with subpatent P. falciparum infections. The study used 2,685 RDT-negative samples randomly selected from 18,688 dried blood spot (DBS) samples collected in 2021 at 100 health facilities (HFs) in 10 regions and four communities from four additional regions. Parasite DNA was extracted from DBS by tween-chlex method and P18S gene was amplified by quantitative real-time polymerase chain reaction (qPCR). Generalized linear model was used to relate predictors to the risk of subpatent P. falciparum infections. Overall, 524 samples (19.5%) were positive by qPCR, with the positivity ranging from 6.0 to 36.7% across different regions. The median parasite density was 1,030 (IQR=318 – 5,150) copies/µL. Individuals with fever at presentation (axillary temperature ≥ 37.5°C) had reduced risk of subpatent infections by 67% (OR=0.33, 95% CI 0.25-0.45; p < 0.001). Individuals sampled in community surveys had higher risk of subpatent infections than patients enrolled at HFs (OR=3.94, 95% CI 2.60 - 5.97; p < 0.001). Patients from regions with low/very low transmission intensities had significantly higher risk of subpatent infections compared to those sampled from high/moderate transmission regions (OR=2.67, 95% CI 1.87-3.83; p < 0.0001). Age and sex did not significantly affect the risk of subpatent infections. The findings show high prevalence of subpatent infections in 14 regions of Tanzania and higher risk of such infections in low/very low transmission regions that are currently targeted for malaria elimination. Thus, more sensitive detection methods are urgently needed to identify carriers of undetectable parasites to effectively guide targeted intervention strategies to support malaria elimination by 2030.

INCIDENCE RATES OF MALARIA, MENINGITIS AND MORTALITY IN CHILDREN UNDER FIVE YEARS OF AGE IN GHANA AND KENYA PRIOR TO THE ROLL-OUT OF THE MALARIA VACCINE

Patrick Odum Ansa4, The RTS,S Epidemiology EPI-MAL-002 study group

1Navrongo Health Research Centre, Research and Development Division of the Ghana Health Service, Navrongo, Ghana

Although advances have been made in reducing the global burden of malaria, morbidity and mortality due to the disease remain very high, especially among children less than 5 years of age in sub-Saharan Africa. In 2021, the WHO recommended widespread use of the RTS,S/AS01E malaria vaccine for children living in regions with moderate to high Plasmodium falciparum transmission. This recommendation was based on results from the ongoing Malaria Vaccine Implementation Programme (MVIP) that introduced the RTS,S/AS01E vaccine in selected areas of Ghana, Kenya and Malawi in 2019. Within MVIP areas, several studies are
being conducted by the RTS, S epidemiology group to evaluate vaccine safety, effectiveness and impact. As knowledge gaps on background disease incidence rates may hamper this evaluation, the present study (NCT02374450; EPI-MAL-002 study) was designed to assess incidence rates of malaria, meningitis, death and other health outcomes leading to hospitalization in children less than 5 years of age enrolled before the implementation of the RTS, S/AS01E malaria vaccine. The same outcomes will be monitored and compared in the post-vaccine implementation safety and impact study (NCT03855995, EPI-MAL-003 study). Final analysis results of the EPI-MAL-002 study conducted in Ghana and Kenya during 2016–2022 are presented here. This analysis includes 23,601 children who were either followed-up through prospective cohort event monitoring (9,041 children enrolled in the 6–12 weeks and 9,854 in the 5–17 months age groups) or through hospital-based disease surveillance (4,703 children). In the 5–17 months age group, the incidence rates of meningitis and cerebral malaria, within an at-risk period of 1 year after a virtual schedule mimicking RTS, S/AS01E vaccination, were both equal to 28 (95% confidential interval [CI]: 9–65) per 100,000 person-years; the all-cause mortality rate was 643 (95% CI: 531–771) per 100,000 person-years. This is the first study to provide a broad assessment of the burden of disease in children less than 5 years of age in Ghana and Kenya including malaria, meningitis and mortality prior to the roll-out of the malaria vaccine.

6850 USING MORTALITY AUDITS TO IDENTIFY FACTORS INCREASING MALARIA MORTALITY IN KARAMOJA, A HIGH-BURDEN REGION OF UGANDA

Stephen Kigongo1, Derrick Nabongho2, Christine Ludungoko3, Badru G. Walimbwa4, Constance Agwang1, Dorah Taranta2, Edward Mugwanya2, Benjamin Birigwa2, Irene Ochola2, Mariam Bahova3, Asma Qureshi4, Sandra D. Incardona1

1MCD Global Health, Silver Spring, MD, United States, 2John Snow Inc, Boston, MA, United States, 3Asma Qureshi, Sandra D. Incardona1

6851 PREVALENCE OF FALCIPARUM AND NON-FALCIPARUM MALARIA IN THE 2014-15 RWANDA DEMOGRAPHIC HEALTH SURVEY

Claudia Gaither1, Rebecca Kirby2, Corine Karrema3, Sam White1, Hillary Topazian4, David Giesbrecht5, Kyaw Thwai6, Koby Boyten6, Tharsicce Muryameza7, Jean de Dieu Butera8, Jeffrey A. Bailey6, Jean-Baptiste Mazarati9, Jonathan J. Juliano1

1Institute for Global Health and Infectious Diseases, University of North Carolina, Chapel Hill, NC, United States, 2Department of Pathology, Brown University, Providence, RI, United States, 3Quality Equity Health Care, Kigali, Rwanda, 4Imperial College, London, United Kingdom, 5National Reference Laboratory, Rwanda Biomedical Center, Kigali, Rwanda, 6INES-Ruhengeri, Rwanda

Malaria remains a major cause of morbidity in sub-Saharan Africa despite advances in treatment. Most malaria infections are caused by Plasmodium falciparum. However, evidence suggests that non-falciparum malaria are becoming more common as falciparum rates decline. This is important as first-line therapy for falciparum infection is ineffective against the dormant stages of Plasmodium ovale and vivax parasites. We assessed 4,597 individuals in the 2014-2015 Rwanda Demographic Health Survey (DHS) for asymptomatic falciparum and non-falciparum malaria. Using dried blood spots, (DBS) real time PCR for four species of Plasmodium parasites (falciparum, malariae, ovale, and vivax) was done. Samples (n= 2,255) were drawn from 56 clusters with high malaria prevalence (at least 15% positivity for malaria via rapid diagnostic testing). In addition, a random sample of 2,342 DBS from 401 remaining clusters in lower transmission areas were assessed. Of the 4,597 DBS, 1,233 individuals were positive for P. falciparum, 248 for P. ovale, 168 for P. malariae, and 7 for P. vivax. We are currently determining national and regional malaria prevalence estimates for each species and assessing for associations between DHS covariates and malaria infection. While P. falciparum malaria remains the most common form of malaria in Rwanda, P. ovale and P. malariae cause a significant number of asymptomatic infections. A better understanding of the distribution of P. falciparum and non-P. falciparum infection in Rwanda, as well as risk factors associated with infection, will help the national malaria control program target interventions to reach malaria elimination.

6852 UNDERSTANDING MALARIA BEHAVIORAL RISK FACTORS IN SEASONAL MIGRANT WORKERS IN SELECTED MID-HIGHLAND AND LOWLAND DISTRICTS OF NORTHWEST AMHARA REGION, ETHIOPIA

Berhan Tesfay1, Henry Ntuku1, Melkamu Tiruneh1, Adem Agmas1, Asefaw Getachew1, Laura Merriman1, Belay Bezaibi2, Gudissa Assefa1, Hiwot Solomon3, Dereje Dillu1, Asnakew Yeshiwondim1, Gezahegn Tesfaye1, Belendia Serda1, Caterina Guinovart4, Amir Siraj1, Adam Bennett1

1PATH Malaria Control and Elimination Partnership in Africa (MACEPA), Addis Ababa, Ethiopia, 2Amhara National Regional State Health Bureau, Bahir Dar, Ethiopia, 3Ministry of Health, Addis Ababa, Ethiopia, 4PATH Malaria Control and Elimination Partnership in Africa, Barcelona Institute for Global Health, Barcelona, Spain

The Ethiopia Ministry of Health is aiming for countrywide malaria elimination by 2030. However, population movement represents a challenge for the successful implementation of malaria control and elimination activities. Every year, in Amhara Region, close to 500,000 seasonal migrant workers move from the low malaria-risk highlands to the high malaria-risk western lowlands, seeking work on agricultural farms. This population movement is considered one of the key factors contributing to and maintaining malaria transmission in low-transmission areas. To inform the design of targeted intervention strategies, we conducted a qualitative study to better characterize the risk profiles of seasonal migrant workers, including movement patterns, exposure, and behaviors. Focus group discussions (FGD) and key informant interviews (KII), coupled with direct observations of the livelihood of migrant workers were conducted in both lowlands and
Malaria and helminth co-infection is among the global health public burden. Various studies have reported malaria helminth co-infection targeting school-age children in Tanzania. However, there is a paucity of data on malaria and helminth co-infection among pregnant women. This study investigated the co-infection burden of malaria and helminth -infections and risk factors among pregnant women in the Morogoro and Geita regions in Tanzania. A hospital-based cross-sectional study was conducted among 559 consented pregnant women from two Morogoro and Geita regions. A pretested se questionnaire was used to collect demographic data and risk factors. Malaria infection was diagnosed by light microscopy, soil-transmitted helminths (STH) were determined by formal-ether concentration followed by microscopy and Schistosomiasis was detected by filtration techniques followed by microscopy. The determination of malaria and helminth parasite intensity was done using WHO standards. The overall prevalence of malaria was 7.2%, Schistosoma mansoni (2.0%), and Schistosoma haematobium (6.3%) while STH was the most common infection at 53.5%. Overall, malaria and any helminth co-infection prevalence was 7.0%, Pf malaria and STH was 5.9%. However, the majority of malaria and helminth infections were of moderate and light intensity respectively. Multivariate analysis revealed younger pregnant women (18-30 years), having primary or no education had a higher risk of malaria infection. In addition, bed net sharing, farmers, primigravidae women, second-trimester attendants, and those lacking malaria knowledge showed similar high risk. On the other hand, unwashed hands, uncovered pit latrines, soil-eating behavior, fishing, and drinking water from wells were associated with helminth infections. This study showed that malaria and helminth co-infection in Tanzania is substantial among pregnant women. Calling for improved targeted efforts in the provision of health education on the preventive and control measures towards malaria and helminths infections among pregnant women.

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COINFECTION BURDEN AND RISK FACTORS OF MALARIA AND HELMINTH INFECTIONS AMONG PREGNANT WOMEN IN TANZANIA

Felista Walafried Mwingira1, Dennis Massue2, Winfrida Kidima3, Deokary Joseph1
1University of Dar es Salaam- DUCE, Dar es salaam, Tanzania, United Republic of; 2University of Dar es Salaam- MCHAS, Mbeya, Tanzania, United Republic of; 3University of Dar es Salaam, Dar es salaam, Tanzania, United Republic of

Malaria and helminth co-infection is among the global health public burden. Various studies have reported malaria helminth co-infection targeting school-age children in 1 Tanzania. However, there is a paucity of data on malaria and helminth co-infection among pregnant women. This study investigated the co-infection burden of malaria and helminth -infections and risk factors among pregnant women in the Morogoro and Geita regions in Tanzania. A hospital-based cross-sectional study was conducted among 559 consented pregnant women from two Morogoro and Geita regions. A pretested se questionnaire was used to collect demographic data and risk factors. Malaria infection was diagnosed by light microscopy, soil-transmitted helminths (STH) were determined by formal-ether concentration followed by microscopy and Schistosomiasis was detected by filtration techniques followed by microscopy. The determination of malaria and helminth parasite intensity was done using WHO standards. The overall prevalence of malaria was 7.2%, Schistosoma mansoni (2.0%), and Schistosoma haematobium (6.3%) while STH was the most common infection at 53.5%. Overall, malaria and any helminth co-infection prevalence was 7.0%, Pf malaria and STH was 5.9%. However, the majority of malaria and helminth infections were of moderate and light intensity respectively. Multivariate analysis revealed younger pregnant women (18-30 years), having primary or no education had a higher risk of malaria infection. In addition, bed net sharing, farmers, primigravidae women, second-trimester attendants, and those lacking malaria knowledge showed similar high risk. On the other hand, unwashed hands, uncovered pit latrines, soil-eating behavior, fishing, and drinking water from wells were associated with helminth infections. This study showed that malaria and helminth co-infection in Tanzania is substantial among pregnant women. Calling for improved targeted efforts in the provision of health education on the preventive and control measures towards malaria and helminths infections among pregnant women.

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NOVEL METHODS TO ESTIMATE THE LIKELIHOOD OF MIXED-SPECIES INFECTIONS AND RELATIVE SPECIES ABUNDANCE IN MALARIA

Kristan Alexander Schneider1, Kanika Verma2, Douglas J. Perkins3, Praveen K. Bhatt1
1University of Applied Sciences Mittweida, Mittweida, Germany; 2ICMR-National Institute of Malaria Research (ICMR-NIMR), New Delhi, India, 3University of New Mexico Health Sciences Center, Albuquerque, NM, United States

Previously neglected human malaria species are gaining greater importance in endemic areas striving for malaria eradication. Because of hypnozoites, Plasmodium vivax and P. ovale sp. are more resilient than P. falciparum. Evidence of recrudescence from sequestered blood-stage parasites might also render P. malariae a resilient species. Additional challenges are posed by non-human primate transmission reservoirs of P. knowlesi that render the species difficult to control in humans. The occurrence of mixed-species infections has become more evident because of the sensitivity offered by PCR-based diagnostics. However, such infections likely remain undetected by light microscopy since P. falciparum tends to dominate concurrently infecting malaria species. Consequently, light-microscopy results in underestimates of mixed-species infections. Since PCR-based diagnostics are not always available in resource-limited settings, results from light-microscopy can be combined with PCR diagnostics to estimate the distribution of mixed-species infections and the relative abundance of malaria species. As such, we developed a novel maximum-likelihood framework to estimate (i) the distribution of mixed-species infections and (ii) their relative abundance for different sample designs. In a study design in which all P. falciparum(-) infections detected by light microscopy are subjected to PCR, it is possible to derive an explicit maximum-likelihood estimator for the desired quantities. It can be proven that the estimator is asymptotically unbiased, efficient, and consistent. Because it has an explicit form, bias correction can be straightforward. As an illustration, we apply the method to data from India and Kenya. We further discuss generalizations for a variety of sampling designs. The methodology introduced has promising statistical properties to monitor the relative abundance of human malaria species in endemic areas, particularly those aiming for malaria eradication. Importantly, the method per se is also applicable to non-human malaria.

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MALARIA TRANSMISSION IN GOLD MINING AREAS, A CHALLENGE TO OVERCOME IN THE BRAZILIAN AMAZON REGION

Mariana Aschar1, Pacoia Vieira2, Daniel Ward3, Maria de Jesus Costa-Nascimento4, Ronaldo Cesar Borges Gryschek1, Susana Campino5, Silvia M. Di Santi6
1University of Sao Paulo, Sao Paulo, Brazil, 2Health Department, Belém, Brazil, 3London School of Hygiene and Tropical Medicine, London, United Kingdom, 4Health Department, Sao Paulo, Brazil

It is widely known that mining activities lead to deforestation, an increase in Anopheles breeding sites and close contact between humans and vectors, promoting an increase in the malaria burden, as observed in the Brazilian Amazon. The state of Pará showed a 7% increase in malaria cases in 2022. Cases associated to gold mining areas increased by 7%. We collected 238 blood samples and 306 Anopheles in Jacareacanga, a municipality located in the southwest of Pará with an Annual Parasitic Index of 1,101. After informed consent, a form was applied to study participants. Thick blood smear (TBS) showed a positivity of 57%, with 77% diagnosed as Plasmodium vivax, 22% as P. falciparum and one mixed infection. Among 98 samples tested by RDT, 11.2% were positive, with parasitemias ranging from 240-1440 p/µl/microliter. TBS revealed 11.6% of positive samples among those negatives by RDT. All P. falciparum infections were treated with ACTs, according to the Brazilian guidelines. As for treatment for P. vivax, 83% received chloroquine plus primaquine and 15.6% ACTs plus primaquine, due to the short period of time since the last malaria episode
Morbidity and mortality due to malaria are of public health concern in Sub-Saharan Africa (SSA). Household, maternal, paternal and environmental factors have been implicated in high malaria. We determined the prevalence of malaria and associated factors amongst children under five years in SSA using data over a 12-year period. Geospatial covariate data and women's health education is essential.

Prevalence of Malaria Amongst Children Under Five and Associated Factors in Sub-Saharan Africa: A Pooled Analysis Covering 33 Countries, 2000 - 2022

Magdalene Akos Odiko1, Williams Kwarah1, Kwasi Torpey1, Margaret Larkey2, Frances Baaba da-Costa Vroom1, Duah Dwomoh1, Ernest Kenu1, Samuel Bosomprah1

1School of Public Health, Accra, Ghana, 2Korle-Bu Teaching Hospital, Accra, Ghana

Morbidity and mortality due to malaria are of public health concern in Sub-Saharan Africa (SSA). Household, maternal, paternal and environmental factors have been implicated in high malaria. We determined the prevalence of malaria and associated factors amongst children under five years in SSA using data over a 12-year period. Geospatial covariate data and women's health education is essential.

Primaquine (PQ) kills Plasmodium vivax hypnozoites but can cause haemolysis in patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency. We did two systematic reviews: the first used data from clinical trials to determine the spectrum of definitions and frequency of haematological serious adverse events (SAE) related to PQ treatment of vivax malaria. The second used data from prospective studies and case reports to describe the clinical presentation, management and outcome of severe PQ-associated haemolysis necessitating hospitalisation. In the first review, SAEs were reported in 70 of 249 clinical trials. There were 34 haematological SAEs amongst 9,824 patients with vivax malaria treated with PQ, 9 of which necessitated hospitalisation or blood transfusion. Criteria used to define SAEs were diverse. In the second review, 21 of 8,487 articles screened reported 163 patients hospitalised following PQ radical cure; 79.9% (123/154) of whom were prescribed PQ at ≥0.5mg/kg/day. Overall, 101 patients were categorised as having probable or possible severe PQ-associated haemolysis, 96.8% of whom were G6PD deficient (<30% activity). The first symptoms of haemolysis were mostly reported on day 2 or 3 (45.5%) and all patients were hospitalised within 7 days of PQ commencement. 57.9% (77/133) of patients had blood transfusion. Seven (6.3%) patients with probable or possible haemolysis died. Even when G6PD testing is available, enhanced monitoring for haemolysis is warranted following PQ treatment. Clinical review within the first 5 days of treatment may facilitate early detection and management of haemolysis. More robust definitions of severe PQ-associated haemolysis are required.

Trends of Malaria Prevalence and Associated Risk Factors Among School Age Children in Mainland Tanzania: Cross-Sectional Surveys from 2015 - 2021

Frank Chacky1, Susan F. Rumisha2, Patrick G.T. Walker2, Fabrizio Moltinis3, Proper Chaki4, Mbaraka John Remyii5, Sijenunu Aaron1, Samwe1, N. Nhiga1, Joseph T. Hicks3, Naomi Serbantez6, Erik Reeves7, Billy Ngesala8, Bruno Mmbando9, Robert W. Snow10, Jean-Pierre Van Geertruyden11

1Ministry of Health, Dodoma, Tanzania, United Republic of, 2Telethon Kids Institute, Malaria Atlas Project, Western Australia, Australia, 3Imperial College London, London, United Kingdom, 4Swiss Tropical and Public Health Institute, Dar-es-salaam, Tanzania, United Republic of, 5Itsekira Health Institute, Dar es Salaam, Tanzania, United Republic of, 6U.S. President’s Malaria Initiative, U.S. Agency for International Development, 7President’s Malaria Initiative, Dar es Salaam, Tanzania, United Republic of, 8National Institute for Medical Research, Tanga, Tanzania, United Republic of, 9KEMRI-Wellcome Trust Research Programme, Nairobi, Kenya, 10Global Health Institute, University of Antwerp, Antwerp, Belgium

In most countries, including Tanzania, malaria surveillance efforts categorize vulnerable populations as children under-five years and pregnant women. Moreover, as they represent approximately one-third of the general
population and often exhibit a low uptake of insecticide treated nets; schoolchildren play an important role as a plasmodium reservoir and mostly asymptomatic though still have patent infections. Lack of appropriate strategies targeting this age group masks or slow down elimination efforts. The School Malaria Parasitology Survey (SMPs) implemented biennially conducted from 2015 till 2021, covering 890 public primary schools, 241,670 schoolchildren in all 184 councils in 26 regions of mainland Tanzania, was designed to provide understanding on the landscape of malaria profile for school children to guide designing locally-appropriate interventions. This work assessed malaria prevalence trends and associated risk factors in mainland Tanzania. Generalized linear modelling was conducted to determine factors associated with malaria prevalence across survey rounds and temporal trends. We found statistically significant reductions in malaria prevalence in 2017, 2019 and 2021 by 32%, 39%, and 52% respectively compared to baseline (2015). Asymptomatic malaria carriage was associated with age (p=8.10.001) and inversely associated with elevation (p=0.001) whereby schoolchildren living in areas below 750m above sea level (asl) had a malaria prevalence of 23% (2015) which dropped to 12.3% (2021) compared those living in areas above 1750 m asl with malaria prevalence reduced from 4% (2015) to 0.4% (2021). Malaria prevalence was higher among children with a fever history (AOR=1.32, CI: 1.26-1.38, p=0.001) in 2015 and (AOR=1.65, CI:1.47-1.85, P=0.001) in 2021. Sleeping under a mosquito bed net had a protective effect across the four survey rounds in 2015, 2017, 2019, and 2021 with the AOR reduction risk estimated at 33%, 26%, 20%, and 44% respectively against children not sleeping under nets. Our results highlight the benefits of SMPS in providing more granular insight into malaria infection trends by age and region.

**MOLECULAR SPECIATION OF PLASMODIUM FROM THE TWO LARGEST POPULATION CENTERS OF CAMEROON**


1Faculty of Medicine and Pharmaceutical Sciences, University of Douala, Douala, Cameroon. 2Yale School of Medicine, New Haven, CT, United States. 3Yale School of Public Health, New Haven, CT, United States. 4Douala Military Hospital, Douala, Cameroon. 5Douala Military Hospital School of Nursing, Douala, Cameroon. 6Malaria Research Service, Centre Pasteur de Cameroon, Yaoundé, Cameroon. 7Faculty of Medicine and Biomedical Sciences, University of Yaoundé, Yaoundé, Cameroon.

Cameroon is one of 11 WHO-designated high burden to high impact regions in sub-Saharan Africa. Although Plasmodium falciparum is the dominant circulating species, little is known about the prevalence of non-falciparum species due to the poor sensitivity of rapid diagnostic tests and microscopy in detecting minor species. We used molecular methods to speciate the two most populated centers in Cameroon. All four parasites were detected demonstrating the circulation of four major human malaria parasites in the two most populated centers in Cameroon. All four parasites were detected among symptomatic patients at hospitals in Douala and on the island of Manoka. At a single hospital in Yaoundé, all species aside from P. ovale were detected. The presence of non-falciparum malaria, and P. vivax in particular, highlights the need to revisit current diagnostic and treatment algorithms in Cameroon in order to ensure appropriate case management and continue progress towards elimination.

**REDUCTION OF MALARIA CASE INCIDENCE FOLLOWING THE INTRODUCTION OF CLOTHIANIDIN-BASED INDOOR RESIDUAL SPRAYING IN PREVIOUSLY UNSPRAYED DISTRICTS: AN OBSERVATIONAL ANALYSIS USING HEALTH FACILITY REGISTER DATA FROM COTE D’IVOIRE, 2018-2022**

Emily R. Hilton1, Ndombour Gning-Cisse2, Auguste Assi3, Mathieu Eyakou4, John Koffi2, Barthelemy Gnakou2, Bernard Kouassi12, Cecilia Flatley2, Joseph Chabi13, Constant Guy N’Guessan14, Gbalegba2, Serge Alex Aimain2, Colette Yañez Kukasset15, Antoine Mea Tanoh16, Sylvain Koffi N’Gotta17, Francine Octavie Yao18, Hugues Assi Engou19, Philomène Konan20, Kelly Davis21, Edi Constant22, Allison Belemvire23, Patricia Yepsass-Zembrou24, Pascal Zinzindohoue24, Blaise Kouadio25, Sarah Burnett26


Indoor residual spraying (IRS) using neonicotinoid-based products (clothianidin and clothianidin combined with deltamethrin) was deployed for the first time in two districts of Côte d’Ivoire in 2020 and 2021 to complement standard pyrethroid insecticide-treated nets. This retrospective observational study is among the first to assess the impact of neonicotinoid-based IRS on malaria case incidence. Health facility malaria case data were abstracted from registration reports for September 2018-April 2022 in two IRS districts and two control districts that did not receive IRS. Community health worker-reported cases confirmed by rapid diagnostic test were obtained from district reports and DHS2. Health facility missing registers for >3 months each year were excluded. Controlled interrupted time series models were used to estimate the effect of IRS on monthly all-ages population-adjusted confirmed malaria cases and to estimate cases averted by IRS. Models controlled for climate, transmission season, proportion of confirmed cases reported by CHWs, proportion of confirmed out of suspected cases, and non-malaria outpatient visits. The estimated mean annual malaria incidence over 24 months pre-IRS was 308.2 cases per 1,000 population in control areas, and 420.8 in IRS areas. The month after 2020 IRS deployment, incidence decreased by 26.7% (IRR=0.73, 95%CI=0.59-0.92) in IRS areas and increased by 16.1% in control areas (IRR=1.16, 95%CI=0.97-1.39). An estimated 64.1 cases per 1,000 population (95% CI=34.1-104.5) were averted in IRS areas over 12 months post-IRS. After IRS in 2021, incidence in IRS areas immediately decreased by 37.2% (IRR=0.63, 95%CI=0.47-0.84), and increased by 12.6% in control areas (IRR=1.13, 95%CI=0.89-1.42). Over 8 months post-IRS, an estimated 82.4 cases per 1,000 population (95% CI=76.9-86.7) were averted in IRS areas. The difference in incidence change between IRS and control areas was significant both years (P<0.05). Neonicotinoid-based IRS appeared to substantially reduce malaria case rates following campaigns in 2020 and 2021 and remains a priority intervention of the Côte d’Ivoire National Malaria Program.
URBAN AND PERI-URBAN MALARIA: NEW EPIDEMIOLOGICAL LANDSCAPE OF MALARIA TRANSMISSION IN VENEZUELA

David A. Forero-Peña1, María E. Grillet2, Hélbani S. Carrión-Nessi3, Juan C. Gabaldón-Figueira4, Jorge E. Moreno5, Natasha A. Camejo-Avila1, Andrea Maricuto1, Javier Lezaun5

1Biomedical Research and Therapeutic Vaccines Institute, Ciudad Bolivariana, Venezuela, Bolivarian Republic of; 2Vector and Parasite Biology Laboratory, Tropical Ecology and Zoology Institute, Faculty of Sciences, Centro Universitario de Venezuela, Caracas, Venezuela, Bolivarian Republic of; 3Instituto de Salud Global de Barcelona (ISGlobal), Hospital Clinic, University of Barcelona, Barcelona, Spain, 4Centro de Investigaciones de Campo “Dr Francesco Vitanza”, Tumeremo, Venezuela, Bolivarian Republic of, 5Institute for Science Innovation and Society, University of Oxford, Oxford, United Kingdom

Malaria epidemic epicenter in Venezuela is the mining regions south of the Orinoco River, where illegal gold mining and associated deforestation have rapidly increased and expanded over the last decade in southern Venezuela. Recent interventions by international agencies to control this epidemic have focused their efforts on the areas of highest transmission, but local internal migration generated by mining has led to a resurgence of this disease in other urban and peri-urban areas where viable anopheles vector populations exist. We conducted a cross-sectional study with data on diagnosed malaria cases from the main sentinel site of Ciudad Bolívar between 2019-2023. Out of 1,297 patients analyzed, 1,082 (83.4%) had P. vivax, 131 (10.1%) P. falciparum, and 84 (6.5%) had mixed malaria (P. vivax/P. falciparum) infections. A total of 588 (45.3%) cases were related to illegal mining. The main non-mining malaria occupations were heads of household (n = 210, 16.2%), agriculture/livestock (n = 116, 8.9%), and worker (n = 85, 6.6%). The median age was higher in the non-mining group compared to the mining group (33 vs. 30, p = 0.006). In the mining group, there was a higher predominance of men than in the non-mining group (72.3% vs. 51.8%, p < 0.001). The median P. vivax parasitaemia were significantly higher in the mining group compared to the non-mining group (3,700 vs. 3,175, p = 0.001). The probable area of infection in the non-mining group was concentrated mainly in urban and periurban parishes of Angostura del Orinoco municipality (n = 273, 41.9%), such as Marthanta (n = 125, 19.2%). In contrast, the most frequent probable area of infection in the mining group was Sifontes municipality (n = 255, 45.4%), mainly in the Dalla Costa parish (n = 171, 30.4%). Malaria not related to illegal mining represents the majority of the cases studied, therefore, our results provide sound evidence of autochthone malaria transmission in peri-urban and urban areas in southern Venezuela. In the transition towards the elimination of malaria in the Americas, urban or peri-urban malaria can become an epidemiological scenario that is not expected but at the same time difficult to combat.

REDUCING MALARIA CAUSED ABSENTEEISM AMONG ORPHANS AND VULNERABLE CHILDREN IN CAMEROON: A CROSS SECTIONAL STUDY AMONG TEACHERS AND CAREGIVERS

Joseph Lewinski1, Leslie Chingong2, Joel Wanyoike2, Emeka Anjere3, Rachel Laure Nguela1, Akinola Shonde3

1Catholic Relief Services, Baltimore, MD, United States, 2Catholic Relief Services, Yaounde, Cameroon, 3Catholic Relief Services, Abuja, Nigeria

Malaria is a major cause of morbidity and mortality in Cameroon, especially among children. It has a direct impact on efforts to improve education, protection, and socio-economic outcomes among children living with and affected by HIV, their caregivers, and families. Catholic Relief Services (CRS), along with national partners, identified malaria as a leading cause of school absenteeism in efforts to provide educational subsidies to improve educational access, progression, and completion. CRS conducted a baseline cross-sectional study from February 2022 to December 2022 to determine patterns of malaria among OVCs (orphans and vulnerable children) and its impact on school attendance and other proximal factors. The study had two concurrent arms. The first arm of the study enrolled educators and 18 schools (10 public, 5 private religious, and 3 private seculars) located in the same six health districts (catchment areas). Among the 18 schools enrolled, 41% of absenteeism in school was due to suspected malaria cases and 55% of absenteeism lasts between 3-5 days. The study found a positive association between malaria and school absenteeism. Analysis shows that there is a statistically significant relationship between malaria prevalence and school absenteeism among OVCs [OR 6.18, 95% CI 5.79-6.57]. Qualitatively, 89% of teachers witnessed cases of suspected malaria amongst pupils and 83% said it had an impact on educational performance. The second arm of the study enrolled 1,318 children among 524 OVC households and in the same six health districts to determine the proximal impact of malaria and HIV status. Among caregivers of OVCs living with HIV, 69% reported malaria-HIV coinfection worsens illness and 22% reports it affects HIV drug adherence. Adjusted incidence of malaria-caused absenteeism by OVC status will be presented. These results suggest that expansion of OVC-support agents to test for malaria will help further the education subsidies provided to OVCs and the need to and improve coordination with community health workers to provide malaria case management services within schools.

EPIDEMIOLOGICAL CHARACTERISTICS OF MALARIA PARASITES IN SOKOTO STATE, NORTHWEST NIGERIA

Bassey A. Orok1, WELLINGTON OYIBO2, Chimere O. Agomo1, Musa Babalola3

1College of Medicine of the University of Lagos, Nigeria, Lagos, Nigeria, 2Centre for Transdisciplinary Research in Malaria and Neglected Tropical Diseases, College of Medicine of the University of Lagos, Nigeria, LAGOS, Nigeria

Community malaria surveys provide data on malaria epidemiology and dynamics of transmission for monitoring given interventions being deployed. This study was undertaken to determine the epidemiology of malaria in three communities of Sokoto state namely; Doruwa, Kambarna and Kwargaba, Sokoto State, Nigeria. It was a cross-sectional community-based survey involving 689 participants. Malaria parasitaemia was by light microscopy using standard protocol. Participants were of the age group 5-10 years 216 (31.8%), 11-15 years were 124 in number representing 18.3% and >20 years were 227 (33.4%). Age groups 16-20 years, 63 (9.3%) were the least participants in the study. About 51 (7.55%) of 103 (76.3%). P. malariae 4 (3.0%), P. ovale 4 (3.0%), P. falciparum + P. malariae 14 (10.4%), P. falciparum + P. ovale 1 (0.7%). Across the three communities, P. falciparum as the predominant species detected, 103(76.3%). Also, Gender was not statistically associated with malaria (P=0.758; 0.197) although males 62 (20.3%) had more malaria than females 103(76.3%). Malaria is hyper-endemic in these communities requiring concerted interventions for intentional burden reduction.
Effective malaria elimination requires an understanding of how resistance mutations arise, establish, and spread in parasite populations. Drug resistance in the malaria parasite Plasmodium falciparum has recurrently evolved first in Southeast Asia and South America, geographic regions with low transmission. Retrospective genetic analyses found causal mutations on multiple distinct genomic backgrounds in both these regions, suggesting that recurrent de novo mutation may drive resistance emergence. In real time, such repeated mutational events are challenging to detect and track as they evade most genomic surveillance methods. Here, we tailor a selection testing framework to the specific scenario of recurrent de novo mutation. To circumvent the high background relatedness of small, inbred parasite populations, we employ identity-by-descent analysis to define haplotype blocks, track recombination events, and identify targets of both hard and soft sweeps. Once haplotypes are defined, we identify de novo mutations shared among multiple distinct haplotypes. We apply the approach to two whole-genome data sets: recent data from over 800 P. falciparum blood-stage infections sampled from the Guiana Shield of South America and Southeast Asian parasites previously sequenced by the MalariaGEN consortium. In recent decades, both parasite populations experienced strong selection and phenotypic adaptation due to changes in drug usage, diagnostic/treatment rates, and vector habitat availability. We use previously identified resistance-associated alleles (Kelch13 C580Y and CRT C350R) as benchmarks and find additional candidates of recurrent de novo mutation in other resistance-associated genes. Overall, the results support the hypothesis that small Plasmodium populations are not mutation-limited, as the same de novo mutations appear on distinct haplotypic backgrounds. This observation should inform the methods used to monitor, track, and contain emerging drug resistance.

**GENETIC DIVERSITY OF PLASMODIUM VIVAX IN HIGH-RISK MALARIA AREAS IN CORDOBA, COLOMBIA**

Carlos J. Castro, Virginia C. Rodriguez, Maria F. Yasnot, Linda M. Chams

**Universidad de Córdoba, Montería, Colombia**

Plasmodium vivax is the most widely distributed species of human malaria, causing significant morbidity worldwide. The genetic diversity of P. vivax populations is an important factor in the epidemiology, pathogenesis, and transmission of malaria. The Pvmsp-3α gene is one of the most polymorphic genes in P. vivax, and its variability has been used to study the genetic diversity of this species. In this context, PCR-RFLP analysis of the variable region of the Pvmsp-3α gene has emerged as a useful tool to investigate the genetic diversity of natural populations of P. vivax parasites. The genetic diversity of natural populations of P. vivax parasites in areas of high malaria risk in Córdoba-Colombia was studied by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) of the variable region of the Pvmsp-3α gene. A total of 125 whole blood samples were collected on filter paper from patients with P. vivax residing in the municipalities of Tierralta and Puerto Libertador. Samples confirmed as P. vivax were subjected to PCR-RFLP for the Pvmsp-3α gene, the restriction enzymes used were Elu and Hha I. Of the 125 samples analyzed by nested PCR for the Pvmsp-3α gene, 116 amplified successfully, confirming molecularly in these samples the single infection by P. vivax. The size of the PCR products of the Pvmsp-3α gene showed the circulation of three different genotypes, type A (1900 bp), type B (1500 bp) and type C (1100 bp), with genotype A being the most frequent (88%). 97.4% (113/116) of the samples showed single infections and 2.6% (3/116) polyclonal infections, one by type A and C and two by types A and B. Digestion of the PCR products of the Pvmsp-3α gene showed 10 different restriction enzyme digestion patterns and 9 with the Hha I enzyme. The results of the enzymatic restriction of the 113 samples analyzed revealed that 40/113 (35.3%) and 47/113 (41.6%) of these samples showed polyclonal infections when digested with Alu I and Hha I enzyme, respectively. The Pvmsp-3α gene exhibited high polymorphism and the results suggest that this gene can be used in Colombia as an epidemiological molecular marker for P. vivax genotyping.
GENOME-WIDE SINGLE NUCLEOTIDE POLYMORPHISM (SNP) ANALYSIS OF PLASMODIUM FALCIPARUM DRUG RESISTANCE-ASSOCIATED LOCI IN AREAS OF DIFFERENT MALARIA ENDEMICITY IN TANZANIA

Beatus Lyimo1, Celine Mandara1, Misago Seth1, Rashid Madobe1, Catherine Bakari1, Dativa Pereus1, David Giesbrecht2, Zachary Popkin-Hall3, Jonathan Juliano43, Jeffrey Bailey2, Deus Ishengoma1

1National Institute for Medical Research, Dar es salaam, Tanzania, United Republic of; 2Brown University, Providence, RI, United States; 3University of North Carolina, Chapel Hill, NC, United States

Genetic diversity of Plasmodium falciparum drives its ability to adapt environment changes, develop resistance to antimalarial drugs, and evade the host immune response; these enable the parasite to continue to persist despite massive intervention. Understanding the P. falciparum genetic diversity across populations of different malaria transmission is critical for the implementation of new strategies to eliminate malaria in Tanzania. A total of 300 samples were collected between May 2014 and January 2015 from three districts of Tanzania (Muheza – a low, and Muleba and Nachingwea – both high transmission areas) as part of the MalariaGEN Community Project. High-quality SNPs retained after quality checks were analyzed for the complexity of infections, population structure, and signatures of selection. The complexity of infections was different across the study population, ranging from 0.2 (most polygenomic) to 1.0 (monogenic). Based on principal component and identity by state analyses, P. falciparum populations clustered into a single cluster with no significant population structure. Genome-wide analysis showed a signature of differential selection on positions of genes for drug resistance-associated loci. The highest density of selection was observed in chromosome 7 around the chloroquine resistance transporter (PfCRT) locus in all populations. While highest density for dihydrofolate reductase (Pfdhfr) and dihydropteroate synthase (Pfdhps) loci around chromosomes 4 and 8 respectively were observed in Muheza and Muleba compared to Nachingwea. The signature of selection was also observed in the immune-related locus Pftrap around chromosome 13 of P. falciparum from Muheza and Muleba. These results suggest that drugs and immune pressure are dominant selective forces against Pfalciparum in Tanzania but their effect on the parasite genome varies geographically. Thus, interventions interacting with these genome variants need to be monitored as malaria elimination strategies are implemented.

IMPACT OF PLASMODIUM FALCIPARUM INFECTION ON DNA METHYLATION OF CIRCULATING IMMUNE CELLS

Dareen Almojil
New York University Abu Dhabi, Abu Dhabi, United Arab Emirates

Epigenetic modifications are known to regulate cell phenotypes during the course of infection. However, it remains largely unknown whether epigenetic modifications play a role in the host immune response in human malaria. In this study, we investigate the dynamics of genome-wide in vivo DNA methylation profiles of 66 children in Burkina Faso, West Africa, sampled longitudinally before infection, during symptomatic Plasmodium infection, and after malaria treatment (n = 189 genome-wide DNA methylation profiles). Temporal analysis of the data revealed major and statistically significant changes in the epigenetic profiles of children in response both to infection and treatment and identified differentially methylated probes and regions (FDR<0.01). The analysis revealed a widespread hypomethylation of Cpgs upon infection that revert back to before infection state following malaria treatment. The significant methylation changes observed implicate the parasite to continue to persist despite massive intervention. Understanding the P. falciparum genetic diversity across populations of different malaria transmission is critical for the implementation of new strategies to eliminate malaria in Tanzania. A total of 300 samples were collected between May 2014 and January 2015 from three districts of Tanzania (Muheza – a low, and Muleba and Nachingwea – both high transmission areas) as part of the MalariaGEN Community Project. High-quality SNPs retained after quality checks were analyzed for the complexity of infections, population structure, and signatures of selection. The complexity of infections was different across the study population, ranging from 0.2 (most polygenomic) to 1.0 (monogenic). Based on principal component and identity by state analyses, P. falciparum populations clustered into a single cluster with no significant population structure. Genome-wide analysis showed a signature of differential selection on positions of genes for drug resistance-associated loci. The highest density of selection was observed in chromosome 7 around the chloroquine resistance transporter (PfCRT) locus in all populations. While highest density for dihydrofolate reductase (Pfdhfr) and dihydropteroate synthase (Pfdhps) loci around chromosomes 4 and 8 respectively were observed in Muheza and Muleba compared to Nachingwea. The signature of selection was also observed in the immune-related locus Pftrap around chromosome 13 of P. falciparum from Muheza and Muleba. These results suggest that drugs and immune pressure are dominant selective forces against Pfalciparum in Tanzania but their effect on the parasite genome varies geographically. Thus, interventions interacting with these genome variants need to be monitored as malaria elimination strategies are implemented.

GENETIC DIVERSITY OF PLASMODIUM FALCIPARUM RETICULOCYTE BINDING HOMOLOGUE-5 (PFHRH5) IN REGIONS OF DIFFERENT MALARIA TRANSMISSION IN TANZANIA

Angelia Julius, Beatus Lyimo, Deus Ishengoma, Dativa Pereus, Celine Mandara, Ruth Boniface, Misago Seth
National institute for medical research, Dar es salaam, Tanzania, United Republic of

Recently, the Plasmodium falciparum reticulocyte binding-like protein homologue family (PFHRH) specifically the pfhrh5 gene has been the focus of vaccine development as it is a key determinant of erythrocyte invasion. However, little is known about the extent of genetic variability of pfhrh5 gene in P. falciparum isolates from areas of varying transmission intensity. This study assessed the genetic diversity of pfhrh5 gene by testing the hypothesis that the pfhrh5 gene locus is conserved in regions of different malaria endemicity in Tanzania. A total of 300 whole genome sequences generated from samples collected between May 2013 and January 2014 from three districts in Tanzania (160 in Muheza, 61 in Muleba and 79 in Nachingwea) were retrieved from MalariaGEN project Database. Bioinformatic tools were used to study within-host diversity, evidence of natural selection, population differentiation and structure. Results showed high within-host diversity (Fst<0.95) in Muleba (42.6%) compared to Nachingwea (39.2%) and Muheza (36.9%). Nucleotide diversity in the pfhrh5 gene was high in Muleba (0.00102) compared to Muheza (0.00096) and Nachingwea (0.00094). No evidence of genetic diversity in the pfhrh5 gene was observed...
across all the districts (Fst mean = 0.00238). Tajma's D analysis was done to look for signatures of selection and showed direction selection in both Muheza (-1.055) and Nachingwea (-0.676) while Muleba showed neutrality (Tjd= 0.151). More analysis is under way to map the diversity of the pfhr5 gene in all the districts. The preliminary results reveal low levels of genetic variation in the pfhr5 gene across the districts, these results correspond to the findings from previous studies conducted in 2020 in Nigeria. However, a broader investigation is required in some other parts of the country to support the potential of pursuing pfhr5 gene as a malaria vaccine antigen.

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POPULATION GENOMICS OF PLASMODIUM FALCIPARUM AND MALARIA CONTROL: IMPLICATIONS IN ABIDJAN (CÔTE D’IVOIRE)

Desire N Ehouni1, Abibatou Konate1, Steven G Nyanjom2, Amadou Ouattara3, William Yavo1
1Malaria Research and Control Center, NIPH, ABIDJAN, Côte d’Ivoire, 2Department of Biochemistry, College of Health Sciences, Jomo Kenyatta University of Agriculture and Technology, NAROKI, Kenya. 3Malaria Research Program, Center for Vaccine Development and Global Health, University of Maryland School of Medicine, BALTIMORE, MD, United States

The onset of Plasmodium falciparum (P. falciparum) resistance to antimalarial drugs requires careful surveillance of African parasite population. Genomics tools are implemented to detect evolutionary changes that could impact on malaria control and elimination strategies. Here, we evaluate the genome-wide pattern of selection and sequence variation of P. falciparum populations in Abidjan, Côte d’Ivoire. The study was conducted in three localities of Abidjan from 2013 to 2014. We collected 70 blood samples following a written informed consent from patients above two years of age. After extracting P. falciparum and human DNA from isolates, we performed Whole Genome Sequencing and used population genomics approaches to investigate genetic diversity, complexity of infection and identify loci under positive directional selection. We observed an excess of rare variants in the population showing a clear mutation process in the isolates. Moderate Fst estimates (0.3) was detected for surfin, an immune invasion gene family. Seven iHS regions that had at least two SNPs with a score greater than 3.2 were identified. These regions code for genes that have been under strong directional selection. Two of these genes were the chloroquine resistance transporter (ctr) on chromosome 7 and the dihydropteroate reductase (dhp5) on chromosome 8. Our analyses showed a recent selective sweep in the erythrocyte membrane protein (Pfempr1). In conclusion, our analyses identified genes under selective drug pressure and balancing selection on protective immune-specific genes. These findings demonstrate the effectiveness of genomics analyses to follow malaria parasite evolution of parasite and adopt appropriate strategies to eliminate malaria in Côte d’Ivoire.

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ASSESSING TRANSMISSION DYNAMICS AND RELATEDNESS OF PLASMODIUM FALCIPARUM ON BIOKO ISLAND, EQUATORIAL GUINEA

Thomas C. Stabler1, Ankit Dwivedi2, Olivier T. Donfack3, Carlos A. Guerra2, Guillermo A. Garcia1, Claudia Daubenberger1, Joana C. Silva2
1Swiss Tropical and Public Health Institute, Allschwil, Switzerland, 2Institute for Genome Sciences, Baltimore, MD, United States, 3Medical Care Development Global Health, Malabo, Equatorial Guinea, 4Medical Care Development Global Health, Silver Spring, MD, United States

In 2019, the Bioko Island Malaria Elimination Project (BIMEP) conducted their annual malaria indicator survey on Bioko Island (BI), Equatorial Guinea, revealing 13.4% malaria prevalence by RDT. The challenge facing BIMEP as BI approaches pre-elimination, is defining the sources that contribute to the persistence of malaria in the island. To this end, we are investigating transmission dynamics of Plasmodium falciparum (Pf), the predominant malaria species on BI, both within BI and between BI and continental Africa. Dried blood spot samples from participants with reported fever and a positive RDT for Pf were selected for selective whole genome amplification and sequencing (n=74). Utilizing a variety of population genetics metrics and analyses, including nucleotide diversity (π), FST, admixture and identity-by-descent (IBD), the genetic diversity and population structure of the BI parasite population were compared to continental African countries. Initial results show BI parasites cluster with, and have similar ancestral background to, samples from its geographical neighbor Cameroon. This further supports previous epidemiological evidence of malaria importation to BI via human migration, and the observation of mixing between island and continental strains, despite a geographical barrier. Next, to determine whether Pf in BI forms a panmictic population, relatedness between BI samples was measured using IBD to characterize on-island transmission dynamics. Overall, BI samples appeared mostly unrelated (average IBD = 0.003). However, stratification by urban and rural communities revealed some differentiation (FST = 0.03) and higher IBD among rural infections (average IBD = 0.008) than among urban (average IBD = 0.002) consistent with a partially structured population. If progress continues, rural communities may be amenable to elimination interventions without the fear of constant reseeding from urban environments. Further investigation using epidemiological and genetic data will be utilized to provide greater detail of BI transmission among epidemiological subgroups within the context of malaria control management.

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PLASMODIUM FALCIPARUM POPULATION STRUCTURE IN SOUTHWESTERN AFRICA, USING WHOLE GENOME SEQUENCE DATA: INITIAL GENOME-WIDE SEQUENCE DATA FROM ANGOLA

Wilson Tavares1, Ankit Dwivedi2, Thomas Stabler2, Samyukta Rao3, José Martins4, Filomeno Fortes1, Ana Paula Arez1, Joana Morais5, Joana Carneiro da Silva6
1Global Health and Tropical Medicine, GHTM, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa, UNL, Lisboa, Portugal, 2Institute for Genome Sciences, University of Maryland School of Medicine, Baltimore, Maryland, USA, 3Medical Care Development Global Health, Malabo, Equatorial Guinea, 4Programa Nacional de Controlo da Malária, PNCM, Luanda, Angola, 5Instituto Nacional de Investigação em Saúde, INIS, Luanda, Angola, 6Department of Microbiology and Immunology, University of Maryland School of Medicine, Baltimore, MD, United States

Malaria continues to be the principal cause of morbidity and mortality in Angola, primarily due to Plasmodium falciparum (Pf) infection. Angola ranked 9th worldwide in number of malaria deaths in 2021 but the distribution of disease is remarkably heterogeneous, with provinces varying between <1% and >50% malaria prevalence. Despite malaria's heavy toll on public health in Angola, Pf population genetic questions, parasite DNA was isolated from 150 dried blood spots collected in 2022, and subjected to selective whole genome amplification, and sequencing in an Illumina NovaSeq 6000 platform. The sequencing data was mapped against the P. falciparum reference genome, single nucleotide polymorphisms (SNPs) were identified according to best practices, and joint SNPs calling was done together with WGS data from several hundred publicly available Pf samples from East, West and Central Africa, as well as Brazil and French Guiana. A Principal Component Analysis (PCA) done on the SNP calls revealed that Pf samples from Angola cluster with others from Central Africa. Admixture analyses are still ongoing, to determine the extent to which the ancestry of the Angolan Pf population differs from those of neighboring countries. In addition, average multiplicity of infection and overall nucleotide diversity will be estimated for each province, and...
population differentiation between provinces will be estimated with Wright's fixation index (FST). To test isolation by distance, FST will be compared with geographic distance using a Mantel test.

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**SEQUENCE POLYMORPHISMS IN THE PF547 6-CYSTEINE PROTEIN IN PLASMODIUM FALCIPARUM ISOLATES FROM ANGOLA, 2019**

Marko Bajic, Julia Kelley, Sophie Allen, Piper Shifflett, José F. Martins, Ana L. Cándido, Filomeno de Jesus Fortes, Mateusz M. Plincinski, Eldin Talundzic

1Centers for Disease Control and Prevention, Atlanta, GA, United States, 2Emory University, Atlanta, GA, United States, 3Programa Nacional de Controle de Malária, Luanda, Angola, 4Instituto Nacional de Investigação em Saúde, Luanda, Angola, 5Institute of Hygiene and Tropical Medicine, Nova University of Lisbon, Lisbon, Portugal, 6U.S. President's Malaria Initiative, Malaria Branch, US Centers for Disease Control and Prevention, Atlanta, GA, United States

The survival and transmission of the Plasmodium falciparum parasite is dependent on the gene encoding the 6-cysteine protein Pf547 (PF3D7_1346800) that plays a crucial role in the parasite avoiding the mosquito vector's immune system. Allelic variants of the Pf547 sequence constitute haplotypes that vary globally and are adapted to evade the immune system of specific Anopheles mosquitoes found in each region. We utilized a targeted amplicon deep sequencing (TADS) protocol to evaluate the highly polymorphic Pf547 gene for its ability to determine the geographic origin of P. falciparum. A total of 56 samples from three provinces in Angola (11 from Benguela, 14 from Lunda Sul, and 31 from Zaire) were individually sequenced and evaluated for single nucleotide polymorphisms (SNPs) in Pf547. From these, 52 samples (11 from Benguela, 14 from Lunda Sul, and 27 from Zaire) had sufficient alignments to Pf547 (PF3D7_1346800) and were evaluated further. There was a total of 12 haplotypes observed in Angola made up of 11 unique amino acid changes. Two haplotypes were exclusively found in Zaire, two in Lunda Sul, and one in Benguela. Major SNPs, those with a weighted allele frequency of 25% or more, were P194H, N272I, E188D, E27D, and P669H. Apart from E27D (Domain 1) and P669H (Domain 3), the other three polymorphisms occur in Domain 2 of Pf547. Interestingly, E27D is most prevalent in Lunda Sul, E188D is most prevalent in Zaire, and N272I is more prevalent in Zaire and Benguela than in Lunda Sul. Finally, we utilized 535 publicly available consensus sequences for Pf547 (99 from Asia, 59 from Americas, and 377 from Africa) and compared them with our Angola samples. Through hierarchical clustering, the representative haplotype for Angola is most similar to haplotypes found in Western African countries, as expected. This evaluation strengthened the observation that E27D and N272I are distinctly African SNPs. Although Pf547 haplotypes can discern the geographic origin of P. falciparum among Africa, Asia, and the Americas, more power is needed to evaluate whether Pf547 polymorphisms can discern parasite origin among countries or provinces/regions.

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**DRUG RESISTANCE PROFILE OF PLASMODIUM FALCIPARUM IN THE COMMUNITIES OF CONDORCANQUI, AMAZONAS, PERU**

Julio Sandoval-Bances, Milagros Saevedra-Samiliani, Luis M. Rojas, Carmen I. Gutierrez, Rafael Tapia-Limonchi, Stella M. Chenet

1Instituto de Enfermedades Tropicales, Universidad Nacional Toribio Rodríguez de Mendoza, Chachapoyas, Peru, 2Laboratorio Referencial de Salud Pública Amazonas, Chachapoyas, Peru, 3Instituto de Investigaciones en Ciencias Biomédicas, Universidad Ricardo Palma, Lima, Peru

Malaria is a serious health problem in the native communities of Condorcanqui in the Amazonas region of Peru, reporting a 2.5-fold increase in the number of cases since 2019. Resistance to antimalarial drugs hampers malaria control and elimination; suspected resistance to artemisinin, chloroquine, sulfadoxine, pyrimethamine, and mefloquine in Plasmodium falciparum can be explored by analyzing polymorphisms in the PFK13, Pfcrt, Pfmdhps, Pfmdhr, and Pfmdr1 genes, respectively. In this study, P. falciparum positive cases, collected during 2019 to 2022, from native communities of Condorcanqui were evaluated. Genomic DNA was isolated from fifty-one blood samples on filter paper, collected from 2019 to 2022 and species confirmation was performed by real-time PCR. Polymorphisms of PFK13, Pfcrt, Pfmdhps, Pfmdhr and Pfmdr1 genes were analyzed by nested PCR followed by Sanger sequencing. Electropherograms were analyzed in Geneious Prime and then compared to the 3D7 reference sequence obtained from the NCBI database. All samples had the same genotype, carrying mutant alleles for Pfcrt (C72S and K76T), Pfmdhfr (A16V, C50R, N51I and S108N/T), Pfmdhp (A437G, K540E, A581G) and Pfmdr1 (Y86N and Y184F). However, no mutations were found in the PFK13 propeller domain. These results are consistent with a recent clonal expansion, due to a P. falciparum outbreak reported in the area in 2019. Continued surveillance of polymorphisms associated with antimalarial resistance is recommended to guide the formulation of rational drug policies and the mitigation of risk of P. falciparum artemisinin resistance.
A MORE EFFECTIVE MULTIPLICITY OF INFECTION: INCORPORATING WITHIN-HOST RELATEDNESS AND GENOTYPING ERROR TO OBTAIN MORE ACCURATE ESTIMATES OF PLASMODIUM WITHIN-HOST DIVERSITY AND POPULATION ALLELE FREQUENCIES

Maxwell Murphy1, Bryan Greenhouse2
1Division of Biostatistics, University of California, Berkeley, Berkeley, CA, United States, 2Department of Medicine, Department of Medicine, University of California, San Francisco, San Francisco, CA, United States

Analysis of Plasmodium genetic data is complicated by the unique biology of malaria where multiple genetically distinct strains may be present in a single infection. Naïve approaches to estimating allele frequencies without accounting for the number of distinct infecting strains, known as multiplicity of infection (MOI), and genotyping error, may result in biased estimates. Undercounting strain contribution leads to overestimating and underestimating rare and common allele frequencies respectively, biasing downstream metrics that rely on these estimates. Analysis of malaria also often assumes a panmictic population, however the phenomena of co-transmission, where multiple strains within a single individual are transmitted by a single mosquito, results in highly related progeny in downstream infections. This within-host relatedness can bias estimates of MOI that assume infecting parasites are independent and complicates the concept of MOI. For example, the presence of multiple unrelated strains suggests a different epidemiological origin compared to multiply highly related strains, despite having the same MOI. To address these issues, we developed a Bayesian approach for the simultaneous estimation of individual level MOI, within-host relatedness, and population allele frequencies from polyclonal data subject to genotyping error. We introduce a new concept of “effective MOI” (eMOI), a continuous metric that captures the interplay of MOI and within host relatedness. Using a high diversity amplicon sequencing panel, we are able to accurately recover eMOI as high as 12 from simulations with genotyping error, even in the absence of any within sample relatedness where eMOI = MOI. We applied our approach to previously collected data from northeastern Namibia containing 2585 samples from 29 clinics across 4 health districts genotyped at 26 microsatellite loci, revealing substantial within host relatedness (median eMOI = 1.55 (IQR: .26), median MOI = 5.16 (IQR: 3.46), median relatedness = 0.77 (IQR: .26)). Estimated MOI was lower when not allowing for relatedness (median MOI = 1.0263 (IQR: 1.40)).

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STRUCTURAL AND FUNCTIONAL ANNOTATION OF A UNIQUE HYPOTHETICAL PHOSPHATASE OF PLASMODIUM VIVAX

Madison Tarallo, Ashley Valenzuela, Amy Ibarra, Kevin Cruz, Lizbeth Cruz, Christopher Campbell
AdventHealth University, Orlando, FL, United States

The protozoan parasite Plasmodium vivax is one of the causative agents leading to life threatening malaria cases worldwide. Infection with P. vivax can lead to severe and fatal infections, contributing to significant global morbidity and mortality. Due to increasing drug resistance, conventional antimalarial medicines are losing their efficacy, necessitating the urgent need for alternative and more potent antimalarial medications or vaccines. Recent breakthroughs in sequencing technologies provide vital information about the parasite’s entire genome to aid in the discovery of novel drugs or the development of vaccines, but much more must be uncovered due to its insufficient proteome annotation. Conserved hypothetical proteins with unknown functions pose a unique challenge in understanding the complex lifecycle of these parasites. Computational analysis provides a general prediction of biochemical function establishing a foundation for further direct experimentation. Specifically, domain identification and subsequent homology modeling allow us to develop a greater understanding of the potential roles of these proteins in signal transduction and stage-specific gene expression during intraerythrocytic development. In this study, a putative characterization of the hypothetical protein PVX_090120 evaluated its potential as a target for future chemotherapeutic strategies.

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BUZZWORTHY: NOVEL HIVE SEQUENCING TECHNOLOGY MAKES SINGLE-CELL SEQUENCING POSSIBLE FOR MALARIA FIELD ISOLATES

Erim Saufe, Pieter Guetens, Pieter Monsieurs, Johanna Helena Kattenberg, Anna Rosanas-Urgell
Institute of Tropical Medicine Antwerp, Antwerp, Belgium

While single-cell sequencing has been revolutionary for molecular biology and understanding gene expression of infectious diseases, its application to Plasmodium has been limited due to a lack of single-cell preservation options compatible with collecting Plasmodium field isolates. Until now, preservation methods for single-cell sequencing have utilized probes only designed for the human transcriptome or rely on cryopreservation which results in the loss of fragile Plasmodium life stages. Here we apply HIVE single-cell sequencing to Plasmodium parasites, a new technology that separates the sample capture and processing into two steps that can be performed at different locations. The cell capture can be completed in low-resource field settings to isolate single Plasmodium parasites in individual wells where they are preserved for transportation to a lab facility for processing and library preparation. We optimized sample preparation and processing using P. knowlesi and P. falciparum in vitro cultures to simulate capturing P. vivax and P. falciparum isolates in the field. The initial sequencing analysis shows that we recovered 1000+ cells and identified clear biomarkers for our cell clusters. More detailed analysis is ongoing and we will present how these preserved gene expression profiles compare to the publicly available Malaria Cell Atlas data in this proof-of-concept study. This new single-cell sequencing technology can transform Plasmodium research by making it possible to perform single-cell studies on field isolates. This is of particular importance for species that cannot be cultured in vitro, such as P. vivax, and for minority subpopulations often missed by bulk RNAseq. Studies will also be able to investigate gene expression profiles of individual parasites in heterogeneous populations including mixed life stages and polyclonal infections, which will lead to advancements in Plasmodium biological systems such as drug resistance and invasion.

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ASSESSING THE INTERACTION BETWEEN NATURALLY-ACQUIRED PFCSP-SPECIFIC HUMORAL IMMUNITY AND THE PROTECTIVE EFFICACY OF THE ANTI-MALARIAL MONOCLONAL ANTIBODY CIS43LS

Hamidou Cisse1, Hyeseon Cho1, Jeff Skinner1, Youngsul Seo1, Shanping Li1, Mary Peterson1, Cherrelle Dacon2, Azza H. Idris3, Aisssata Ongoioba4, Safiatou Dourbon4, Kassoum Kayentao4, Boubacar Traore4, Joshua Tan2, Robert A. Seder5, Peter D. Crompton1
1Malaria Infection Biology and Immunity Section, Laboratory of Immunogenetics, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States, 2Antibody Biology Unit, Laboratory of Immunogenetics, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States, 3Vaccine Research Center, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States, 4Mali International Center of Excellence in Research, University of Sciences, Techniques and Technologies of Bamako, Bamako, Mali

CIS43LS is an antimalarial monoclonal antibody that targets a conserved junctional epitope on the Plasmodium falciparum circumsporozoite protein (PfCSP). We recently reported that a single 40 mg/kg dose of CIS43LS given intravenously to adults before the 6-month malaria season in Mali provided 88.2% protective efficacy against P. falciparum infection. It is important to understand whether baseline levels of naturally acquired PfCSP-specific antibodies impact CIS43LS efficacy, and conversely, whether CIS43LS impacts the subsequent PfCSP-specific antibody
response during malaria transmission. Moreover, it is important to investigate how GCS43LS-mediated protection from blood-stage infection effects levels of pre-existing blood-stage-specific antibodies. To address this, we are measuring levels of PICSP- and blood-stage-specific antibodies in serum collected from study participants (N=330 adults) at baseline before GCS43LS/placebo administration, 84 and 168 days later. We are performing bead-based, multiplexed analysis of various antibody isotypes IgG1-4, IgA, IgM specific for full length PICSP and various components thereof (N-term, NAP repeat region, C-term, and junctional peptides) as well as the blood-stage antigens including PFMSP1. The relationship between pre-existing antibody levels and GCS43LS efficacy will be determined, and naturally acquired antibody responses to PICSP and blood-stage antigens in those who received GCS43LS, or placebo will be compared. This analysis may provide insight into whether GCS43LS efficacy varies with background levels of PICSP-specific humoral immunity (i.e., vary with age and malaria transmission intensity) and the extent to which naturally acquired humoral immunity to the pre-erythrocytic and erythrocytic stages of P. falciparum might be impacted by GCS43LS.

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THE PROTECTIVE ROLE OF MATERNALLY DERIVED ANTIBODIES AGAINST SYMPTOMATIC MALARIA IN THE FIRST YEAR OF LIFE

Nicholas Zehner1, Isaac Ssewanyana2, Anthony Kyimba2, Erick Okek2, Abel Kakuru4, Chris Drakely2, Kevin Tetteh3, James Beeson4, Harriet Adrama2, Teddy Andra2, Richard Kajubi2, Melissa Conrady2, Felistas Nankya2, Tamara D. Clark4, Bryan Greenhouse4, Moses Kamya2, Isabel Rodriguez-Barraquer5, Grant Dorsey5, Prasanna Jagannathan4

1Stanford University, Stanford, CA, United States, 2Infectious Disease Research Collaboration, Kampala, Uganda, 3London School of Hygiene & Tropical Medicine, London, United Kingdom, 4Burnet Institute, Melbourne, Australia, 5UCSF, San Francisco, CA, United States

While Plasmodium falciparum infection during pregnancy is common in endemic settings, there is significant uncertainty as to how transplacental malaria exposure and neonatal antibody repertoire at the time of birth affect the incidence, symptomatology, and severity of malaria infections in the first year of life. To interrogate these questions, which may provide new avenues for novel therapeutics, we enrolled a birth cohort of children from Busia, Uganda, an area with very high and perennial malaria transmission, and followed them through one year of age. Mothers were enrolled during pregnancy, and at the time of birth, cord blood samples were collected from 678 infants. Both mothers and children received all care at a study clinic, including artesiminin-based therapy for smear-positive cases of malaria, and followed them through one year of age. Mothers were enrolled during pregnancy, and at the time of birth, cord blood samples were collected from 678 infants. Both mothers and children received all care at a study clinic, including artesiminin-based therapy for smear-positive cases of malaria, and followed them through one year of age. Mothers were enrolled during pregnancy, and at the time of birth, cord blood samples were collected from 678 infants. Both mothers and children received all care at a study clinic, including artesiminin-based therapy for smear-positive cases of malaria, and followed them through one year of age.

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DENGUE AND MALARIA IMMUNE CROSS-TALK:
UNDERSTANDING THE IMPORTANCE OF CO-INFECTIONS IN ENDEMIC REGIONS

Rosa I. Gálvez1, E. Alexandar Escarrega1, Christina Descherrmeier1, Thomas Jacobs2, Daniela Weiskopf1

1Center of Infectious Disease and Vaccine Research, La Jolla Institute for Immunology, La Jolla, CA, United States, 2Diagnoses Development Laboratory, Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany, 3Protozoa Immunology, Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany

40 % of the world’s population lives in the tropics, areas characterized by poverty, but also by the presence of multiple pathogens. This is a special challenge for the immune system which deals with multiple infections, often at the same time. Although theoretically there is a strong geographic overlap between Malaria and Dengue Fever, there is scarce data on the interaction of these two diseases. The interruption of Malaria transmission has led to malaria-free areas in Asia and Latin America and these areas report increasing Dengue fever outbreaks over time. In contrast, sub-Saharan Africa is still a Malaria hyper-endemic area where Dengue fever incidence is largely underdetermined, and there is no scientific data that can explain the widespread absence of Dengue fever outbreaks. Our study aims to understand the interaction between these two infections in samples from a pediatric cohort in Ghana. We first determine Dengue Virus incidence using a newly developed serological test for endemic areas, allowing high specificity for different Flaviviruses. We performed a phenotypical analysis of Dengue-specific T cells in the context of previous or acute Malaria infection using high content flow cytometry. This is to our knowledge the first study addressing this immunological crosstalk. Furthermore, we shed light on how the history of previous and/or concurrent infections shapes the immune response and thereby influences disease outcomes in real-life settings.

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T CELL RESPONSES AGAINST LIVER STAGE PLASMODIUM FALCIPARUM ANTIGENS IN UGANDAN CHILDREN EXPOSED TO MALARIA

Gonzalo R. Acevedo1, Sophie Samiee2, Mikias Ilala3, Justine Levan4, Meagan Olive5, Mary Prah1, John Rek6, Emmanuel Arnaitwe7, Mary K. Muhindo7, Abel Kakuru4, Moses R. Kamya4, Grant Dorsey5, Margaret E. Feeney1

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

Experimental vaccine models show that the CD4+ T cell response against liver stage (LS) Plasmodium is critically important for immunity against reinfection. However, the identity of the relevant antigens and epitopes targeted by these cells remains largely unknown. In addition, this protection is partially abrogated by blood stage (BS) infection. CD4+ T cells feasibly play a role in this phenomenon, given their ability to regulate and polarize other effectors of immunity. We aimed to (1) identify the targets of the CD4+ immune response at the LS; and (2) analyze the size and phenotype of circulating T cell subsets with liver homing properties in infants with diverging histories of malaria exposure. To overcome the data gap on antigen expression in LS P. falciparum, we used a data mining approach to prioritize parasite genes on the basis of their stage-selective expression in other Plasmodium species. We selected 11 LS and 3 BS candidate antigens, and HLA-DRB1 binding peptides were predicted on conserved segments of their encoded proteins. Seventy-eight peptides were tested for T cell recognition using PBMC from malaria-exposed infants recruited for the PRISM study. In vitro T cell expansion revealed an IFN-γ response against sequences from all BS and at least 5 LS antigens in multiple donos. In parallel, we defined a set of phenotypic markers based on their reported relevance for liver homing and implication in protective anti-malarial T cell responses. These were combined with lineage, memory, and subset markers in a spectral flow cytometry panel, which we used to characterize circulating T cell subsets in Ugandan infants. Preliminary characterization

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of specimens from the Tororo Child Cohort study showed significant differences in the populations defined by these markers within the dβ and yβ T cell compartments associated with diverging levels of malaria exposure (>35 episodes vs ≤2 episodes since recruitment). We are currently extending this characterization to infants in the PROMOTE BC1 study, who underwent intermittent or continuous chemoprevention affecting primarily BS parasites.

**OFF-TARGET ANTIBODY RESPONSES INDUCED BY RTS,S/AS02A/1B IN MALARIA-NAÍVE ADULTS ASSOCIATED WITH PROTECTION AGAINST CONTROLLED HUMAN MALARIA INFECTION**

DeAnna J. Friedman-Klabanoff1, Travis L. Jensen2, Casey E. Gelber1, Johannes B. Golf1, Richard Pinapati1, John C. Tam1, Gregory A. Deys1, Jason A. Regules2, Elke S. Bergmann-Leitner3, Matthew B. Laurens1, Mark A. Travassos1, Shannon Takala-Harrison1, Andrea A. Berry1

1Center for Vaccine Development and Global Health, University of Maryland School of Medicine, Baltimore, MD, United States, 2Biomedical Data Science and Bioinformatics Department, The Emmes Company LLC, Rockville, MD, United States, 3Nimble Therapeutics, Inc., Madison, WI, United States, 4Parasitology and International Programs Branch, Division of Microbiology and Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States, 5Walter Reed Army Institute of Research, Silver Spring, MD, United States

The RTS,S vaccine targets the circumsporozoite protein (CSP), which coats the Plasmodium falciparum sporozoite surface. Although some studies have demonstrated an association between anti-CSP repeat region IgG antibody titers and vaccine-induced protection from RTS,S, the relationship does not seem to be linear or suggest an absolute protective threshold. New data suggests that off-target/cross-reactive antibody responses may also be associated with protection. We examined IgG responses in malaria-naïve adults vaccinated with RTS,S who were either protected (n=18) or unprotected (n=17) against controlled human malaria infection to examine the association between off-target responses and protection. Baseline and post-vaccination sera were probed on a pre-erythrocytic peptide array with representation of diverse variants of 127 proteins previously associated with vaccine-induced or naturally acquired protective immunity as 16-amino acid peptides overlapping by 15. Protected adults were seropositive for more peptide variants in regions of CLAMP, GSK3, DOCC2 and MSPP5 compared to unprotected adults. Protected adults also had higher reactivity than unprotected adults to peptide variants in regions of CLAMP, GSK3, DOCC2, LRR9, and MSPP5, with some peptides of CLAMP, GSK3, and MSPP5 being targets of both increased seropositivity and reactivity. BLAST between areas of differential responses and CSP identified sequence similarities between a CLAMP peptide and the CSP central repeat region and a CLAMP peptide and a DOCC2 peptide. These sequence similarities were present in sera from both protected and unprotected adults. The findings support emerging data that off-target antibody responses induced by RTS,S may be important for protection, either through direct action or as an indicator of higher quality CSP antibodies. Further work to isolate cross-reactive antibodies and examine their binding and function is warranted.

**ROLE OF MALARIA AND EPSTEIN-BARR VIRUS IN PEDIATRIC CANCER DEVELOPMENT IN MALAWIAN CHILDREN**

Gabriela Samayoa-Reyes1, Elshafa M. Ahmed2, Corner Jackson3, Robert Basiocchi2, Robert Newton3, Rosemary Rochford1

1University of Colorado, Denver, CO, United States, 2The Ohio State University, Columbus, OH, United States, 3University of York, York, United Kingdom

Endemic Burkitt Lymphoma (eBL), an aggressive B-cell non-Hodgkin lymphoma, is the most common childhood cancer in sub-Saharan Africa (SSA), with a peak occurrence at 7-years of age. Its distribution has been linked to chronic and intense holoendemic Plasmodium falciparum malaria transmission. Epstein Barr virus (EBV) is also a known cofactor for eBL development; both malaria and EBV infection explain the geographic concentration of eBL in malaria-endemic regions where EBV is ubiquitous. We wanted to investigate if children with eBL have a higher proportion of EBV infection. Additionally, among the children with an EBV infection there is a difference in EBV viral load between children with eBL and children with other malignancies. Lastly, is there an effect of sex or age on EBV viral load. Participants were part of a childhood cancer study conducted at the main hospital in Blantyre, Malawi, between July 2005 and July 2006. All 572 children aged 15 years or younger with a provisional diagnosis of cancer admitted to the pediatric oncology ward in Blantyre were recruited. Cancer diagnosis was based on clinical presentation and confirmed by histology and cytology. Only HIV-negative children were included as part of this study. EBV DNA was quantified by measuring the BALF5 gene using quantitative polymerase chain reaction (qPCR). We found a significant relationship between cancer type and EBV positivity, where children that developed eBL are more likely to have detectable levels of EBV than children with other cancers (p-value < 0.01). Of the children with detectable virus, 223 children with eBL and 141 children with other cancers, we found that children with eBL had a significantly higher EBV viral load (p-value < 0.01). Next, we wanted to determine if there was an effect of age or sex on EBV viral load, both in children that developed eBL as well as children with other cancers. When looking at the effect of age on EBV viral load we found a significant effect of age with viral load (p-value = 0.04). Together these results suggest that EBV viral load is directly correlated to eBL development furthermore, increased age is associated with increased EBV viral load.

**OPSONIC PHAGOCYTOSIS OF PLASMODIUM FALCIPARUM MEROZOITES IS ASSOCIATED WITH PROTECTION FROM CLINICAL MALARIA IN AN AREA OF LOW AND UNSTABLE MALARIA TRANSMISSION**

Elui O. Odhiambo1, George Ayodo2, Chandy C. John3

1Department of Microbiology and Immunology, Indiana University School of Medicine, Indianapolis, IN, United States, 2Jaramogi Oginga Odinga University of Science and Technology, Bondo, Kenya, 3Ryan White Center for Pediatric Infectious Diseases & Global Health, Indiana University School of Medicine, Indianapolis, IN, United States

Immunoglobulin G (IgG) antibodies to Plasmodium falciparum (Pf) antigens are known to be important in protection from clinical malaria, but immune correlates of protection from clinical malaria are still sub-optimally characterized. Most characterization has been done in areas of moderate to high malaria transmission, but data is limited for areas of low transmission due to a need for prolonged evaluation periods. It is unclear if immune correlates of protection are identical in areas of high vs. low malaria transmissions. In areas of high malaria transmission, studies suggest that antibody-dependent cellular phagocytosis (opsonic phagocytosis, OPN) strongly correlates with protective immunity, but this has not been investigated in areas of low malaria transmission. In a highland area of low and unstable malaria transmission in western Kenya, we conducted a nested case-control study in which we collected plasma samples from 5753 individuals in 2007, and followed these individuals through 2017
for the development of clinical malaria. Individuals who developed clinical malaria were defined as cases and compared to age- and village-matched controls who did not develop clinical malaria. OPA of merozoites was higher in cases (30.9%, n=56) than controls (28.6%, n=56, p=0.051). The data suggest that the OPA of merozoites may be an immune correlate of long-term protection in low malaria transmission areas. Future studies will assess OPA of merozoites and selected Pf antigens in a larger sample of cases and controls to determine specific antigen targets for OPA and examine whether OPA is a better immune correlate of protection from clinical malaria in low malaria transmission settings than IgG, IgG1, or IgG3 antibody levels to Pf antigens

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QUANTIFYING THE EFFECT OF NUMBER OF INFECTIONS ON MALARIA IMMUNITY

Cassia Wagner1, Jared Honeycutt2, Sophia Maxfield2, Saki Takahashii, Joaniter Nankabirwa4, Abel Kakuru4, Mary Muhindo4, John Rek4, Jessica Briggs2, Moses Kamya4, Grant Dorsey2, Prasanna Jagannathan5, Trevor Bedford2, Isabel Rodriguez-Barrarquii, Bryan Greenhousei

1University of Washington, Seattle, WA, United States, 2University of California San Francisco, San Francisco, CA, United States, 3Johns Hopkins University, Baltimore, MD, United States, 4Infectious Diseases Research Collaboration, Kampala, Uganda, 5Stanford University, Palo Alto, CA, United States

Naturally acquired immunity provides near complete protection from symptomatic falciparum malaria, yet the mechanisms underlying its development remain poorly understood. Increasing age and transmission intensity are associated with multiple measures of anti-disease and anti-parasite immunity, including lower parasite densities, lower probability of symptomatic malaria, and higher fever thresholds. It is unknown to what extent these trends can be explained by cumulative exposure versus other factors, such as parasites’ genetic diversity, complexity of infection (COI), and age-dependent immunity. In this study, we aim to quantify the relationships between number of unique infections, COI, age, annual entomological inoculation rate (eIIR) and anti-disease and anti-parasite immunity. We performed amplicon sequencing of AMA1 in longitudinal blood samples from 158 individuals in birth cohorts in Uganda collected via active and passive detection. Individuals ranged from 1mo to 10yrs of age, and were enrolled in the study for 3.6 years on average. eIIRs during the study ranged from 30-1000 infectious bites per year. We successfully genotyped 889 samples, 6 samples per individual on average, representing 89% of a person’s symptomatic malaria episodes. Haplotypes per sample ranged from 1-11 (avg=2). This relatively low value is consistent with frequent, symptomatic infections cleared by treatment. From genotyping, we can infer unique infections per individual and apply a mixed effects model to quantify the impact of number of infections and predictors, like COI, age, and eIIR, on measures of immunity, including probability of symptomatic malaria, parasite density, and fever threshold. Preliminary results indicate that higher eIIR and lower age are associated with higher probability of symptomatic disease as previously observed. In symptomatic infections, higher COI is associated with lower parasite densities, or fever thresholds, while, in asymptomatic infections, higher COI is associated with higher parasite density. This work provides an important foundation to future studies on the development of P. falciparum immunity.

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ITERACTIVE SUBTRACTIVE BIOPANNING OF A PLASMODIUM FALCIPARUM SEXUAL STAGE (I-III) PHAGE DISPLAY LIBRARY IDENTIFIES A POTENTIAL TARGET OF ANTI-GAMETOCYTE IMMUNE RESPONSES


Brown University, Providence, RI, United States

Decades of malaria research have identified highly promising vaccine candidate antigens capable of eliciting protective immune responses at multiple malaria life-cycle stages. These include antigens such as PICSP (pre-erythrocytic stage), PfRh5 (blood-stage), and Pfsl230 (classical transmission-blocking activity in the mosquito mid-gut). Additional antigens however are likely to be discovered to augment combinatorial vaccines that target multiple life cycle stages. Gametocytes, the asexual form of the parasite responsible for transmission from human to mosquito vector, may also allow opportunities for intervention. To identify sexual stage antigens capable of eliciting by gametocyte controlling immune responses, we have performed a whole proteome differential screen of a Plasmodium falciparum phage display constructed from early stage gametocytes (Stages I-III). Employing an iterative subtractive bio-panning approach in conjunction with plasma previously collected from a western Kenya treatment-reinfection study (n=143), we performed out initial screen with highly matched pools (n=10/each) of resistant plasma (low gametocytemia[R]) and susceptible plasma (high gametocytemia[S]), matched for a host of variables including asexual parasitemia, age, Hgb, % HgbAS trait, exposure, etc. to identify 12 clones uniquely recognized by RP but not SP. Next, we performed a whole cohort validation to determine whether or not antibody titers to each antigen measured 2 weeks post treatment but prior to reinfection in all members of the cohort, predicted gametocyte indices over the 18 weeks of follow-up. In multivariate linear regression and generalized estimating equation models, antibody titers were significantly inversely correlated with gametocytemia (P ≤ 0.04), in numerous outcomes. In silico analysis of this predicted large 300kD protein, identified a Plasmapain IX cleavage site, indicating a potential nphagy organelle secreted protein. In conclusion, we have a identified a novel antigen, potentially capable of eliciting gametocyte controlling immune responses.

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CYTOMEGALOVIRUS VECTOR PROLONGS LIVE MALARIA VACCINE IMMUNITY THROUGH INNATE AND ADAPTIVE MECHANISMS

Komi Gbedande1, Sarrad A. Ibitokou2, Monique L. Ong2, Mariapia A. Degli-Esposti3, Michael G. Brown1, Robin Stephens1

1Rutgers New Jersey Medical School, Newark, NJ, United States, 2Department of Internal Medicine, Division of Infectious Diseases, University of Texas Medical Branch, Galveston, TX, United States, 3Centre for Experimental Immunology, Lions Eye Institute, Nedlands, Western Australia, Australia, 4Infection and Immunity Program and Department of Microbiology, Biomedicine Discovery Institute, Monash University, Clayton, Victoria, Australia

Immunity to Plasmodium infection or vaccination is known to decay. In mouse models, this decay correlates with loss of parasite-specific T cells, not antibody. Whole live parasite vaccines, such as “infection and drug cure”, protect. However, sterile protection from live P. chabaudi vaccination lasts less than 200 days. Persistent infection itself protects, and generates both effector (Teff,) and effector memory T cells (Tem). Both CD4+ Teff and Tem contribute to protection, as we recently showed for Plasmodium infection. Cytomegalovirus (CMV) is a promising chronic vaccine vector that can induce sustained T cell responses. CMV vectors contribute to protection against SIV, tuberculosis and liver-stage malaria through CD8 T cells. As CMV promotes Tem and Teff, we tested murine CMV encoding P. chabaudi MSP-1 epitope B5 (MCMV-B5), as a booster to prolong live vaccine-induced protection from P. chabaudi infection. The
Opoka4, Keisuke Kawata5, Dibyadyuti Datta1, Chandy John1
Dorsey5, Margaret Feeney5, Will Greenleaf1, Prasanna Jagannathan1

into mechanisms driving incomplete acquisition of natural antimalarial following malaria exposure. All together, this work provides needed insight and -extrinsic mechanisms contributing to reduced V T cell function are in progress. In parallel, we established in vitro stimulation with CRISPR knock outs to confirm the role of target genes in modulating Vδ T cell function. Experiments using HAVCR2, TIGIT). Footprinting analysis further identified distinct transcription factor activities associating with malaria incidence. Experiments using ATAC-Seq and RNA-Seq. By using multiple samples from children (n=20) enrolled in longitudinal cohorts in Tororo, Uganda, δ T cell chromatin accessibility and immune function associates with prior malaria incidence.

Kathleen Dantzler Press1, Sandy Klemm1, Midhuna I. Joseph Maran2, Fabian Müller2, Derek Chen3, John Rek4, Felistas Nankya3, Isaac Ssewanyana3, Moses Kamya4, Bryan Greenhouse3, Grant Dorsey3, Margaret Feeney3, Will Greenleaf4, Prasanna Jagannathan1

1Stanford University, Stanford, CA, United States, 2Saarland University, Saarbrücken, Germany, 3Infectious Disease Research Collaboration, Kampala, Uganda, *Makerere University College of Health Sciences, Kampala, Uganda, 4University of California at San Francisco, San Francisco, CA, United States

Repeated malaria infection results in children in malaria-endemic regions gaining the ability to tolerate parasitemia without symptoms. One mechanism driving immune tolerance and the shift towards asymptomatic parasitemia is the attenuation of cytotoxic, pro-inflammatory responses by innate immune cells. Aiming to better understand the processes underlying changes in Vβ2+ T cell function, we analyzed Vβ2+ T cells isolated from children (n=20) enrolled in longitudinal cohorts in Tororo, Uganda, by paired ATAC-Seq and RNA-Seq. By using multiple samples from the same children, including before and after a district-wide insecticide campaign that dramatically reduced malaria transmission, we could assess differential chromatin accessibility and transcription associating with year, prior incidence of clinical malaria, or exposure to infected mosquitoes. We identified differential sites enriched for genes involved with immune signaling (e.g. IL-19, CD8, CXCR6, STAT1) and regulation (e.g. BCL2, KLRC1, HAVCR2, TIGIT). Footprinting analysis further identified distinct transcription factor activities associating with malaria incidence. Experiments using CRISPR knock outs to confirm the role of target genes in modulating Vβ2+ T cell function are in progress. In parallel, we established in vitro stimulation assays to simulate the in vivo context; results support both cell-intrinsic and -extrinsic mechanisms contributing to reduced Vβ2+ T cell responsiveness following malaria exposure. All together, this work provides needed insight into mechanisms driving incomplete acquisition of natural antimalarial immunity and could be applied to novel therapeutics targeting innate immune responses.

ELEVATED LEVELS OF CEREBROSPINAL FLUID NEURON-SPECIFIC ENOLASE ARE ASSOCIATED WITH LONG-TERM NEUROLOGIC AND COGNITIVE IMPAIRMENT IN CHILDREN WITH CEREBRAL MALARIA

Alejandro Soto1, Adnan Gopinadhan2, Paul Bangirana2, Robert Opoka3, Keisuke Kawata1, Dibyadyuti Datta1, Chandy John1

1Indiana University School of Medicine, Ryan White Center for Pediatric Infectious Disease and Global Health, Indianapolis, IN, United States, 2Indiana University School of Medicine, Department of Microbiology and Immunology, Indianapolis, IN, United States, *Makerere University College of Health Sciences, Department of Paediatrics and Child Health, Kampala, Uganda, 3Indiana University School of Medicine, Center for Global Health, Bloomington, IN, United States

Cerebrospinal fluid neuron-specific enolase (CSF NSE) is a known biomarker of adult and pediatric neurologic disorders, including stroke and encephalopathy. In severe malaria, one study with a small sample size has investigated CSF NSE in adults and none in children. We evaluated admission CSF NSE levels in 144 children, 1.5-12 years of age with cerebral malaria (CM), and examined associations with blood-brain barrier (BBB) disruption measured using CSF to plasma albumin index, and neurologic deficits (ND) and cognitive impairment (NCI) tested at discharge, 6, 12, and 24-months follow-up. Mean (SD) CSF NSE levels in children with CM (5.01 (7.67) ng/mL) were significantly higher than CSF NSE levels in children in two cohorts of children without neurologic symptoms (1.52 (1.01) and 2.12 (0.30) ng/mL, N=37 and 32, P<0.001 for both comparisons). CSF NSE levels were elevated in children with CM and BBB impairment (n=39, median 4.22 ng/mL [95% CI 2.16, 5.47]) compared to 103 children with CM and no BBB impairment (median 2.19 [95% CI 1.43, 4.40], P<0.002). Children with CM and persistent ND at 12 and 24-month follow-up had elevated admission CSF NSE compared to children with CM and no persistent ND (all P<0.04). The predictive value of admission CSF NSE for ND increased over time with AUC of 0.59, 0.70, 0.77, and 0.86, at discharge, 6, 12, and 24-months. CSF NSE levels were also associated with worse z-scores for working memory over 24 months follow-up in children who were <5 years of age at CM episode and at follow-up cognitive testing (β [95% CI] -0.26 [-0.47, -0.06], P=0.01), and with worse z-scores for overall cognitive impairment over 24 months follow-up in children who were <5 years of age at CM episode but >5 years at follow-up testing (β [95% CI] -1.13 [-1.93, -0.32], P=0.007). Elevated levels of the neuronal injury marker CSF NSE at admission are associated with persistent neurologic and cognitive impairment in pediatric CM.

ELEVATED RENIN PREDICTS MORTALITY IN CHILDREN WITH SEVERE MALARIA

Daniel Adan1, Ruth Namazzi2, Anthony Batte3, Robert Opoka2, Chandey John1, Andrea Conroy4

1Indiana University School of Medicine, Ryan White Center for Pediatric Infectious Disease and Global Health, Indianapolis, IN, United States, 2Makerere University College of Health Sciences, Department of Paediatrics and Child Health, Kampala, Uganda, 3Makerere University College of Health Sciences, Child Health and Development Centre, Kampala, Uganda, 4Indiana University School of Medicine, Center for Global Health, Indianapolis, IN, United States

Renin has been identified as a biomarker of acute kidney injury (AKI) in critical illness. In the present study, we assessed plasma renin levels in children with severe malaria (SM) from a two-site prospective cohort study and their association with inpatient mortality and endothelial and immune activation. Plasma renin was measured in enrollment samples from 594 Ugandan children with SM and 120 age-matched community children using Luminex bead assay. Community children renin levels were used to establish a population reference with the 99th percentile as the cut-off for elevated renin. The mean age of children was 2.1 years and 44.3% were female. Children with SM had substantially higher renin levels than community children, with 26.9% of children having elevated renin. Children with elevated renin had a mortality rate of 8.9%, corresponding to a 5.4-fold increased odds (95% CI, 2.8 - 10.4, p<0.0001) of mortality compared to children without elevated renin. 62.8% of children that died had elevated renin (p<0.0001). Furthermore, 117 children (19.7%) had severe (stage 2/3) AKI. Among the 10.9% of children that had both elevated renin and severe AKI, there was a 10-fold increase in mortality (29.2%) compared to when neither condition was present (2.9%). Clinical complications associated with elevated renin included signs of poor tissue perfusion (acidosis) and kidney injury (severe AKI, elevated BUN, blackwater fever). To further delineate the role of renin in malaria pathogenesis, we evaluated multiple pathways of organ injury as well as endothelial and immune activation. As elevated...
renin was strongly associated with severe AKI, we stratified models by AKI to evaluate renin-specific pathways. Children that had elevated renin without severe AKI showed evidence of endothelial activation and an increase in selected markers of kidney injury and hemolysis. In conclusion, renin is elevated in children with SM and strongly associated with mortality, particularly when it occurs concurrently with AKI. Additional studies are needed to understand whether interventions targeting renin could be beneficial in severe malaria.

ASSOCIATION OF XANTHINE OXIDASE LEVELS AND DEVELOPMENT OF SEVERE MALARIAL ANEMIA

Marilyn Vasquez1, Margaux Sica1, Roth Namazzi2, Robert Opoku3, Julian Sherman4, Dibyadyuti Datta4, Miquel Duran-Frigola4, John Ssenkusu5, Andrea Conroy3, Ana Rodriguez1

1NYU School of Medicine - Viscic Institute of Graduate Biomedical Sciences, New York, NY, United States, 2Department of Pediatrics, Makerere University College of Health Sciences, Kampala, Uganda, 3Ryan White Center for Pediatric Infectious Disease and Global Health, Indiana University School of Medicine, Indianapolis, IN, United States, 4Ersilia Open Source Initiative, Cambridge, United Kingdom, 5Department of Epidemiology and Biostatistics, Makerere University School of Public Health, Kampala, Uganda

Malaria is a potentially fatal infectious disease caused by Plasmodium parasites, with most casualties occurring in children under the age of 5 infected with P. falciparum. P. falciparum infection can manifest with a variety of different complications, one of which is severe malarial anemia (SMA). SMA is caused primarily by the loss of uninfected red blood cells (RBCs). Although the mechanisms underlying the loss of uninfected RBCs during Plasmodium infection are not yet fully understood, oxidative stress has been proposed as a potential contributor to SMA. To further investigate the contribution of oxidative stress to the development of this complication, we quantified the levels of xanthine oxidase (XO), an oxidative enzyme that is upregulated during malaria, in serum samples from a cohort of 552 Ugandan children with severe P. falciparum malaria. We found that patients with SMA had significantly higher levels of XO activity in circulation, compared to patients with non-SMA severe malaria. Moreover, we observed a significant negative correlation between XO levels and RBC hemoglobin, suggesting a potential role for this enzyme in the development of SMA. XO levels did not correlate with serum levels of immune complexes or anti-phosphatidylserine antibodies, two factors that contribute to SMA. This suggests that XO may contribute to the development of this complication independently of these previously established mechanisms. In future experiments we will further clarify the role of XO in the development of SMA using a murine model of malarial anemia, where mice will be treated with allopurinol, an inhibitor of this enzyme. Lastly, we also observed that XO is associated with other severe manifestations of malaria including acidosis and kidney injury, among others. Since patients with severe malaria can present with multiple complications, and inhibitors of XO are affordable and already approved for use in humans, corroborating the role of XO as a causative agent for SMA and other malarial complications may facilitate the use of XO inhibitors as a potential novel treatment for these life-threatening complications.

LONGITUDINAL CLONAL PARASITE DYNAMICS FOLLOWING ARTEMETHER-LUMEFANTRINE TREATMENT FOR MALARIA IN HIV-INFECTED AND HIV-UNINFECTED CHILDREN IN UGANDA

Justin Goodwin1, Richard Kajubi2, Martina Wade1, Francis Orukan3, Moses Whalen4, Francesca T. Aweke2, Norah Mwebaze5, Sunil Parikh1

1Yale School of Medicine and Public Health, New Haven, CT, United States, 2Infectious Disease Research Collaboration, Kampaala, Uganda, 3University of California San Francisco, San Francisco, CA, United States

Sub-Saharan Africa has seen continent-wide shifts in susceptibility patterns to artemisinin-based combination therapy (ACT) partner drugs and now faces emerging artemisinin resistance. High transmission settings require consideration of post-treatment parasite dynamics and the role of waning drug exposure and multicolonial infections in the selection and spread of resistance. In the context of a 42-day study of 3 versus 5-day artemether-lumefantrine (AL), we used 18S and SBP1 RT-PCR alongside amplicon-sequencing (amp-seq) to distinguish post-treatment clonal dynamics. Weekly samples were analyzed from 76 HIV-infected and 227 HIV-uninfected Ugandan children in a high-transmission setting. 18S RT-PCR revealed a dramatic submicroscopic parasite burden after AL, with nearly 70-75% of children having detectable 18S RNA throughout 42-day follow-up. The ring-stage marker, SBP1, was notably positive in 10% and 17% of HIV-uninfected children at 7 and 14 days post-treatment, respectively. We performed amp-seq of clone, spp., and clp to distinguish and quantify parasite clones at a relative abundance of 0.1%. Clone diversity was high, with a multiplicity of infection (MOI) of 4.3 prior to AL treatment that notably increased upon recurrent parasitemia (day 28 MOI 5.2). Our dataset revealed a pattern of clonal persistence following therapy with ring-stages detectable for up to two weeks after AL. Finally, stratification by HIV status demonstrated that the prevalence and density of ring-stage parasites was significantly lower over the course of follow-up in HIV-infected children, which we attribute to daily trimethoprim-sulfamethoxazole prophylaxis. Our next step will be to assess the relationship between clonal persistence/re-infection and lumefantrine levels, available from this same cohort. Our data represents the first use of amp-seq paired with molecular parasite density and drug exposure data to study longitudinal post-treatment clonal dynamics and represents the largest and longest follow-up of ring-stage specific RNA following AL in Africa. Such novel data are critical as we strive to extend the useful therapeutic life of ACTs.

INVESTIGATING MICROBIOME AND TIGHT JUNCTION INTEGRITY IN THE GUT DURING PLASMODIUM KNOWLESI INFECTION IN MACAQUES

Ttryphena Adams1, Noelle G. Allen1, Ryan M. Kelly1, Mariko S. Peterson2, Chester J. Joyner3, Mah-HPIC Consortium4, Rabindra Tirovanziam5, Alberto Moreno2, Sanjeev Gumber2, Mary R. Galinski5, Regina Joice Cordy1

1Wake Forest University, Winston Salem, NC, United States, 2Emory University, Atlanta, GA, United States, 3University of Georgia, Atlanta, GA, United States

Gastrointestinal illness is one of the more common manifestations of infection with Plasmodium falciparum and P. knowlesi. The involvement of the gut during malaria and how it enhances the severity of the disease is key to understanding overall malaria pathogenesis, yet uncertainty exists regarding these mechanisms. Previous work in murine models has demonstrated that inflammation in the gut during malaria is associated with disruptions to the microbiome and gut barrier integrity. Limited information exists, however, in terms of whether similar phenomena occur in human and non-human primates. Recent work by our group has demonstrated elevated levels of mucosal microbes in rectal swabs collected from rhesus macaques at the acute phase of malaria infection with P. cynomolgus. To validate that this finding also occurs in a model that exhibits sequestration in
the gut tissue and pathology of the gastrointestinal system, we performed a follow-up study using *P. knowlesi*, which exhibits cytoadherence through the expression of the SICAvar gene family and is known to cause gut pathology. To investigate whether similar microbiome changes were seen in this context, we performed shotgun metagenomics of rectal swabs from macaques infected with *P. knowlesi*. Here, we detected an enrichment of mucosal microbes, specifically Helicobacter macacae, during the acute phase of the *P. knowlesi* infection compared to pre-infection. This result mirrored that which was seen in our *P. cynomolgi* study. To further investigate the gut, we performed immunohistochemistry on colon tissue from these same animals and labeled tight junction proteins Claudin-5 and Occludin, which regulate intercellular permeability in the gut epithelium. These studies revealed reduced expression of tight junction proteins in colon samples collected from the acute phase of the infection, suggesting that there may also be a reduction in gut barrier integrity alongside the disruption of the gut microbiome. The study adds to a broader goal to comprehend the mechanisms underlying gastrointestinal pathology and the role of gut microbiota in malaria pathogenesis.

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THE SEVERE MALARIA TRIUMVIRATE: AN INVESTIGATION OF ABO BLOOD GROUP, ROSETTE FORMATION, AND PFEMP1 TYPE

William A. Cromwell, Iset Vera, Kami Kim
University of South Florida, Tampa, FL, United States

Malaria kills approximately 430,000 people every year, mostly children under 5 years old in sub-Saharan Africa. Many of these lethal cases are due to cerebral malaria (CM), a condition caused by Plasmodium falciparum and characterized by cerebral inflammation, blood-brain barrier (BBB) breakdown, brain swelling and death. The exact mechanisms that lead to CM are unknown. Rosetting is a phenomenon occurring in malaria infections in which an infected red blood cell (RBC) is surrounded by non-infected RBCs, bound by surface ligands. Rosetting has been associated in previous studies with CM severity. ABO blood group may also be related to CM development with non-O blood group patients being more likely to develop cerebral malaria, although studies have been contradictory on this topic. Further, previous studies have shown an association between non-O blood groups and rosetting. Plasmodium falciparum erythrocyte membrane protein 1 (PFEMP-1) variants have binding domains specific to rosetting phenotypes. PFEMP-1 is an important virulence factor in *P. falciparum* encoded by around 60 different var genes. Some of these var genes are potentially critical in CM development and in rosetting. To our knowledge, relationships between all three of these potential factors in CM development (var genes, ABO blood type group, and rosette formation) have not been investigated. In this work, malaria patient blood samples from a clinic in Blantyre, Malawi were analyzed via rt-qPCR to detect var genes (especially those associated with rosetting) and correlate these with blood type and CM status. We hope this study will better elucidate the relationship between ABO blood group, rosette formation, and cerebral malaria development.

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DEDUCING THE EFFECT OF VARIABLE OXYGEN CONCENTRATIONS ON PLASMODIUM FALCIPARUM GROWTH

Dinah Nahid1, Lauren Childs2, Regina Cordy1
1Wake Forest University, Winston-Salem, NC, United States, 2Virginia Tech, Blacksburg, VA, United States

Elucidating within-host infection dynamics is crucial to figuring out the underpinnings of how malaria infections progress. Understanding the role of bodily oxygen concentrations on Plasmodium parasite multiplication rate (PMR) may provide more insights into this progression. Over the course of its life cycle, Plasmodium falciparum cytoadheres to the endothelial lining of the capillaries within various organs in the body that have differing oxygen concentrations. The effect of this difference in oxygen on the parasite’s growth is not well understood and previous modeling work generally does not consider variable oxygen levels. We tested two different oxygen concentrations (1% and 13%) and monitored parasite growth for three complete cycles of development within human red blood cells. This was carried out with considerations for variabilities in blood from different donors. Our data supports a significantly higher parasitemia in cultures grown in 1% oxygen compared to those grown at 13% oxygen. In order to improve understanding of the parasite dynamics in vitro, we constructed a stage-structured mathematical model composed of a series of ordinary differential equations (ODEs) incorporating empirically determined parameters such as PMR. Assuming prolonged exposure to differing oxygen concentrations, we found parasitemia peaked later in our simulations before crashing in 13% oxygen. As the quantity of red blood cells is known to undergo a shift within-host due to bone marrow production and splenic elimination, we extend the model to include red blood cell production and elimination to make predictions about in vivo parasite population level response. Better understanding of the effect of differing oxygen concentrations on parasite growth and development could lead to the improvement of understanding of chronic malaria infections and the mechanisms mediating them.
Although fatty acid-based lipids are crucial for regulating Plasmodium falciparum parasite maturation, replication, and sexual commitment during the intraerythrocytic developmental stage, our current understanding of the interplay between lipid metabolism and infection in vivo is limited. Here we apply an integrative multi-omics approach combining high-resolution analysis of complex human serum lipids (999 complex lipid molecules) and host-parasite co-transcriptomics (12,375 host and 1,923 parasite transcripts) to document for the first time lipid metabolism and transcriptomic perturbations in 100 malarial children from Burkina Faso sampled before and after Plasmodium falciparum natural infection (n = 200) and assessed their impact on parasite development and disease progression. Our results provide a detailed in vivo blueprint of infection-triggered lipidomic changes and identify sub-classes of polyunsaturated phospholipids and triglycerides associated with parasitemia. Integrative lipidomic-transcriptomic analysis identified parasite gene expression changes in core parasite lipid remodeling and biosynthesis pathways that are activated by host-derived lipids. We experimentally validate the crucial role of these phospholipid subclasses in supporting the parasite’s asexual and sexual development in vitro. These findings expand our understanding of the role of polyunsaturated fatty acid and phospholipid metabolism in P. falciparum parasite pathogenesis and transmission biology. Furthermore, our identification of specific gene-lipid networks offers potential therapeutic targets for future antimalarial interventions and control strategies.

**PROFILE OF ENDOTHELIAL BIOMARKERS (ANGIOPOIETIN-1 AND ANGIOPOIETIN-2) IN PATIENTS WITH UNCOMPLICATED MALARIA IN LAGOS**

**Azuka Ike**

1College of Medicine of the University of Lagos, Nigeria, Lagos, Nigeria, 1Centre for Transdisciplinary Research in Malaria and Neglected Tropical Diseases, College of Medicine of the University of Lagos, Nigeria, LAGOS, Nigeria

Malaria related mortality is associated with significant host endothelial activation such as host angiogenic factors such as angiopoietin-1 (Ang-1) and angiopoietin-2 (Ang-2). This study profiles the levels of plasma Ang-1 and Ang-2 which are critical regulators of endothelial activation and integrity with some haematological parameters such as total white blood cell counts (WBC), haemoglobin level (Hb), packed cell volume (PCV), and malaria parasite density in uncomplicated Plasmodium falciparum malaria patients. This study was conducted in four hospital sites: Ijede, Ijebu, Ijebu-Ode, and Ori-Okuta in Ikorodu Local Government Area, Lagos, Nigeria. A total of 83 participants with the age ranged from 2 years - 79 years; the mean age was 22.6 ± 16.3 years. The study population comprised 49 uncomplicated malaria patients and 34 non-malaria patients. Plasma levels of Ang-1 and Ang-2 of the study participants were measured using an enzyme-linked immunosassay. The haematological parameters were determined using the WHO standard. Pearson correlation was used to evaluate the correlation between plasma levels of each biomarker in malaria patients. A p-value of < 0.05 was considered significant. There was no significance difference observed in PCV, Hb and WBC parameters but there was a significance difference (p < 0.001 and p < 0.05) observed in the levels of Ang-1 and Ang-2 in malaria positive patients when compared with non-malaria patients. A significant decrease was seen between the Ang-1 levels of uncomplicated malaria, and non-malaria patients; however, significant increase was seen in Ang-2 levels between uncomplicated malaria and non-malaria patients (p < 0.05). The ratio of Ang-2: Ang-1 showed a significant increase (p = 0.001) between malaria positive patients and non-malaria patients. Endothelial activation of angiopoietins may be involved in the pathogenesis of malaria. This study suggests that Ang-1 and Ang-2 may be used as biomarkers to determine the severity of malaria infection.

**THE ROLE OF PSYCHOSOCIAL FACTORS IN NET USE IN KENYA: FINDINGS FROM THE KENYA MALARIA BEHAVIOR SURVEY**

**Joseph Millward**

1Breakthrough ACTION Project, Johns Hopkins Center for Communication Programs, Baltimore, MD, United States, 2Division of National Malaria Programmes, Ministry of Health, Nairobi, Kenya, 3U.S. President’s Malaria Initiative, Nairobi, Kenya, 4Breakthrough ACTION Kenya Project, Johns Hopkins Center for Communication Programs, Nairobi, Kenya

Research shows that psychosocial factors can influence net use, yet, their role has rarely been studied in Kenya. Kenya’s Division of National Malaria Program (DNMP) and Breakthrough ACTION Kenya conducted the Malaria Behavior Survey in 2022 in 8 malaria-endemic counties. Interviews were conducted in 1,456 households and included information on 7,573 household members, 4,196 mosquito nets, 1,787 women, and 466 of their male partners. Key outcomes included insecticide treated net (ITN) access (one ITN per two household members), ITN use the night before the survey among those with access, and consistent self-reported daily use of nets among those in households with at least one net (consistent use). 88% of household members had access to an ITN, yet only 80% with access reported sleeping under an ITN the night before the survey. Most (89%) respondents reported that they consistently use a net. Multivariable logistic regression models were fit to examine the socio-demographic and psychosocial factors associated with consistent net use. Perceived confidence to use a net (Adjusted odds ratio (AOR): 6.6, 95% confidence interval (CI): 4.8-9.2), favorable attitudes towards net use (AOR: 2.2, 95% CI: 1.6-3.0), net care (AOR: 1.6, 95% CI: 1.2-2.1), and weekly radio listenership (AOR: 1.5, 95% CI: 1.1-2.2) were positively associated with consistent net use. Respondents aged 20 years and above had 2 to 3 times increased odds of consistent net use compared to those aged 15-19. Those in households with at least one net per 2 household members also had significantly increased odds of reporting consistent net use (AOR: 3.3, 95% CI: 2.1-5.1). Common unfavorable attitudes towards ITNs included perceptions that insecticide smell disrupts sleep (41%); net care is a tedious task (36%); purchased ITNs are more effective than free ITNs (31%); and dislike of sleeping under an ITN during warm weather (29%). The results highlight Kenya’s high net use rate and suggest that increasing access to ITNs, promoting favorable attitudes towards ITN use and care, boosting confidence in one’s ability to use ITNs, and radio programming targeting adolescents may increase and sustain it.
A FIVE-ARM TRIAL COMPARING ARTESUNATE-AMODIAQUINE AND ARTEMETHER-LUMEFANTRINE-AMODIAQUINE WITH OR WITHOUT SINGLE-DOSE PRIMAQUINE TO REDUCE PLASMODIUM FALCIPARUM TRANSMISSION IN MALI

Alhamamoudou Mahamamar1, Leen R. Vanheer1, Koualy Sanogo1, Merel Smit2, Youssouf Sinaba2, Sidi M. Niambale2, Oumar M. Dicko1, Richard S. Diarra3, Makonon Diallo1, Seydina O. Maguiraga1, Ahmadou Youssouf1, Adama Sacko1, Sekouba Keita1, Siaka Samake1, Adama Dembélé1, Yahia Dicko1, Sekou F. Traoré1, Chris Drakeley2, Teun Bousema3, Allassane Dicko1, William R. Stone2

1Malaria Research & Training Center, Faculty of Pharmacy and Faculty of Medicine and Dentistry, University of Science, Techniques and Technologies of Bamako (USTTMB), Bamako, Mali, Mali, 2Department of Infectious Biology, London School of Hygiene & Tropical Medicine, London, UK, WC1E 7HT, London, United Kingdom, 3Vanke School of Public Health, Tsinghua University, Beijing, China, 4Ministry of Health, Juba, South Sudan

Artemisinin Combination Therapies (ACT) are the first-line treatment for uncomplicated Plasmodium falciparum malaria. ACTs rapidly clear asexual P. falciparum parasites, responsible for clinical symptoms, but have limited activity against mature gametocytes, which are the only life stages capable of transmitting to mosquitoes. To reduce P. falciparum transmission, the World Health Organisation recommends the addition of a single low dose of primaquine (0.25 mg/kg), a potent and fast-acting gametocytocidal drug, to ACT. Although Artesunate-Amodiaquine (ASAQ) is a commonly used ACT, limited available data suggest poor activity against gametocytes and the added benefit of SLD PQ in combination with ASAQ remains unknown and Artemether-Lumefantrine (AL) showed more gametocyte clearance and transmission reduction efficacy compared to Dihydroartemisinin-Piperaquine (DP). Recently, triple artemisinin-based combination therapies (TACT) such as Artemether-Lumefantrine plus Amodiaquine (ALAQ) have been proposed to delay the emergence of drug resistance and to provide efficacious treatment for multi-drug resistance P. falciparum infections. To determine transmission reducing efficacy of ASAQ with and without SLD PQ and AL with and without Amodiaquine (AQ) and Primaquine (PQ), we conducted a five arm, single-blind, randomized controlled trial in Quelbessebougou, Mali. Participants aged 10-50 years with asymptomatic P. falciparum malaria with gametocytes detected by blood smear were randomised in a 1:1:1:1:1 ratio, to receive either ASAQ, ASAQ-PQ, AL, ALAQ or ALAQ-PQ. The primary efficacy endpoint, analysed in all infected patients with at least one infectivity measure before and after treatment, was median within person percent change in mosquito infection rate in infectious individuals from baseline to day 2 post treatment, assessed by membrane feeding. The recruitment is completed, and data cleaning is ongoing. Full results on the safety and malaria transmission blocking effect of these combinations will be presented.

A QUASI-EXPERIMENTAL STUDY TO ESTIMATE EFFECTIVENESS OF SEASONAL MALARIA CHEMOPREVENTION IN AWEIL SOUTH COUNTY IN NORTHERN BAHR EL GHAZAL, SOUTH SUDAN

Jamshed Khan1, Denis Mubiru1, Francis Okot Lokang1, Abubaker Rom Ayue1, Jonathan Magocola1, Maria Suau Sans2, Christian Rassi2, Craig Bonnington2, Kevin Baker2, Sol Richardson2, Chucks Njaji2, Sikai Huang3, Ahmad Julia4

1Malaria Consortium, Juba, South Sudan, 2Malaria Consortium, London, United Kingdom, 3Vanke School of Public Health, Tsinghua University, Beijing, China, 4Ministry of Health, Juba, South Sudan

Seasonal malaria chemoprevention (SMC) is an effective intervention to prevent malaria in children, in locations where transmission is seasonal. Due to sulfadoxine-pyrimethamine (SP) resistance concerns in East and Southern Africa (ESA), SMC was implemented in the Sahel region of West and Central Africa. Growing evidence suggest that SMC with SP and amodiaquine retain high level of effectiveness in ESA despite these concerns. This study aims to generate evidence on effectiveness of SMC when delivered under programmatic conditions in an area with an unknown antimalarial drug resistance in the Northern-Bahr-el-Ghazal. Five SMC cycles were delivered in Aweil South between July and November 2022. We conducted a non-randomised quasi-experimental study comparing intervention county with control county. Data were obtained from repeated cross-sectional household surveys of caregiver’s children aged 3–59 months using cluster sampling. Wave1 survey took place in both counties before SMC implementation; Waves2 and 3 took place after cycles2 and 4. Difference-in-differences analysis was performed by fitting logistic regression models with interactions between county and wave. Estimates of effect of SMC expressed as odds ratios (OR) with 95% confidence intervals (CIs). A total of 2,760 children sampled in study across three survey waves in both study counties. Children in the intervention arm had 70% lower odds of caregiver-reported fever compared to those in control-arm during one-month period prior to Wave2 (OR:0.30, 95%CI:0.12–0.70, p=0.003) and 47% reduction in Wave3 (OR:0.63, 95%CI:0.22–1.59, p=0.306) after controlling for baseline difference between counties in Wave1. Odds of caregiver-reported RDT-confirmed malaria were 82% lower in previous one-month period prior to Wave2 (OR:0.18, 95% CI: 0.07–0.49, p=0.001) and Wave3 (OR:0.18, 95%CI:0.06–0.54, p=0.003). These results indicate that SMC is an effective intervention for malaria prevention in children in Northern-Bahr-el-Gazhal. More research is necessary for a better understanding of the role of drug resistance in determining SMC effectiveness and scalability in region.

DIGITAL TRANSFORMATION IN HEALTH: GUINEAN EXPERIENCE OF USING A NATIVE ANDROID APPLICATION COUPLED WITH DHIS2 IN THE 2022 NATIONAL ITN DISTRIBUTION CAMPAIGN

Fatoumata Battouly Diallo1, Lawson Agossa Charles Lebon1, Conde Mohamed Saran1, Soua Gomou1, Ibrahim Kail Keita2, Abdourahamane DIALLO1, Moustapha CAMARA1, Aloune CAMARA2, Chrestien Yameni1, Suzanne Van Hulle1

1Catholic Relief Services, Conakry, Guinea, 2National Malaria Control Program, Conakry, Guinea

From 2013 to 2019, Guinea carried out three ITN distribution campaigns using paper-based data collection systems. Based on lessons learned from the 2021 digitalization of Guinea’s seasonal malaria chemoprevention campaign, the 2022 ITN campaign was digitalized. This abstract describes the technological approach used for the digitalized ITN campaign and key challenges encountered. The digitalization was based on a customized Android application “MILDA 2” designed by HISP WCA to collect individual data centralized in DHIS2. The application was installed on 7785 tablets, which were managed by a Mobile Device Management (MDM) system. A national server and a cloud-based system were configured to host the data. The first step in the digitalization process involved development of the designer’s guide and the integration of the micro-planning tree into DHIS2. The campaign data was collected by scanning the QR code of a physical coupon provided to the households, followed by completing electronic and physical coupons. An Excel database was used for comparative analyses. A back-scan of unrecognized coupons was performed to improve the completeness of the data. MDM protection limited tablet losses to 0.04%. The completeness of the enumeration data in DHIS2 was 95% for the population, 95% for households and 90% for the ITNs, compared to the physical count data. For distribution, 70% of households were covered and 75% for ITNs. The advantages of digitalization included shorter data collection period, data availability for multiple programs and higher data quality. The major difficulties were the instability of the application, the server and the poor network/internet coverage. The digitalization of the ITN distribution campaign in Guinea has been a hopeful experience for the digital transformation of the health sector.
In Burkina Faso, children under 5 years of age face a 72% risk of malaria deaths. Seasonal malaria chemoprevention (SMC) is a strategy to prevent malaria in children under 5 years old. Its expected benefits are to prevent approximately 75% of malaria episodes and probably reduce the incidence of moderate-severe anemia. This study aimed to assess hemoglobin rate and Plasmodium falciparum infection prevalence after large-scale SMC implementation in one village of Sabou health district. The study was conducted in Sabou Health District where malaria is hyperendemic and seasonal. SMC was delivered as sulfadoxine-pyrimethamine plus amodiaquine (SP-AQ) at monthly intervals from July to October 2020 to 182 children. Five cross-sectional surveys were conducted at baseline, before each SMC and endpoint which place 2 months after SMC. During each survey, malaria parasitemia and anemia were assessed. We noticed a significant increasing prevalence of Plasmodium falciparum infection across the different rounds of SMC. Malaria infection varied from 13.3% to 16.8% at baseline, 15.5% to 20.3% during the pic of transmission, and 17.5% to 20.8% at the endline survey. Malaria parasitemia and anemia prevalence in under 5 year old children receiving SMC decreased by 27% (85,520/320,765) in ACCESS-supported districts and 27% (145,094/668,327) nationally; coverage was 17% (59,574/347,562) in the 45 non-ACCESS-supported districts. In 2019, IPTp3 coverage was 22% (145,094/668,327) nationally; coverage was 17% (59,574/347,562) in the 45 non-ACCESS-supported districts. In 2022, IPTp3 coverage was 51% (340,669/671,191) nationally: 56% (216,388/388,148) in the districts supported by ACCESS—meeting the national target—and 44% (124,281/283,043) in the districts not supported by ACCESS. This represents an increase of 39 percentage points of IPTp3 coverage in ACCESS-supported districts between 2019 and 2022, compared to a 17-percentage-point increase in IPTp3 coverage in non-supported districts, suggesting that the intervention package was successful in improving IPTp3 coverage and expanding this model could further improve coverage. A difference-in-differences analysis controlling for potential confounders is recommended to provide further evidence for the effectiveness of ACCESS’s interventions.
Since 2021 the National Malaria Control Program and the Expanded Program on Immunization, supported by their partners, have collaborated in six districts to combine seasonal malaria chemoprevention (CPS) and immunization activities. The objective of this study was to share the innovative measures and results achieved during the seasonal malaria chemoprevention campaign, with a focus on improving vaccination coverage and malaria case management in the community. A descriptive study was conducted between October 3 and 11, 2022, during the fourth round of the SMC campaign in 10 health districts of Guinea. The preparatory phase consisted of organizing meetings, identifying strategies and needs, and designing tools. The implementation phase included distributing SMC drugs, identifying children and women to be caught up, providing immunization, and treating all confirmed malaria cases in the community. The final phase involved synthesizing achievements, results sharing, and drawing lessons learned. In the implementation phase of the SMC campaign, for a target of 522,698 children, the administrative coverage of the fourth round was 95% (92-98%). For catch-up immunization, 23,817 EPI cards were identified at the health center level, and 18,474 children were located and immunized in the community by SMC distribution agents, i.e., 78% coverage. For pregnant women, 4,656 ANC cards were identified at the health center level, and 3,899 pregnant women were located and caught up by agents, for a coverage of 83%. The agents tested 3,172 suspected malaria cases in the community and treated 1,542 RDT-confirmed malaria cases. In conclusion, the mutualization of SMC activities with catch-up immunization has contributed to improving immunization coverage and reduced malaria cases in the community level. The next step should be to replicate in all the 17 eligible districts for SMC in Guinea.

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**THE IMPORTANCE OF QUANTIFICATION TO OPTIMIZE RESOURCE MOBILIZATION FOR A CONTINUOUS SUPPLY OF ANTIMALARIAL COMMODITIES IN MADAGASCAR**

Tiana Ravelonarivo1, Serge Ramahazomanana1, Aline Mukerabiron1, Joelson Soca-Naivo1, Jane Briggs2, Luz Razafimbelo2, Timon Anjaramijoro3, Yvette Razafimaharo2, Soza Andriamarovesetra2, Haingomala Razafrandimby2, Hasina Rabarijaona2, Rija Razafindrade2, Jeanine Raharilala2, Ony Andriamanalina2, Tantely Rajaoblina2, Laurent Kapesa2, Lantonirina Ranarison2, Elisoahasina Rafaimanana2

1Management Sciences for Health, IMPACT Program, Antananarivo, Madagascar; 2Management Sciences for Health, Arlington, VA, United States; 3Management Sciences for Health, ACCESS Program, Antananarivo, Madagascar; 4National Malaria Control Program (NMCP), Antananarivo, Madagascar; 5Population Services International (PSI), Antananarivo, Madagascar; 6The U.S. President’s Malaria Initiative (PMI), USAID Antananarivo, Madagascar, Antananarivo, Madagascar; 7InterAide, Antananarivo, Madagascar

Correct quantification of needs is important for resource mobilization to ensure availability of antimalarial commodities. Since 2019, the USAID IMPACT program in Madagascar supports the Ministry of Public Health (MOPH) to improve the process of quantifying medicines and supplies to test and treat malaria. The country had few experts to conduct accurate forecasting and supply planning, limited financial resources to procure forecasted quantities and lack of visibility and coordination on procurement of commodities from multiple funding sources. In 2018, IMPACT provided technical assistance to a Procurement Supply Management (PSM) Committee led by the National Malaria Program (NMP). Since 2019, IMPACT provided training and coaching to PSM committee members on data collection and adjustment methods, forecasting and supply planning and the use of quantification tools, mainly the supply plan tool Pipeline to enable them to analyze data and correctly project needs. From 2019 to 2021, 42 quantification staff were trained: 13 from NMP, 14 from MOPH, 4 from SALAMA (central medical store), and 11 stakeholders. Quantification exercises were conducted annually with semester reviews to adjust the needs and mobilize financial resources to fill gaps. The quantification process contributed to increased mobilization of financial resources, dedicated to procuring malaria medicines and supplies to cover 81% of needs forecasted for 2021 ($8,092,744) and 100% for 2022 ($6,419,125) through sources of funding including the PMI, the Global Fund, UNICEF, and the Malagasy government. Madagascar experienced no central-level stock outs of antimalarials and diagnostic tests in 2021 and 2022 despite the national and global logistics disruptions due to COVID-19. In July 2022, stock levels at SALAMA were sufficient to meet quarterly needs forecasted by the 114 districts of Madagascar with a maximum stock level of 6 months. Improvement of the central quantification process has contributed to improving the country’s supply of antimalarial commodities. Other components of the supply chain remain to be strengthened for better availability of health commodities.

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**PSYCHOSOCIAL FACTORS ASSOCIATED WITH INTENTIONS FOR SEEKING EARLY ANTENATAL CARE (ANC) DURING A FUTURE PREGNANCY: FINDINGS FROM THE KENYA MALARIA BEHAVIOR SURVEY**

James Andati1, Christine Wayua2, Jacinta Opondo2, Daniel Wacira3, Joseph Millward4, Jayme Hughes5, Jeremiah Ochieng6, Grace Miheso7, Jennifer Boyle8, Anna McCartney-Melstad9, Carol Underwood2, Zoe Hendrickson10

1Breakthrough ACTION Project, Johns Hopkins Center for Communication Programs, Nairobi, Kenya; 2Division of National Malaria Programme, Ministry of Health, Nairobi, Kenya; 3U.S. President’s Malaria Initiative, USAID, Nairobi, Kenya; 4Breakthrough ACTION Project, Johns Hopkins Center for Communication Programs, Baltimore, MD, United States

Despite recent gains, only 38% of pregnant women in the malaria-endemic Lake region of Western Kenya received all three doses of intermittent preventive treatment during pregnancy (IPTp) according to the 2022 Kenya Demographic Health Survey. Given the association between antenatal care (ANC) and IPTp uptake, there is a need to understand the psychosocial factors associated with ANC intentions to inform future malaria programming. Kenya’s Division of National Malaria Program (DNMP) and Breakthrough ACTION Kenya fielded the Malaria Behavior Survey in 2022 and interviewed 1,787 women of reproductive age from 1,456 households. Psychosocial factors, including attitudes towards seeking ANC and IPTp, were assessed based on agreement to a series of statements. Responses were scored and summed into factor-specific scales. Bivariate and multivariable logistic regression models examined socio-demographic, psychosocial, and structural factors associated with ANC intentions among women of reproductive age. Among women who had a child in the last two years who also intended to have more children, 70% intended to attend ANC early, 90% intended to attend 4 or more ANC visits, and 24% intended to attend 8 or more ANC visits in their next pregnancy. Attendance at ANC visits was significantly and positively associated with uptake of one, two, three or more IPTp doses (p<0.01). In the final adjusted logistic regression model, respondents with favorable attitudes towards seeking ANC as well as favorable attitudes toward IPTp had significantly increased odds of early ANC intentions in their future pregnancy (Adjusted odd ratios (AOR): 3.7; p<0.01). Having seen or heard a malaria message in the last six months was also significantly associated with 1.9 higher odds of early ANC intentions (AOR: 1.9; p<0.05). The findings highlight the need for better understanding of the drivers of favorable attitudes towards ANC and IPTp to inform the design of social and behavior change activities to improve women’s intentions to seek ANC early during their next pregnancy and ultimately, their receipt of IPTp.
IMPACT OF COMMUNITY-BASED PROMOTION OR FOCUSED MALARIA IN PREGNANCY TRAINING FOR HEALTH PROVIDER ON THE COVERAGE OF INTERMITTENT PREVENTIVE TREATMENT IN PREGNANCY IN SAN, MALI

Sory Diawara1, Julie R. Gutmann2, Boureima Kone1, Samba Diarra1, Celia Woodfill3, Jules Mihigo4, Aliou Diallo1, Philippe Mutwara5, Renior Saye6, Beh Kamate7, Moussa Niangaly8, Moussa Djimde9, Mohamed Keita1, Balla Bagayoko1, Abraham Tembely1, Mamadou Samake1, Almamy Traore1, Issaata Ongboia1, Siatatou Niane1, Fady Toure1, Fatimata Sidibe1, M’Fa Adama Diallo1, Addoulaye Djimde1, Boubacar Traore1, Kassoum Kayentao1

1University of Sciences, Techniques and Technologies de Bamako, Bamako, Mali; 2President’s Malaria Initiative, Malaria Branch, Center for Global Health, U.S. Centers for Disease Control and Prevention, Atlanta, GA, USA; 3Abuja, Nigeria; 4MRC Centre for Global Infectious Disease Analysis, Infectious Disease Epidemiology, Imperial College London, London, United Kingdom

Malaria in pregnancy (MP) is associated with poor maternal and newborn outcomes. The World Health Organization recommends all women in their second and third trimester of pregnancy receive intermittent preventive treatment during pregnancy using sulfadoxine-pyrimethamine (IPTp-SP). In Mali, IPTp-SP is delivered through antenatal care (ANC) visits for eight ANC contacts. However, infrequent, late or non-attendance at ANC has been associated with less IPTp-SP uptake. Between April 2020 and September 2022, we conducted a cluster randomized trial in 30 community health centers in San, Mali, to assess whether community-based promotion of IPTp or MIP focused training and supervision for ANC health workers could increase IPTp uptake. Intervention arm 1 (IA1) is a community-based promotion campaign to improve women’s awareness of and demand for at least three doses of IPTp-SP (IPTp3), while intervention arm 2 (IA2) consists of enhanced MIP focused training and supervision for health workers in ANC clinics. In the third (control) arm (CA), the current standard training and IPTp implementation strategy were maintained. The intervention impact was assessed using data collected from a baseline household survey, conducted in February-March 2020 prior to the study, and an endline survey in September 2022. Differences between proportions of women with IPTp3+ were tested using the chi² test. A total of 2,195 women were interviewed at baseline. Overall, about 40.4% (37.9%-42.9%) received IPTp-SP at baseline; coverage was similar among treatment arms (41.2% in IA1, 36.9% in IA2, and 42.4% in the control arm) (p=0.213). At endline, among 1,464 women interviewed, IPTp3 coverage increased in both intervention arms, to 51.1% in IA1 and 50.0% in IA2, while coverage in the control arm declined slightly to 41.8%, representing an increase of 9.8% in IA1 (p=0.02) and 13.1% (p=0.001) in IA2. Both interventions significantly improved IPTp-SP uptake, although coverage remains lower than the national target. Rolling out MIP focused training more widely could help Mali to achieve national IPTp3 coverage targets.

NEW TREATMENT REGIMEN OF SEASONAL MALARIA CHEMOPREVENTION A PROMISING ALTERNATIVE TO DISRUPT PLASMODIUM TRANSMISSION IN THE FIELD

Dari F. Da1, Frédéric Guignma1, Inés L. Paré1, Issaka Zongo1, Abdoulaye D. Congo1, Léa D. Paré1, Thomas S. Churcher1, Roch K. Dabi1

1Institut de Recherche en Sciences de la Santé, Bobo-Dioulasso, Burkina Faso; 2SECRETARIAT Permanent pour l’élimination du paludisme, Ougadougou, Burkina Faso; 3District Sanitaire de Karagasso Vigué, Bobo-Dioulasso, Burkina Faso; 4MRC Centre for Global Infectious Disease Analysis, Infectious Disease Epidemiology, Imperial College London, London, United Kingdom

Seasonal malaria chemoprevention (SMC) intervention is based on antimalarial treatment for a target population to cure any asymptomatic infection and preventing new infections over a period of time. In some endemic countries, the SMC focuses on under 5 years old children for 5 months in the year with satisfactory outcomes when compared to no interventions. However, this target population is currently reinfected few weeks post intervention as some important reservoirs remain unperturbed. Asymptomatic Plasmodium carriage is important in children up to 15 years old and no large-scale collaborative curative action is carried out in children aged 5 to 15 years, making them the indefectible reservoirs of parasite. Also, malaria infection remains higher after the last SMC treatment leaving numerous asymptomatic carriers who remain infected during the dry season and ensure parasite transmission at the next wet season when the vectors become abundant. However, SMC effectiveness could be optimized with lasting synergistic effects if it was implemented integrating the major epidemiological variables such as human reservoirs and the temporal variations of the vector density. We investigated the potential additional benefits of SMC implemented taking account some parameters. In a pilot study, standard regimen SMC has been implemented but targeting up to 10 years old children and covering 6 months period with an observed versus unobserved regimen in 2 villages. In a sample of about 800 children (5-10 years old), the SMC efficacy for the Plasmodium infection was significantly lower during the peak raining season (about 10%) compared to this observed at the end of the wet season (70%). This finding correlated with the vector abundance, highlighting the important role of infectious mosquitoes which continuously reinfests the children after the protection period. Importantly, the negative individuals after last treatment remained uninfected until dry season. The next step of investigations focuses on follow up of this group to provide more information about the potential long-term impact of the new regimen SMC on the incidence of malaria infection.

MULTI-LEVEL AND EVIDENCE-BASED ADVOCACY SUPPORTED INCREASED UPTAKE OF INTERMITTENT PREVENTIVE TREATMENT OF MALARIA AMONG PREGNANT WOMEN OF CROSS RIVER STATE, NIGERIA

Oluwatobiloba Akerele1, Augustine Firima1, Olugbenga Mokuolu2, Chinwe Nwaeze3, Linda Lawrence3, Eben Etu4, Kenechukwu Ugiebene4, Akpasa Aniefok5, Victor Bassey6, Olayato Abikoye7, Abimbola Olayemi8, InAbasi Inglass9, Uchenna Nwokenna10, Arja Huestis11, Ifeanyi Kalu12, Kenneth Takimi13, Erkwagh Dagba14, Veronica Momoh15, Jules Mihigo15

1United States President’s Malaria Initiative for States, Management Sciences for Health, Cross River, Nigeria; 2United States President’s Malaria Initiative for States, Management Sciences for Health, Arlington, VA, United States; 3United States President’s Malaria Initiative for States, Management Sciences for Health, Abuja, Nigeria; 4Nigerian Interfaith Action Association, Abuja, Nigeria; 5State Malaria Elimination Programme, Cross River, Nigeria; 6United States President’s Malaria Initiative, United States Agency for International Development, Abuja, Nigeria

Sulphadoxine-Pyrimethamine (SP) is an affordable, effective drug used for intermittent preventive treatment of malaria in pregnancy (IPTp) yet remains largely unavailable. In Cross River State, Nigeria, 75% and 33% of pregnant women received two and three doses of IPTp respectively from October 2020 to November 2021, well below the national target of 80%. The U.S. President’s Malaria Initiative for States (PMI-S) project adopted systems advocacy to foster action from community to state government actors to commit to SP procurement across health facilities. PMI-S convened multi-sectoral and multilevel stakeholders to develop evidence-based advocacy briefs highlighting the major issue of SP unavailability. The briefs were data-based, pre-tested and adapted to suit stakeholders with decision-making power. They were used as tools to streamline advocacy activities which commenced in November 2021. The advocacy briefs were given to malaria actors at the state, community, and health facility level to ensure adequate supply of SP and support for malaria interventions in Cross River. Data from the National Health Management Information System was analyzed to assess the impact of the advocacy activities and found that they helped increase donation of SP commitments by chairman of Local Government Areas, ownership of procurement and management of SP by health facility
Managers, and Ward Development Committee donations of SPs. In fact, IPTp 2 coverage was over 80% by August 2022, meeting its target and 37% for IPTp3, closing the gap to target. These findings show that tested, tailored and multi-level advocacy lead to successful resource mobilization and support preventive treatment of malaria in pregnancy.

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COMPARISON OF DIARY-REPORTED BEDNET USE ACTIVITIES AND ACCELEROMETER-BASED BEDNET DATA FROM COTE D’IVOIRE

Soro Dramane1, Laurence Yao1, Benjamin Koudou1, Paul J. Krezenoski2
1Centre Suisse de Recherches Scientifiques in Côte d’Ivoire, Abidjan, Côte D’Ivoire, 2University of California, San Francisco, San Francisco, CA, United States

Malaria has not declined as expected in some countries despite the widespread distribution of long-lasting insecticide treated bednets (LLINs). One explanation could be overestimated LLIN use or the timing of use may not correspond with the timing of vector exposure. Questionnaires about LLIN use may be unreliable as measures of LLIN use, since they are prone to recall and social desirability bias, and only give a snapshot of LLIN use the previous night thus missing any temporal variation. Electronic monitors of LLINs use can provide hourly objective measurements of whether an LLIN is in use or not. This study is an early analysis of households enrolled in an observational study using electronic monitors of LLIN use in the peri-urban town of Tiassale, Côte d’Ivoire. In this analysis, 4 households were followed for 10 nights each in February 2023 (40 nights of monitoring). Accelerometer-based LLIN monitors were attached to one bednet in the household and inhabitants were asked to keep a diary of the times during the night that the LLIN was unfurled, folded up and whenever anyone entered or exited the LLIN. On average, the households reported unfurling the net for sleep at 2150 in the evening and folding up the net at 634 in the morning, with an average of 1.7 net entries (SD: 1.4) and 1.81 exits per night (SD: 1.4). Overall agreement between a previously developed machine learning algorithm for classifying LLIN activities based on accelerometer data and the participant diaries was 80.0% (116/145). This varied by LLIN activity: net put down (82.5%), net put up (79.5%), entry into net (79.5%) and exit from net (77.8%). This analysis suggests that accelerometer-based monitors of LLIN use have good agreement with reported use and may be a useful tool for monitoring LLIN use in malaria endemic settings over time.

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ESTIMATING THE IMPACT OF LLIN USE PATTERNS ON ANOPHELES MOSQUITO EXPOSURE AMONG SCHOOL-AGED CHILDREN IN UGANDA

Kelly Walters, Paul J. Krezenoski
University of California, San Francisco, San Francisco, CA, United States

School-aged children (SAC) in Africa have high prevalence of malaria, yet they are often the least likely to use long-lasting insecticide treated bednets (LLINs). Existing interventions to improve LLIN use in this age group include school-based education and universal distribution programs. Although low use has been attributed to a lack of access, little is known about LLIN use patterns in SAC who have access to LLINs. SmartNet is a validated tool for electronically measuring LLIN use over time. The aim of this study is to characterize LLIN use patterns among SAC and estimate the impact on malaria exposure. Data was collected from 99 individuals living in 20 households in the Tororo District of Uganda from May to October 2019. Time in and out of bed were measured by self-report. Mosquito burden was measured with CDC light traps every two weeks, and hourly distribution of mosquito exposure throughout the night was calculated using human landing catches in nearby houses. SAC were defined as children 5 to 15 years old. Compared with other ages, SAC were less likely to use their LLIN at all overnight (OR 0.42, p = 0.014) and used LLINs for 1.1 fewer hours per night (p = 0.001). Without any LLIN use, SAC were exposed to an average of 2.48 mosquitoes per night. Based on SmartNet data, measured LLIN use would prevent 69.7% of exposures to an average of 0.75 per night. If all SAC unfurled LLINs by 7 PM, 98.7% of exposures could have been prevented, 87.9% if unfurled by 8 PM, and 71.7% if unfurled by 9 PM. This study shows that mere access to LLINs may be insufficient to decrease mosquito exposure in SAC, and provides insight into what temporal use patterns, including earlier LLIN coverage, may be the most effective in preventing exposure.

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IMPACT EVALUATION OF SEASONAL MALARIA CHEMOPREVENTION THROUGH ANALYSIS OF LARGE, AGGREGATED ROUTINE COVERAGE SURVEYS IN NIGERIA, BURKINA FASO, CHAD, TOGO AND MOZAMBIQUE (2020-2022)

Sol Richardson1, Sika Huang1, Taiwo Ibinijie2, Benoit Sawadogo3, Esseboé Sewu4, Alberto Zunza5, Olusola Oresanya2, Narcisse Tounaikok3, Cheick Compaore3, Mercia Sitoe2, Chuks Nnaji7, Kevin Baker7
1Vanke School of Public Health, Tsinghua University, Beijing, China, 2Malaria Consortium Nigeria, Abuja, Nigeria, 3Malaria Consortium Burkina Faso, Ouagadougou, Burkina Faso, 4Malaria Consortium Togo, Lome, Togo, 5Malaria Consortium Mozambique, Nampula, Mozambique, 6Malaria Consortium Chad, N’Djamena, Chad, 7Malaria Consortium UK, London, United Kingdom

Previous studies on seasonal malaria chemoprevention (SMC) with sulfadoxine-pyrimethamine (SP) and amodiaquine (AQ) suggest it is highly effective in reducing risk of Plasmodium falciparum malaria infections among eligible children in areas with seasonal transmission. As SMC has expanded geographically and monitoring surveys established, there is growing potential for aggregated datasets based on routine surveys to address new research questions which may previously have been technically infeasible using Health Management Information Systems data or financially infeasible as primary research. Malaria Consortium has built a harmonized dataset including all end-of-round annual SMC coverage surveys since 2020 with data on children, their caregivers and households. These surveys were representative of SMC target populations and conducted by independent contractors. Using these data we conducted an analysis on 47,345 eligible children aged 3-59 months in five countries to test the association between receipt of SMC (SPAQ on Day 1) in the penultimate cycle of each SMC round and caregiver-reported rapid diagnostic test (RDT) confirmed malaria cases in the subsequent month. We then tested associations between numbers of doses of SP and AQ received and RDT-confirmed malaria in 21,589 children in Nigeria. We fitted random-effects logistic regression models adjusted for child, caregiver and household variables including net use. The results showed that receipt of SMC was significantly associated with 30% lower odds of RDT-confirmed malaria (OR: 0.70, 95% CI: 0.65-0.76, p<0.001). In Nigeria, compared with those who had not received any dose of SMC medicines, there were lower odds of RDT-confirmed malaria among children who received Day 1 SPAQ only (OR: 0.61, 95% CI: 0.35-0.70, p=0.088), Day 1 SPAQ plus one AQ dose (OR: 0.45, 95% CI: 0.31-0.66, p<0.001), and Day 1 SPAQ and both AQ doses (OR: 0.53, 95% CI: 0.47-0.60, p<0.001). The results imply full adherence to the dosing regimen is needed to ensure SMC’s full effectiveness. Next steps will involve a case-control analysis using propensity score matching, and addressing new research questions.
COVERAGE OF INTERRUPMTENT PREVENTIVE TREATMENT OF MALARIA IN INFANTS AFTER FOUR YEARS OF IMPLEMENTATION IN SIERRA LEONE.

Augustin E. Fombeh1, Haily Chen1, Kwabena Owusu-Kyei1, Llorenç Quinto1, Raquel Gonzalez1, Julian Williams2, Mireia LLach Berne1, Myrié Wassenaar1, Abubakar Jaloff1, Joe-Henry C. Sunders2, Maximo Ramirez2, Cesc Bertran-Cobo1, Francisco Saute1, Didier K. Ekouevi4, Valérie Briand5, Anitta R.Y. Kamara6, Berne1, Myrte Wassenaar1, Abubakarr Jalloh2, Joe-Henry C. Tom Sesay7, Mohamed Samai2, Clara Menendez1

1Barcelona Institute for Global Health, Barcelona, Spain, 2College of Medicine and Allied Health Sciences, University of Sierra Leone, Freetown, Sierra Leone, 3Manhiça Health Research Center, Manhica, Mozambique, 4Université de Lomé, Lome, Togo, 5University of Bordeaux, National Institute for Health and Medical Research (INSERM) UMR 1219, Bordeaux, France, 6Ministry of Health and Sanitation, National Malaria Control Program, Directorate of Disease Prevention and Control, Freetown, Sierra Leone, 7Ministry of Health and Sanitation, Directorate of Reproductive Child Health, Freetown, Sierra Leone

Interruption Preventive Treatment of malaria in infants (IPTi) is a malaria control strategy consisting of the administration of an antimalarial drug drug regimen given twice a month. So far, this is being implemented nation-wide in Sierra Leone only; IPTi has been renamed as Perennial Malaria Chemoprevention -PMC-, accounting for its recently recommended expansion into the second year of life. Before starting a pilot implementation on PMC, the currently implemented strategy and malaria prevalence were assessed in young children in selected areas of Sierra Leone. A cross-sectional, community-based, multi-stage cluster household survey was conducted from November to December 2021 in selected districts of the Northern and northwestern provinces of Sierra Leone among 10-23 months old children, whose caretakers gave written informed consent to participate in the survey. Coverage of IPTi and malaria prevalence -assessed with rapid diagnostic tests-, were calculated using percentages and 95% confidence intervals weighted for the sampling design and adjusted for non-response within clusters. A total of 720 children were recruited. Coverage of 3 IPTi doses was 50.57% (968/1970; 95% CI 41.38 – 55.75), while prevalence of malaria infection was 26.19% (95% CI 24.81 – 28.54). Most children had received IPTi1 (80.26%, 574/707; 95% CI 75.30 – 84.44), and IPTi2 (80.09%, 577/707; 95% CI 76.30 – 83.40) and over half of the children also received IPTi3 (77.72%, 420/707; 95% CI 73.20 – 6.21). The uptake of each IPTi dose was lower than that of the vaccines administered at the same timepoint at all contacts. In Sierra Leone, half of the children received the three recommended doses of IPTi indicating an increase in its uptake compared to previous data of just a third of children receiving the intervention. However, efforts need to be made in improving IPTi coverage, especially in the planned expansion of the strategy into the second year of life following recent WHO guidelines.

PERENNIAL MALARIA CHEMOPREVENTION (PMC) STRATEGIES IN WESTERN AFRICA

Manuela Runge1, Semiu Rahman2, Olusua Oresanya3, Monique Ambroise4, Anne Stahlfield5, Ben Kok Toh1, Yahya Hamza6, Caitlin A. Bever7, Perpetua Uhomobhi6, James K. Tibenderana8, Jaline Gerardin1

1Northwestern University, Chicago, IL, United States, 2Malaria Consortium, Abuja, Nigeria, 3Bill and Melinda Gates Foundation, Seattle, WA, United States, 4National Malaria Elimination Program, Abuja, Nigeria, 5Malaria Consortium, London, United Kingdom

Perennial malaria chemoprevention (PMC) is recommended for children living in areas with high and persistent transmission, but it has not yet been widely implemented. Recent recommendations encourage countries to adapt PMC to their local contexts, taking both epidemiological and operational factors into account. However, there are no clear guidelines on how to do this, and it is unclear what the tradeoffs might be, as epidemiological and operational needs do not always align. Compared to delivery via the Expanded program of immunization (EPI) to specific ages of the child, a pragmatic approach to delivery might involve offering additional chemoprevention at every health visit. Through individual-based mathematical modeling, we compare the pragmatic PMC to the EPI-based only delivery schedule in children under the age of two years. We evaluate epidemiological and operational factors that might influence PMC performance, including malaria transmission and exposure, distance to health facilities and health care seeking, as well as coverage, probability of missing doses, and timeliness of doses. We calibrated our model to an ongoing PMC pilot implementation in Osun State, Southern Nigeria, using data from the pilot study and demographic household surveys. With the pragmatic delivery schedule, children received on average twice as many doses as with the EPI-based schedule alone, for a maximum of seven EPI touchpoints. Under ideal conditions (target coverage of 80%, constant care-seeking rates, and no delays), the protective efficacy increased by 25 percentage points. Under operational conditions, the pragmatic delivery approach remained more impactful but varied depending on the different factors affecting coverage. Intervention modeling that disentangles coverage can be used to identify how to customize PMC strategies within countries by assessing the trade-off between epidemiological and operational factors.

ARTEMETHER-LUMEFANTRINE AS A CHEMOPROPHYLACTIC TREATMENT OF MALARIA

Joel Tarning, Lorenz von Seidlein, Arjen M. Dondorp, Nicholas J. White, Richard J. Maude

Mahidol Oxford Tropical Medicine Research Unit, Bangkok, Thailand

Antimalarial chemoprophylaxis for high risk groups in endemic areas of Southeast Asia has the potential to reduce malaria transmission and accelerate elimination. However, the optimal choice of medication and dosing for many potential candidates is not clear. In Southeast Asia, the high prevalence of artemisinin and multidrug resistant Plasmodium falciparum limits the choice of drugs suitable for chemoprophylaxis. Artemether-lumefantrine is one of the six WHO-recommended artemisinin-based combination therapies (ACTs) for treatment of uncomplicated P. falciparum and P. vivax malaria, but is not widely used for prophylactic purposes. As artemether-lumefantrine has not been used previously for prophylaxis, a pharmacometric modelling and simulation approach was used to determine the optimal dosing schedule. Population pharmacokinetic-pharmacodynamic modelling and simulation was used to evaluate different dosing scenarios. Lumefantrine exposures were evaluated for different dosing schedules; (1) a full 3-day treatment course of 480 mg lumefantrine given once a month, (2) a full 3-day treatment course of 480 mg lumefantrine given twice a month, (3) a loading dose of a full 3-day treatment course of 480 mg lumefantrine followed by 480 mg lumefantrine QD given once a week, and (4) a loading dose of a full 3-day treatment course of 480 mg lumefantrine followed by 480 mg lumefantrine BID given once a week. A full 3-day treatment course given twice a month, and twice daily treatment given once a week, resulted in trough concentrations consistently above the therapeutic threshold of 200 ng/mL. However, the most favorable exposure profile, and arguably most practical dosing scenario was an initial 3-day full treatment course followed by twice daily dosing given once a week for the duration of chemoprevention. This dosing was consequently evaluated in a prospective clinical trial in Cambodia and showed excellent safety and protective efficacy.
MODELLING THE PUBLIC HEALTH IMPACT OF PERENNIAL MALARIA CHEMOPREVENTION: CURRENT GUIDELINES AND A PROPOSED AGE-EXPANSION

Swapnomeena Sen1, Lydia Braunack-Mayer1, Sherrie L Kelly1, Josephine Malinga1, Thiery Masserey1, Joerg Moehrle2, Melissa A Junior Voundi2, Joel Ateba2, Erica Mengue1, Annie Michèle Kouakou3, Meredith Center3

Updated guidelines for perennial malaria chemoprevention (PMC) extended the coverage from infants to children up to 24 months, with a flexible contextual dosing schedule, and without the need for sulfadoxine-pyrimethamine (SP)-sensitive parasite genotype based implementation. While decade-long evidence propelled a revival of this underutilized intervention, the WHO also highlights the need for further research, including: evidence of public health impact beyond 15 months of age, expansion to children over 24 months, and assessing if total public health impact outweighs any post-intervention delayed malaria effect. To address these gaps, we applied a validated individual-based model of malaria integrated with pharmacological models of drug impact (OpenMalaria). Current and age-expanded regimens (for children up to 36 months) were modeled by administering seven and nine doses of SP, across a range of transmission and healthcare settings, against partially SP-resistant (prevalent quadruple mutations in Pfdhfr and Pfdhps genes) and fully SP-sensitive parasites. General trends of intervention cost-effectiveness was projected to inform implementation decisions. Consistent with systematic reviews, both current and age-expanded regimens had modest but important public health impact (in settings of 5-67% PfPR2-10: median protective effectiveness over the first three years of life of 14.9% and 17.2% against clinical and 9.1% and 9.7% against severe cases against partially SP-resistant parasites, or to 20.9% and 24.2% against clinical and 9.7% and 13.2% against severe cases in fully SP-sensitive settings). Total program benefits outweighed any risk of rebound delayed malaria in children under five. An age-expanded PMC regimen protects more children with high risk of severe malaria, and may counterbalance modest reductions in impact from drug resistance. Robust healthcare system additional supports mitigating the small risk of delayed malaria. Our economic analysis confirms both PMC regimens implemented via existing EPI delivery platforms are likely to be cost-effective in the recommended transmission levels.

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HARNESSING COMMUNITY HEALTH WORKERS TO IMPROVE PERENNIAL MALARIA CHEMOPREVENTION UPTAKE IN CAMEROON: INSIGHTS FROM THE PLUS PROJECT

Lilly Claire Ekobika1, Charles Ndindjock1, Dominique Bomba2, Junior Youndi1, Joel Ateba1, Erica Mengue1, Annie Michèle Mabaly1, Marguerite M. Clougherty2, Malia Skjefte1, Meredith Center1

1Association Camerounaise pour le Marketing Social (ACMS)/Population Services International, Yaounde, Cameroon, 2Programme National de Lutte contre le Paludisme, Yaounde, Cameroon, 3Population Services International, Washington, DC, United States

Several factors limit access to malaria control services in Cameroon, such as low health seeking behaviors among beneficiaries, limited access to quality care, and data quality issues. Community health workers (CHWs) have proven effective in addressing some of these issues, linking their community to the health system as a first point of contact. CHWs are recruited through a process involving communities, decentralized territorial authorities, health area leaders, and traditional leaders, among others, and are enlisted in Cameroon’s Integrated National Strategy for the implementation of activities under community guidelines. Launched in 2021, the Plus Project supports countries to adapt, implement, and evaluate models of perennial malaria chemoprevention (PMC) using sulfadoxine-pyrimethamine (SP). In Cameroon, the NMCP adopted a five-contact model of PMC in late 2021 and requested the Plus Project to implement an eight-contact model in select districts, increasing the number of doses by adding contacts at 12, 18, and 24 months, and by offering community delivery of SP for PMC starting at six months of age. To date, 275 CHWs from four health districts have been trained and supervised by civil society organizations (CSOs), aligning with the current system of CHW supervision and management. The CHWs sensitize the population on PMC and encourage them to visit their health facility to receive SP for PMC at predefined contact points alongside routine vaccines and vitamin A. The data from CHW-led PMC activities were integrated into the national tools, which have been modified to include the community-based indicators of PMC. These data are reviewed during a monthly data validation meeting chaired by each health area chief before the paper-based record is uploaded into the online HMIS database. As CHW data is currently combined with data from the corresponding health facility, the Plus Project will receive additional reports of doses of SP for PMC delivered by CHWs and track how CHWs contribute to the delivery of this key intervention. Community delivery of SP for PMC is starting in March 2022, with initial data expected in May 2022.

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ADOPTION OF A PERENNIAL MALARIA CHEMOPREVENTION (PMC) STRATEGY IN BENIN: A CO-DESIGN PROCESS

Bienvenu Wakpo1, Firmin Houssou1, William Houndjo2, Cyriaque D. Aftoukou2, Jacques Kouakou2, Marguerite M. Clougherty2, Malia Skjefte1, Meredith Center1


In 2021, the Plus Project was initiated to generate data on the impact, effectiveness, and cost-effectiveness of perennial malaria chemoprevention (PMC) in Benin, among other countries, to support the widespread adoption of PMC to children under two. As a new strategy, key steps were taken to ensure the successful implementation of PMC in Benin and promote the sustainable integration of PMC into the existing health system. A co-design process was used to engage partners, stakeholders and governing bodies during the PMC development process. This included two multi-day strategic workshops with partners at national and subnational levels and the project team to 1) discuss barriers to Benin’s adoption of PMC, 2) determine target geographic areas for implementation, 3) outline the target population and PMC dosing schedule, and 4) identify existing supervision, M&E, and other relevant systems to leverage and integrate with PMC. The workshops included presentations, group work, consensus-building activities, and final decision-making, resulting in key project design elements, including the identification of the target population, implementation areas, and plans for PMC integration with the national HMIS. Participants agreed on an eight-contact model to administer doses of sulfadoxine-pyrimethamine (SP) to children through leveraging the existing immunization schedule, vitamin A and routine visits to health facilities. Participants selected three health zones in the North, Center and South for implementation (Bembéréke-Sinendè, Zogbodome-Bohicon-Zakpota, Klouékannè-Tovkind-Laï), based on an agreed inclusion criteria (weighted for: 1) malaria incidence and mortality in children under five, 2) vaccination coverage, and 3) population under two years) and exclusion criteria (areas where seasonal malaria chemoprevention is already implemented). This process facilitated participatory decision-making, fostered stakeholder engagement, and enabled collaboration among many partners and governing bodies. Lessons learned during the co-design process can be used to guide future country implementation and scale-up of PMC.
DESIGNING COMMUNITY ENGAGEMENT ACTIVITIES USING A HUMAN CENTERED APPROACH TO PROMOTE PERENNIAL MALARIA CHEMOPREVENTION ADOPTION: THE PLUS PROJECT EXPERIENCE IN BENIN, CAMEROON, CÔTE D’IVOIRE, AND MOZAMBIQUE

Jacques Kouakou1, Marguerite M. Clougherty1, Albertina Chihale2, William Hourdjo3, Junier Voundz4, Alan Dagoo5, Malia Skjette6, Lully Claire Ekobika6, Bienvenu Wakpo6, Elsa Nhantumbo6, Hans Bahibo8, Meredith Center9


Perennial malaria chemoprevention (PMC), the updated WHO recommendation replacing intermittent preventive treatment for malaria in infants (IPTi), is shown to reduce clinical malaria by 30% and anemia by 21% in children under two. Given these results, the Plus Project is supporting governments to implement different PMC models in four countries: Benin, Cameroon, Côte d’Ivoire, and Mozambique. The target population for PMC delivery includes children under two, who receive up to eight doses of sulfadoxine-pyrimethamine (SP) during routine visits for immunizations and vitamin A. While vaccination coverage in project countries is high, low attendance at vaccination appointments after the first year of life and inconsistent vitamin A supply at clinics can hinder the effectiveness of this new intervention. To promote consistent attendance and timely PMC delivery, a human-centered community engagement (CE) approach was developed for the Plus Project. CE activities were designed to build empathy, gather insights about potential barriers and facilitators to PMC uptake, and develop prototypes (IEP) for activities and messages. Key to this strategy, the plan was created to ensure that caregivers, community health workers, providers, and community leaders are the drivers of this change. To implement this approach, project teams and NMCP collaborators were invited to a Training of Trainers (TOT) to build capacity on the IEP tools, which included journey mapping, outlining trusted communication networks, and creating target group archetypes. Following the TOT, attendees organized a CE workshop where they conducted IEP activities in a selected district for each country. Insights gathered during these workshops allowed the project team to understand the facilitators and barriers to the uptake of PMC in each country, which will be used to tailor the CE plan to the needs and requests of the community. Through continued use of a human-centered CE approach, additional materials and activities will be implemented throughout implementation to promote improved attendance at vaccination clinics for PMC and other necessary child health services.

IMPLEMENTATION OF PHASE 1 OF PERENNIAL MALARIA CHEMOPREVENTION (PMC) IN CHILDREN UNDER TWO YEARS OF AGE IN ABENGOUROU, CÔTE D’IVOIRE

Hans Bahibo1, Hermann Akissi1, Soro N’wolo1, Stella Abbi1, Sarah Koffi2, Meredith Center3, Jacques Kouakou4, Sadate Soumaborou5, Marguerite M. Clougherty6, Malia Skjette6, Maa Tanch7, Colette Kokrasset7, Marcelin Dougonne7, Thérèse Bleu7, Nicole Lorng7, Paule-valérie Odjouho8


Malaria is the leading cause of morbidity in children under five in Côte d’Ivoire, responsible for 65% of deaths in 2020 with an incidence of 440 (per 1000 people at risk). According to WHO guidelines, perennial malaria chemoprevention (PMC), which consists of administering a full course of antimalarial treatment at predefined intervals to infants or young children, is recommended to prevent disease in areas of moderate to high malaria transmission. With support from the Project Plus, Côte d’ivoire began implementing PMC for children under two years of age in one health district in November 2022. Health centers providing routine immunizations (n=39/42) were included in the PMC activities. The PMC model adapted to Côte d’ivoire provides up to 5 doses of sulfadoxine-pyrimethamine (SP) to children during routine immunizations (DPT2, DPT3, R1, and R2) and vitamin A supplementation at 18 months. Monitoring of implementation is essential to assess progress and detect unforeseen challenges. Key performance indicators for the provision of PMC are based on routine health system data and include the number of doses of SP administered, the number of children experiencing side effects after administration, and the availability of SP. A new supervision module was created to measure provider adherence to PMC protocols, routine monitoring, and data quality processes, with data captured during routine supervision visits. As of February 2023, 9966 doses of SP had been distributed, including 8526 doses of SP1, 1421 doses of SP2, and 19 doses of SP3. No sites reported a shortage of SP and no side effects were documented. All 39 sites were visited by supervisors who found that 100% of the providers were trained and complied with the pediatric SP dosing and DOT strategy. The supervisors’ scores are being recorded and will be used to track changes in the quality of pediatric care delivery over time. The results of this first phase of implementation will be used as documentation for Côte d’ivoire on the sustainability of preventative care and for the revision of national malaria prevention guidelines.

KNOWLEDGE, ATTITUDES, PRACTICES AND ACCEPTABILITY OF DIGITAL PAYMENT BY OPERATORS OF THE INDOOR SPRAYING CAMPAIGN AGAINST MALARIA IN THE HEALTH DISTRICT OF KOUMPENTOUM (SENEGAL)

El Hadji Cheikh Abdoulaye DIOP

District Sanitaire de Koumpentoum, Koumpentoum, Senegal

Digitization of payments could improve worker welfare, patient welfare, and health system performance. The objective of this study was to identify factors associated with the acceptability of digital health payments by indoor malaria spray campaign operators. This was a descriptive and analytical cross-sectional study. Data were collected from operators in the 2022 domestic spray campaign. Collection took place from January 16 to January 28, 2023. We had studied the knowledge, attitudes, practices, and acceptability of digital health payment. Multiple logistic regression was used to identify factors associated with acceptability. Acceptability was 86.16% for the following reasons: perception of safety (68.55%), speed (55.35%), anonymity (46.54%) and time saving (42.14%). 88.31% of the cases were considered very good or good. The reasons for refusal were high shipping/transfer costs (100%), unavailability of service (59.09%), lack of network (54.55%) and handling errors (54.55%). In the multiple logistic regression, favoring digital payment in general (ORa = 8.21 [1.39-49.03]; p = 0.02), favoring digital payment in healthcare (ORa = 8.41 [2.02-34.93]; p = 0.0034), lack of experience using the service (ORa = 21.50 [3.92-118.68]; p = 0.0004), and satisfaction during use (ORa = 37.19 [5.99-230.81]; p = 0.0001) were predictive of acceptability of digital payment in healthcare. In conclusion, the digitization of health care payments is a challenge. It should be factored into health campaigns to improve outcomes, but telephone and internet expansion, co-payment reduction, and claims management must be improved.

astmh.org
Intermittent preventive treatment of malaria in school children (IPTsc) is currently recommended by the world health organisation (WHO) to be implemented in moderate and high endemic areas. However, its wider adoption will depend on acceptability to key stakeholders such as parents, teachers, malaria programs and also the implementation cost. A qualitative research was conducted, following a clinical trial (NCT03640403, N=1566) and an implementation research (NCT04245033, N>73000) assessing the effectiveness of IPTsc in schoolchildren of high endemic areas of Tanga region, north east of Tanzania. We aimed to understand the acceptability, experiences and adoption of IPTsc among parents, schoolchildren, teachers and program leaders on IPTsc using the RE-AIM framework. We also assessed the implementation cost in a real life operational setting including possible synergies with other school health intervention programs. About 108 focused group discussions were conducted (46 to parents and 62 to school children distributed equally by gender). We also conducted 64 in-depth interviews with teachers, community health workers(CHWs) and district and national malaria program officials. Themes obtained included, key factors of intervention acceptability, these included appreciated disease burden and the observed benefits following IPTsc intervention. To most schoolchildren, their acceptability to IPTsc program was positive with a noticeable decline in malaria episodes during the study period. Schoolchildren and parents also accepted the IPTsc delivery approach through their teachers. They described some adverse events following drug administration as mild and tolerable. Program officials and school teachers expressed positive comments on sustainability of the IPTsc programme, readiness for change and overcoming identified barriers to adoption. The average IPTsc delivery cost per round per child was 0.4 USD excluding drug procurement. IPTsc was widely acceptable among communities in the moderate and endemic areas and can be implemented at a minimal cost with a substantial high impact, making it a cost saving intervention.

**INTERMITTENT PREVENTIVE TREATMENT FOR MALARIA IN SCHOOLCHILDREN IN MODERATE AND HIGH ENDEMIC AREAS, NORTH-EASTERN TANZANIA**

Geofrey Makenga1, Edwin Lihelu1, Vito Baraka1, Misago D. Sethi1, Daniel Chala1, Bruno Mbombo1, Filbert Franvis1, Mercy Chiduc1, George Mwote1, Celine Mandara1, Daniel T.R. Minja1, Samwel Gesase1, Abdallah Luwasi2, Frank Chackuy2, Anna David2, Samwel Lazoraro2, Fabrizio Molteni2, Alex Nikayamba2, Jean-pierre Van geertruyden3, John P.A. Lusingu1, Hilde Bastiaens5

1National Institute for Medical Research, Tanzania, Tanga, Tanzania, United Republic of, 2National Institute for Medical Research, Tanzania, Dodoma, Tanzania, United Republic of, 3Swiss Tropical Public Health Institute, Dar es Salaam, Tanzania, United Republic of, 4Tanzania Medicines and Medical Devices Authority, Dar es Salaam, Tanzania, United Republic of, 5Global Health Institute, University of Antwerp, Antwerp, Belgium

**MECHANISMS FOR MANAGEMENT MISSINGNESS OF DATA IN THE HEALTH MANAGEMENT SYSTEM A CASE OF UGANDA MALARIA CONTROL PROGRAM**

David Amwonya1, David L. Smith2, John Cedric Rek1, Austin R. Carter2, Eganuyu Tom3

1Pilgrim Africa, Kampala, Uganda, 2University of Washington, Washington, WA, United States

In many clinical trials or time series setting, data are collected longitudinally over time. The researcher collecting hierarchical data is frequently confronted with incompleteness. Since the processes governing missingness are often outside the investigator's control, no matter how well the experiment or study has been designed, careful attention is needed when analyzing such data. In such studies, missingness, in particular dropout or non-reporting, is an often-encountered phenomenon. In this study we examine missingness in a longitudinal District Health Information systems (DHIS2). The DHIS is an online system used for data management and analysis purposes, health program monitoring and evaluation, facility registries and services. Particularly in this, study utilizes the malaria control program indicators to demonstrate how to manage missing data in informing policies at Ministry of Health Uganda. A number of methods have been or are being developed and tested to be integrated into the Uganda National Malaria Control Division's routine reporting system. We discuss commonly used but often problematic simple methods such as complete case analysis and last observation carried forward and contrast them to a number of viable candidates for a standard analysis, like direct likelihood, multiple imputation and versions of generalized estimating equations. Finally, we conclude with sensitivity analysis to crosscheck the proposed methods. The commonly used methods rest on strong assumptions, including missing completely at random for complete cases and unchanging profile after dropout for last observation carried forward. Such assumptions are too strong to generally hold. Multiple imputation gives a good and more plausible way of imputing missing values other than just using last
observation carried forward despite its computational complexity. Lastly, the selection and pattern mixture models will provide flexible modelling for the outcome and missingness processes at the same time they were used for sensitivity analysis.

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**SYNERGY BETWEEN FACILITY AND COMMUNITY-BASED SURVEILLANCE IN THE MALARIA VACCINE PILOT EVALUATION IN GHANA: BEST PRACTICES, CHALLENGES AND LESSONS LEARNT**

Abraham R. Oduro1, Aaron Kampimi2, Thomas Gyan3, Patrick O. Ansah4, Kwaku P. Asante5

1Research and Development Division, Ghana Health Service, Accra, Ghana, 2Navrongo Health Research Centre, Research and Development Division, Ghana Health Service, Navrongo, Ghana, 3Kintampo Health Research Centre, Research and Development Division, Ghana Health Service, Kintampo, Ghana.

To assess the programmatic feasibility of administering the recommended four doses, impact on mortality and its safety in the context of routine use. The WHO recommended the RTSS vaccine be piloted in countries with moderate to high malaria transmission. Ghana, Kenya and Malawi are participating in the pilot. A combination of facility and community-based surveillance approaches were used to estimate the effect of the vaccine on all-cause mortality, and malaria and gender-specific effects in children aged 5 to 39 months. A cluster randomized design with evenly split implementing and comparator clusters was deployed. The surveillance recorded all under-five deaths and conducted verbal autopsies on them. Vaccination status was determined for each deceased child to enable assess the risk of mortality. A series of engagement meetings were held with stakeholders as part of preparations. At the household, caregivers of the deceased children granted consent for verbal autopsy interview. Community key informants identified all deaths and reported same to verbal autopsy (VA) coordinators who were full-time workers of the health system. All VAs were conducted electronically using WHO 2016 VA instrument. Regional coordinators supervised the work and conducted quality control including re-interviewing randomly generated VAs each month. Overall, about 7361 community volunteers, 132 district verbal autopsy coordinators, 12 regional coordinators, project managers and mortality leads were the personnel involved. As at December 2022, a total of about 7732 deaths were documented with approximately 98% VAs conducted with about 0.6% consent refusals and about 90% cause of death assigned. Documented vaccine records including maternal recalls was over 90%. This presentation highlights the successes, innovations, best practices, challenges, and lessons learnt in this approach.

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**PREDICTION OF RESISTANCE PIPERAQUINE BASED ON ATYPIC PIPERAQUINE CHEMOSUSCEPTIBILITY ISOTOPIC DATA**

Lise Musset, Celia Florimond, Maxime Agranian, Beatrice Volney, Manon Discours, Yassamine Lazrek

Pasteur Institute in French Guiana, Cayenne, French Guiana

Surveillance of antimalarial efficacy is important for the control and elimination of malaria worldwide. Tools have been developed to study this parameter in vitro. Among them, the isotopic susceptibility assay is acknowledged as a gold standard method to evaluate the in vitro drug susceptibility of Plasmodium falciparum strains. However, for the antimalarial drug piperacrine (PPO), susceptibility inquiries often produce incomplete inhibition curves for resistant isolates even at high concentration. This makes phenotypic interpretation difficult and compels researchers to perform other assays deemed more reliable. Nowadays, survival assay represents the most robust method to identify PPO-resistant isolates. However, this method is time consuming. Besides, the large amount of data obtained with the isotopic one would benefit from being analyzable.

In this study, results obtained on 95 isolates collected in an endemic area with PPO resistance were analyzed in parallel through standard isotopic susceptibility assays and piperacrine survival assays. The growth parameters resulting from the isotopic method were then exploited in order to define a predictive approach assessing the in vitro resistance status of an isolate against PPO. Using lumefantrine (LU) drug-response data as reference, isolate growth ratios under PPO vs LU were evaluated and used to classified the isolates. We observed a correlation between survival rate and growth ratio PPOmax/LUmax allowing the selection of a growth ratio threshold of 2.8 beyond which resistance to PPO is strongly suspected. This alternate way to exploit previously stated “uninterpretable data” from isotopic susceptibility assay during longitudinal surveillance allows the prediction of piperacrine resistance phenotype with 75.8% (72/96) of accuracy.

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**UNITED STATES OF AMERICA DOMESTIC MALARIA DRUG RESISTANCE SURVEILLANCE USING TARGETED AMPICON DEEP SEQUENCING (TADS), 2018-2021**

Edwin Pierre-Louis1, Brooke Clemens2, Julia Kelley1, Dhruviben Patel1, My T. Nguyen3, Swarnali Louha1, Je-Hoon M. Oh1, Kimberly E. Mace1, Dragan Ljolje1, Christina Carlson1, Eldin Talundzic1, Susan Madison-Antenucci5

1Centers for Disease Control and Prevention, Atlanta, GA, United States, 2Parasitology Laboratory, Wadsworth Center, New York State Department of Health, New York, NY, United States.

Plasmodium infection and malaria continue to persist worldwide; therefore, global efforts towards control and elimination remains exigent. Some of these efforts include utilizing novel therapeutics and fortifying comprehensive surveillance programs. Despite having eliminated malaria in the mid-1950s, the United States (US) continues to track and support national efforts for public health surveillance of imported malaria cases. The objective of this surveillance activity is to characterize drug resistance mutation profiles of imported malaria cases in the US using molecular methods. For this purpose, we used the previously established targeted amplicon deep sequencing (TADS) and associated bioinformatics pipeline Malaria Resistance Surveillance (MaRsS) to genotype molecular markers of drug-resistance in six full-length P. falciparum (Pf) genes (PfCRT, PfMDR1, PfK13, PfDHPS, PfDHFR and PFCYT) to identify known single nucleotide polymorphisms (SNPs) associated with drug resistance. Data from 1209 imported malaria specimens reported to the Centers for Disease Control and Prevention, in collaboration with the New York State Department of Health Wadsworth Center, from 2018-2021 are presented. We found that 1077 specimens (89%) were positive for Pf. Mutations in the PfDHFR gene (pyrimethamine-resistance) were observed in 88% of specimens while mutations in the PfDHPS gene (sulfadoxine-resistance) were present in 81% of specimens. Additionally, mutations in both PfMDR1 (chloroquine-resistance) and PfCRT (chloroquine-resistance) genes were detected in 60% and 30% of specimens, respectively. Next, mutations in PfcytB gene (atovaquone-proguanil-resistance) were present in 0.1% of specimens. Furthermore, we found 0.9% of specimens carried previously reported mutations in the PfK13 gene; however, these SNPs are not associated with artemisinin-resistance. Overall, our data summarize drug resistance markers among specimens from patients with imported malaria and provides information about molecular resistance to eventually aid with treatment guidelines for travelers and patients in the US.
DIGITALIZATION OF MALARIA CAMPAIGNS IN NIGERIA: IMPACT, CHALLENGES, KEY FACTORS FOR CONSIDERATION AND OPPORTUNITIES

Onyebuchi Augustine Okoro1, Kunle Oreagbagb2, Chukwu Okonkwo1, Oluwaleke Jegede3, Uchenna Igbokev3, Shina Aladeshawe3, Perpetua Uhomoibi4


Malaria remains a major public health issue in Sub-Saharan Africa, with Nigeria contributing 27% of global burden. Among other interventions, malaria campaigns (Insecticide Treated Net (ITN) & Seasonal Malaria Chemoprevention (SMC)) remain proven impactful strategies to reduce malaria burden. In 2018, National Malaria Elimination Programme (NMEP) and supporting partners introduced digitalization as part of efforts to improve malaria campaign activities. While some progress has been made, challenges remain. This work aimed to document progress made with malaria campaign digitalization in Nigeria, challenges and opportunities for improvement. A mix of qualitative & quantitative approaches were utilized to collect relevant information including in-depth key informant interviews, focus group discussions using a structured questionnaire guide and an interviewer-administered survey tool from campaign implementers at national and sub-national levels. Digitalization of malaria campaigns occurred in 27 of 36+1 states with support from 4 donors with no clear plans to scale. Deployment of digital tools for malaria campaigns has enhanced payment of campaign staff and improved visibility during implementation at the sub-national level through real-time monitoring. There are 5 tools deployed for malaria campaign processes. The utilized digital tools are not linked with the National Malaria Data Repository limiting opportunities for decision making. While there are functional platforms for coordination of malaria campaign digitalization, there is no common agenda guiding digitalization efforts. Moreover, efforts are partner-driven and there is no government-led team set up to sustain the digitalization effort. Nigeria has made remarkable progress in its malaria campaign digitalization efforts with over 50% of States in the country digitizing at least one of its malaria campaigns. However, coordination gaps still exist. We recommended that NMEP develop a policy document to guide digitalization efforts across all stakeholders and also set up a digital technology unit to coordinate digitalization efforts by stakeholders.

A ROBUST MALARIA DATA INTEGRATED, STORAGE AND ANALYTICAL SYSTEM CRITICAL FOR ENHANCING SURVEILLANCE


Ministry of Health, Lusaka, Zambia

The Zambia National Malaria Elimination Programme (NMEP) has adopted Surveillance, Monitoring, Evaluation and Operations Research as a core intervention in its subnational elimination and control efforts. It is therefore, important to have a comprehensive and integrated malaria information system that collates all programmatic data into one platform. However, the ministry has three systems that aggregate malaria data without allowing for data linkage. The Health Management Information System (HMIS) for service and disease data, electronic Logistic Management Information System (eLMIS) for commodity data and the Malaria Rapid Reporting System (MRRS) for community level data. In 2019, the NMEP and Zenysis with support from the Global Fund undertook an assessment of the ability of the information system to respond to programme needs. The first step involved conducting interviews with the NMEP and other key stakeholders on the data needs of the programme. The second step involved an assessment of the eLMIS, HMIS and MRRS. The findings reviewed that the three systems were related and all vital for malaria programming but not integrated. It was also difficult to link malaria disease burden to malaria service and malaria commodity data without resorting to manual data manipulations. The coverage of these systems was at different organization unit level. The eLMIS and the HMIS did not capture community level data. On the other hand, the MRRS collected community level data but was at 67% coverage. To that effect, the NMEP implemented a platform that would house malaria data from the three systems. The Platform collated data from these systems using an Application Programming Interface. Through this, the programme is able to link disease burden with availability of malaria commodities and interventions coverage bringing about efficiency and effectiveness in malaria Programming. There is therefore, need to roll out the eLMIS to community level and integrate the HMIS and MRRS. This will facilitate a seamless flow of data from the community to the national level, and ensure that all stakeholders have access to timely and accurate malaria information.

MALARIAGEN AMPICLON TOOLKIT: A GENOMIC SURVEILLANCE TOOL TO SUPPORT MALARIA CONTROL AND ELIMINATION

Cristina V. Ariani, on behalf of MalariaGEN, on behalf of Genomics Surveillance Unit

Wellcome Sanger Institute, Hinxton, United Kingdom

National malaria control programmes, NMCP, need to maximise the impact of their interventions. To achieve this, monitoring emerging drug and insecticide resistance is essential for informed decision making. Our objective is to accelerate genomic data production at scale by providing a public health, research and capacity-building framework to accommodate the growing and changing needs for parasite surveillance data in malaria endemic regions, and putting the power of data generation into the hands of subject matter experts in malaria endemic countries. By generating genomic data closer to the public health decision makers, national priorities can be incorporated at every stage from sampling frameworks to data analysis - revolutionising the delivery of translational research to policymakers. We developed the MalariaGEN Amplicon Toolkit for targeted monitoring of known markers of interest in malaria parasites. Originally implemented at the Wellcome Sanger Institute, this low cost technology platform is now additionally being implemented in 6 countries across Africa and South East Asia and supporting national genomic surveillance of malaria response in 13 countries. By utilising the same platform, these data can be brought together for comparison analysis across borders and around outbreaks, prioritising the needs of public health over research outputs. Translating protocols and tools for multi-laboratory implementation greatly enhances the potential temporal spatial coverage of genomic surveillance, facilitates data interoperability and amplifies the power of the data produced for public health benefit.

STANDARDISATION OF LABORATORY PROTOCOLS AND MULTINATIONAL IMPLEMENTATION TO ESTABLISH GENOMIC SURVEILLANCE CAPACITY IN MALARIA ENDEMIC COUNTRIES

Mozam Ali1, Andrew Mains1, Thomas Pemberton1, Andrews Asante1, Joyce Ngoi2, Kukua Thompson3, Eniyou Oriero4, Nhien Nguyen Thanh Thuy5, Angela Rumase6, Nadia Fatid1, Pinkan Pertwi Karidomido7, Agatha Mia Puipitasari7, Rintis Noviyanti7, Sarah Auborn7, Olivo Miotto7, Gordon Awandare2, Lucas Amenga-Etego7, Alfred Amambua-Ngwa2, Cristina Ariani2, Sónia Gonçalves1, Shawanthi Rajatilleka1

1Wellcome Sanger Institute, Hinxton, United Kingdom, 2West African Centre for Cell Biology of Infectious Pathogens, University of Ghana, Accra, Ghana, 3MRC Unit, The Gambia at the London School of Hygiene and Tropical Medicine, Banjul, Gambia, 4Oxford University Clinical Research Unit, Ho Chi Minh City, Viet Nam, 5Menzies School of Health Research, Darwin,
With the emergence of resistance to control measures, National malaria control programmes need effective tools to monitor and plan effective interventions in the face of increasing drug and insecticide resistance. The Amplicon Toolkit has been developed to meet the needs of genomic surveillance of malaria, targeting genes associated with P. falciparum drug resistance. Implementation and ongoing support accompany protocols and tools at every stage from laboratory to data integration and interpretation. As the implementation of genomic surveillance protocols for routine surveillance operations is particularly dependent on standardised methodology, we worked with teams in laboratories in West Africa and South East Asia, to adapt protocols capable of being implemented in multiple geographical locations. Through harmonisation of genomic surveillance protocols for drug resistance in P. falciparum, and using bespoke end-to-end laboratory implementation plans encompassing establishing workflows, procurement support and tailored laboratory training, we have supported four laboratories in Ghana, The Gambia, Indonesia and Vietnam, in establishing genomic surveillance capacity and thereby establishing inter-laboratory reproducibility. The protocols and training programmes developed, can now be deployed in other laboratories who wish to establish genomic surveillance capabilities. Additionally, leveraging on these previous achievements in capacity building, we aim to establish regional sequencing hubs that will provide National Malaria Control Programmes and Public Health Institutions with timely, actionable genomic surveillance data. Through establishing a Surveillance Hub Training programme, developing logistical processes and strengthening cross-border partnerships, we aim to provide proof of concept of public health impact and an operational model that will lead to multilateral investment in more comprehensive regional surveillance frameworks for malaria which can also be adapted for the surveillance of other endemic diseases.

**EMPOWERING HEALTH DISTRICTS IN SUSTAINING HIGH-QUALITY MALARIA CASE MANAGEMENT AND DIAGNOSTIC SERVICES PER NATIONAL GUIDELINES IN EQUATORIAL GUINEA**

Matilde Riloha Rivas1, Olivier Tresor Donfack2, Ramona Mbata Andeme1, Martin Eka Ondo2, Consuelo Oki Eburi1, Delicia Esono Mba1, Kyle R. DeBoer3, Sandra Incardona3, Carlos A. Guerra2, Wonder P. Phiri4, Guillermo A. Garcia5

1Ministry of Health and Social Welfare, National Malaria Control Program, malabo, Equatorial Guinea, 2MCD Global Health, Malabo, Equatorial Guinea, 3MCD Global Health, Silver Spring, MD, United States

Malaria diagnosis and treatment have been cornerstones of controlling transmission on Bioko Island for the past 20 years. Accessible, high-quality health services are essential for managing uncomplicated and severe malaria cases, enhancing malaria detection, and preventing malaria in high-risk groups including pregnant women. However, health districts face various challenges, including inadequate training, insufficient resources, and a lack of effective monitoring systems. We present a package of interventions that aim to enhance service delivery on Bioko Island in line with national case management guidelines and the operationalization of the district level health system by the Ministry of Health and Social Welfare of Equatorial Guinea. High-standard training manuals for clinical management and diagnosis of malaria, outreach training and supportive supervision (OTSS) manuals and checklists, state-of-the-art training materials, and job aids were developed. Real-time operational dashboards were designed to render OTSS indicators. The District Health Information System (DHIS2) software was customized and deployed to follow key indicators on malaria case management and diagnostic practices. This monitoring and evaluation system will be used for decision-making and adaptive management, from providing evidence for resource allocation to deciding on training and supervision priorities. A cohort of trainers in each district of Bioko Island will be identified and trained in case management and diagnostic guidelines, and supervision practices. Trainers will be evaluated over time on their effectiveness and use of data to adapt their efforts and optimize their support to the health districts. The comparison of indicators before and after implementation of this package will allow to assess changes in case management practices and adherence to guidelines within health districts on Bioko Island, while identifying challenges and areas for further improvement, providing insights into the effectiveness of the training materials and supportive supervision systems.

**THE POTENTIAL OF SATELLITE-DERIVED BUILDING POLYGONS DATA AS A PROXY TO ON-THE-GROUND HOUSEHOLD MAPPING TO PROVIDE DENOMINATOR FOR PUBLIC HEALTH INTERVENTIONS: A PILOT STUDY IN BATA DISTRICT, EQUATORIAL GUINEA**

Jeremias Nzamio Mba Eyono1, Restituto Mba Ngueama Avue1, David Galick1, Victor Mba Micha1, Julia Yumbe Baka1, Jose Osa Nfumu2, Matilde Riloha Rivas2, Carlos A. Guerra2, Guillermo A. Garcia2

1MCD Global Health, Malabo, Equatorial Guinea, 2Ministry of Health and Social Welfare, Malabo, Equatorial Guinea, 3Ministry of Health and Social Welfare, National Malaria Control Program, Malabo, Equatorial Guinea, 4MCD Global Health, Silver Spring, MD, United States

To optimize the implementation of public health interventions at the household level, it is necessary to have accurate denominators. Ideally, programs should have recourse to a household database to facilitate

**MONITORING, MENTORING AND MOTIVATION VISITS TO COMMUNITY HEALTH WORKERS AS A MECHANISM TO SUSTAIN HIGH QUALITY OF CARE IN MALARIA COMMUNITY CASE MANAGEMENT: A CASE OF FOUR HIGH MALARIA BURDEN PROVINCES IN ZAMBIA**

Sarah Shankwaya1, Mathews Monde1, Chabu C. Kangale1, Marie-Reine I. Rutagwera1, Caroline Phiri-Chibawe1, Webby E. Phiri1, Paul Tembo1, Jennifer Somtore2, Busiku Hamainza2

1PATH PAMO Plus, Lusaka, Zambia, 2US President’s Malaria Initiative (PMI)/United States Agency for International Development (USAID), Lusaka, Zambia, 3Zambia Ministry of Health National Malaria Elimination Centre (NMEC), Lusaka, Zambia

Since 2011, 20,448 community health workers (CHWs) have been deployed in Zambia to provide basic malaria diagnostic and treatment services in their respective communities. Of these, 5,765 work in the four President’s Malaria Initiative (PMI) PAMO Plus-supported provinces of Eastern, Luapula, Muchinga, and Northern. In 2020, the National Malaria Elimination Programme (NMEP) introduced monitoring, mentoring, and motivation (MMM) as an approach to sustain clinical quality of care (cQoC) and data quality among CHWs. MMM includes observing CHWs’ clinical skills in testing with a rapid diagnostic test (RDT) and administering the appropriate treatment. This case study describes the MMM process and its importance to sustaining and measuring cQoC at the community level. Twice in 2022, CHWs met at their health facility where district and health facility mentors directly observed the CHWs consulting a suspected malaria patient. Mentors assessed six key variables and provided immediate feedback. Data were collected using Open Data Kit and analyzed in STATA and a score was assigned to each variable. In 2022, 2,033 CHWs with more than a year’s experience participated in MMM. The results show that CHWs’ case management skills are generally high. Overall, 96% of the CHWs scored above 80% (the minimum acceptable score). Of the 2,033 CHWs, 94% identified danger signs correctly, 97% made the correct decision to treat locally or refer patients to a health facility, 96% dispensed the correct treatment, and 95% administered RDTs correctly. The findings of this study indicate that MMM is a promising approach for providing individualized mentorship to improve and maintain high cQoC among CHWs. It also serves to alert mentors should CHW skills decrease, allowing for immediate action to be taken. The 2022 baseline data will be used to monitor cQoC over time.
planning, coordination, implementation, and monitoring of activities. The gold standard is a household mapping system that not only provides a complete list of locations but also allows spatial decision making. Such a system, however, can be very expensive and laborious as it requires geo-locating houses in the field. Satellite imagery promises alternative resources for mapping buildings and generating household denominators. Here, the recently released Open Buildings (OB) data set from Google was tested in Equatorial Guinea (EG). Three communities were randomly selected in Bata district, in mainland EG, to assess the sensitivity (recall) and positive predictive value (precision) of OB against household mapping on the ground. According to OB, a total of 1,174 buildings were identified in the three communities, which were then verified in the field. OB identifies buildings without any distinction of type and three levels of uncertainty of being a building. Field teams were deployed in these communities and could classify each building according to use (i.e., household, business, government offices and other use). They also identified any households not present in the 1,174 polygons. Hence, true positives were considered polygons mapped in OB and verified in the field as households. False positives were those polygons mapped in OB but identified in the field as either buildings for use other than household or polygons that did not correspond to a building in the field. False negatives were households identified in the field that did not have a corresponding polygon in OB. These data will help evaluate OB as a source of household denominators for public health interventions. Equatorial Guinea’s political will to promote public health programs nationwide makes this crucial. Satellite-derived building polygons can be a surrogate for household mapping could save resources, improve intervention planning, and scale.

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GENETIC ANALYSIS OF MALARIA IN PALAWAN, PHILIPPINES REVEALS HIGHLY MONOCLOWAL INFECTIONS IN HIGH TRANSMISSION AREA

Aaron Nicholas Elliott1, Malou Macalino2, Bryan Greenhouse1, Chris Drakely3
1University of California San Francisco, San Francisco, CA, United States, 2LSHTM-Nagasaki University, Nagasaki, Japan, 3LSHTM, London, United Kingdom

The Global incidence of Malaria has decreased in the last 2 decades, but eradication still remains a challenge in many areas. Over 90% of malaria cases occur in the Palawan region of the Philippines. Genetic data can help guide elimination strategies by highlighting sources of infection and key transmission routes. Following an enhanced surveillance approach in Palawan, Philippines using rolling cross-sectional surveys, we combined genetic data with household coordinates to characterize the genetic diversity and transmission dynamics of malaria parasites in the Palawan study site. We successfully genotyped 247 dried blood samples, collected during the years 2016-2018 using a panel of 217 targeted amplicons, specific for P. falciparum. Targets covered areas of drug and diagnostic resistance and regions of high parasite diversity. From the resulting genetic data we estimated Effective COI (eCOI) and allele frequencies using an MCMC based approach, and intersample pairwise relatedness using a probabilistic model (DOCler). Calculated mean eCOI for each individual was 1.03, with an interquartile range (IQR) of 0.308. The data suggests that the majority of the infections are monomodal with 90 samples deemed as polyclonal infections having mean eCOIs >1.1. Our analysis showed that 148 samples had some relatedness to other infections (r >0.6), forming one large cluster (comprising 30 infections) and several smaller clusters. Further analysis will be able to infer specific transmission networks within these clusters. Our clusters were not regionally defined suggesting that any intervention will need to be administered on a larger scale and not local. Overall our investigation of genetic data coupled with corresponding geographic data displays promising methodology in infection mapping and transmission analysis of malaria in the Philippines.

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BENCHMARKING COMMUNITY CASE MANAGEMENT WORKFORCE NEEDS AND MALARIA COMMODITY DEMAND ACROSS SUB-SAHRANAFRICA USING GEOSPATIAL OPTIMIZATION

Justin Miller1, Emily Hilton1, Samantha Herrera2, Danya Rogers1, John M. Miller1, Travis Porter1, Hannah Slater1, Adam Bennett1
1PATH Malaria Control and Elimination Partnership in Africa (MACEPA), Seattle, WA, United States, 2PATH Malaria Control and Elimination Partnership in Africa (MACEPA), Washington, DC, United States, 3PATH Malaria Control and Elimination Partnership in Africa (MACEPA), Lusaka, Zambia

Community health workers (CHWs) provide essential access to malaria treatment in many parts of Africa, especially for populations in hard-to-reach areas with limited access to health facilities. CHWs need to be geographically located so that they are within walking distance of populations in need, with manageable workloads based on the population density and disease burden in their catchment area. In this study, we estimate the number of CHWs needed in each second administrative unit across 31 countries in sub-Saharan Africa based on population density and travel time. Numerical optimization was used to determine the optimal number (and location) of CHWs needed to ensure all populations were within a two-hour walk of a CHW while limiting to a feasible service population size. A modeled malaria incidence surface was used to translate burden into estimated CHW workload, based on expected rapid diagnostic tests (RDTs) per month, to inform feasibility and commodity needs for integrated community case management (ICCM). We also conducted a prioritization exercise using public health facility data to identify the most underserved populations to inform CHW expansion. Our results estimated around 215,000 CHWs would be required to achieve saturation of coverage within two hours of walking travel time, of which about 102,000 (47.7%) would be required for populations greater than 5 km from a health facility. There was a wide range of expected RDTs conducted in a typical month (54.8, IQR: 32.2 - 66), which increased substantially during the peak transmission season (163.7, IQR: 90.5 - 218.5), highlighting the need for context-specific targets for coverage populations per CHW. These estimates provide a benchmark for measuring progress toward universal healthcare coverage goals and a methodological framework for developing country-specific estimates and designing optimal ICCM strategies. By improving access to care for hard-to-reach populations, scaling up CHW programs could have a substantial impact on achieving universal health coverage and the Sustainable Development Goals by 2030.

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SUBNATIONAL TAILORING AND TARGETING OF ANTI-MALARIA INTERVENTIONS IN ETHIOPIA

Amir Siraj1, Gudissu Asfessa2, Hiwot Solomon2, Asefaw Getachew3, Gezahegn Tesfaye3, Achamytesh Sisay4, Kebede Etana4, Ayele Tyou4, Mebrahtom Haile2, Belendia Serda5, Asnakew Yeshiwondim4, Dereje Dillu4, Berhane Tesfay3, Getachew Abebe5, Aranxta Roca-Feltrer4, Adam Bennett4, Hannah Slater1
1PATH Malaria Control and Elimination Partnership in Africa (MACEPA), Seattle, WA, United States, 2Ministry of Health, Addis Ababa, Ethiopia, 3PATH Malaria Control and Elimination Partnership in Africa (MACEPA), Addis Ababa, Ethiopia, 4PATH Malaria Control and Elimination Partnership in Africa (MACEPA), Maputo, Mozambique

Ethiopia has shown steady progress in reducing malaria morbidity and mortality, following a major scale-up of malaria control interventions and an extensive deployment of health extension workers at lower administrative units. Despite these gains, the country is facing a malaria upsurge recently, and there remain many areas still reporting high incidence, including along the western borders where large numbers of seasonal workers migrate annually. Diverse climatic factors, insecticide resistance, and lack of appropriate control interventions targeting migrant workers are among additional complicating factors. In this study, we developed a sub-national tailoring (SNT) process, and classified woredas based on...
their parasite incidence, level of seasonality, level of insecticide resistance, and the presence of seasonal migrant workers to informing the targeting of appropriate control interventions. We then used a malaria transmission model to estimate the impact of scenarios including the use of seasonal malaria chemoprevention (SMC) among children under five, Pyrethroid (PBO) nets in areas with high insecticide resistance, and targeted mass drug administration (tMDA) among migrant workers, with a business-as-usual scenario assuming continuation of the current control interventions, while accounting for seasonal movement between high and low transmission woredas. Results show the largest effect of SMC among resident under-five children in the high transmission areas, while PBO nets had the largest effect among the resident population in targeted low and moderate transmission areas. A combination of tMDA for migrant workers at destination and PBO nets in moderate and low-risk source woredas is estimated to yield the highest reduction in annual clinical incidence. With a model-based SNT leading to an effective mix of targeted interventions, Ethiopia can further reduce malaria transmission and continue progressing toward its elimination goal.

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COMBAT LAO PDR: FEASIBILITY AND EFFECTIVENESS OF COMMUNITY-BASED ACTIVE CASE DETECTION AND TREATMENT

Tim Finn1, Sarah Cassidy-Seyoum1, Keobouaphone Chindavongs2, Viengxay Vanisaveth1, Sengkeo Vongvixay1, Watthana Lasichanh1, Michelle Hsiang1, Chris Cotter1, Adam Bennett1

1University California San Francisco, San Francisco, CA, United States, 2Centre for Malaria, Parasitology, and Entomology (CIMPE) Lao PDR, Vientiane, Lao People’s Democratic Republic

Lao PDR has achieved dramatic success in reducing its malaria burden by implementing robust intervention coverage and surveillance. One current strategy is the 1-3-7 approach to case investigation and reactive case detection (RACD) targeting neighboring households. We conducted a pragmatic randomized controlled trial to evaluate the operational feasibility and effectiveness of enhanced RACD with HS-RDTs against the passive case detection standard of care (SOC). Thirty-two health centers were randomized to conduct RACD or SOC from September 2020-August 2021. RACD was conducted in index households and five neighboring households, with all enrolled receiving a standard RDT and HS-RDT and providing dried blood spots (DBS) for PCR analysis. RACD response coverage was 91% of index cases (n=225 followed-up, with 3,254 individuals in 498 households enrolled. RACD testing coverage was 88.2% of eligible individuals, with fifteen secondary Plasmodium vivax (PV) infections detected by standard RDT, yielding a household test positivity rate (HTPR) of 0.5%. Together, 91.7% of index and RACD cases detected by RDT were PV versus 8.3% P. falciparum (PF). Results from PCR analysis for 2,718 household samples yielded a HTPR of 3.7%. Whereas PCR for index cases found a similar 91.0% PV distribution among positive samples, household members detected through RACD were positive for PV (63%), PF (5%), P. malariae (28%) and P. knowlesi (14%) in mono or co-infections. Mono-infection with Pf or Pk accounted for 30% of all PCR positives, with Pk detected in multiple health centers. Results suggested that RACD is feasible in Lao PDR but is not an optimal use of resources given low sensitivity of standard field diagnostics. Data analysis is ongoing as PCR results were completed in March 2023. This trial yielded valuable data on the utility of RACD in Lao PDR, as well as on speciation and potential health risks to the population after Pf and Pk cases decline from novel malaria infections including Pk that are currently not detected by RDT.

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A PROGRAM EVALUATION OF COMMUNITY HEALTH WORKER-LED REACTIVE CASE DETECTION AND ITS IMPACT ON MALARIA MORBIDITY AND MORTALITY IN ZAMBIA

Ellen Ferriss1, William Sheehan1, Chris Lungu2, Katula Slumber2, John M. Miller1, Hannah Slater1, Adam Bennett1

1PATH, Seattle, WA, United States, 2PATH, Lusaka, Zambia

Reactive case detection (RACD) is conducted by programs globally as a malaria surveillance and elimination strategy, but there is limited evidence of its impact. In addition to improving case detection, RACD may clear asymptomatic infections from the population and reduce time-to-treatment, preventing cases from progressing to severe disease or death. In Zambia, community health worker (CHW)-led RACD has been scaled up since 2013 as part of community case management. This retrospective analysis used routine DHIS2 surveillance data to characterize RACD implementation from 2015 through 2022 and assess RACD impact on malaria hospital admissions and deaths from 2017 through 2021 using multilevel regression modeling. From 2015 through 2022, 12,453 CHWs conducted 4,210,166 reactive tests, identifying an additional 1,201,748 cases. RACD was conducted intensively in low transmission areas like Southern Province and inconsistently or infrequently in higher burden areas. On average, CHWs who performed RACD conducted 4 index case follow-ups per month and 8 tests per follow-up. RACD intensity increased from 2015, peaking at 0.40 reactive tests per passively detected case nationally in 2019. However, by 2022, testing decreased to 0.11 reactive tests per case. Higher passive case counts were associated with both a lower likelihood of follow-up and fewer tests per follow-up conducted. At the district level, each additional reactive test per passively detected case was associated with 7% and 4% reductions in the monthly rate of under-5 and all-age malaria hospitalizations, respectively (under-5: IRR = 0.93, 95% CI = 0.88-0.99; all-age: 0.96, 0.92-1.01), and 8% and 13% reductions in under-5 and all-age malaria deaths (IRR = 0.9, 95% CI = 0.87-1.00; IRR = 0.87, 95% CI = 0.77-0.96), after controlling for vegetation, rainfall, temperature, month, year, urbanicity, incidence, malaria care provider density, and mass drug administration, bed net, and indoor residual spraying coverage. These reductions were modest in comparison with those associated with malaria care provider density, highlighting the greater importance of increasing access to care.

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DOCUMENTING THE EVIDENCE OF ROUTINE DATA QUALITY AUDITS ON MALARIA DATA REPORTING ACCURACY IN ZAMBIA, 2015-2021

Smita Das1, Arantxa Roca-Feltz1, Marie-Reine I. Rutagwera1, Christopher Lungu1, Prudence Malama1, Mathews Monde1, Ignatius Bandia1, Mercy M. Ingwe1, Busiku Hamainza1, Adam Bennett1, Michael Hainsworth1

1PATH, Seattle, WA, United States, 2PATH, Maputo, Mozambique, 3PATH, Lusaka, Zambia, 4Zambia National Malaria Elimination Centre, Lusaka, Zambia

Routine data quality audits (DQAs) are implemented by country malaria programs to assess and improve data reporting accuracy, but they can be resource intensive, and their impact on accuracy over time is rarely documented. We evaluated accuracy in health facilities (HF’s) reporting into Zambia’s weekly malaria rapid reporting system using DQA datasets from 2015-2021. In Southern and Western provinces, DQAs began in 2015 in 155 HFs and expanded to 645 HFs across 33 districts by 2021. HFs were audited 1 to 7 times during the study period. Accuracy was measured using the weighted average percentage error (WAPE), and accuracy strata were defined as high (≥ 85%), medium (70-85%), and low (< 70%). Three data elements were analyzed: outpatient department (OPD) attendance, rapid diagnostic test (RDT)-tested cases, and RDT-positive cases; the mean of the three data element median accuracies determined overall accuracy. Except for RDT-positive cases, median accuracy increased from 2015 to
LOST TIME, LOST LIVES: INVESTIGATING DETERMINANTS OF DELAYED MALARIA TREATMENT IN SUB-SAHARAN AFRICA’S CHILDREN

Jailos Lubinda, Paul Castle, Susan Rumisha, Paulina Dzianach, Daniel Weiss, Peter Gething
Telethon Kids Institute, Malaria Atlas Project, Cockburn City, Australia

Early malaria diagnosis and treatment are critical to prevent death and reduce disease transmission. Evidence from the literature shows that delayed treatment increases the odds of disease progression to severe malaria and/or death, affects drug effectiveness, increases the chance of onward transmission, and could lead to drug resistance. This study analyzed data extracted from demographic health surveys conducted between 2005 - 2022 consisting of about 600,000 sub-Saharan African children treated with antimalarials. We used hierarchical Bayesian modelling frameworks to model the spatio-temporal proportion of children who delayed receiving antimalarial treatment and the associated factors. The study further compared the magnitude of the delayed treatment between the 24-hour and 48 hours policy recommendations, deriving region and country estimates by child age, household wealth, urbanicity and endemicity. During the 18 years period, the study found that delayed treatment was a significant problem in sub-Saharan Africa, with over a quarter of children receiving treatment late. No significant difference was observed between very young (under 2 years) and older children; households in rural areas and those with low wealth are not performing well. However, the gap between rural and urban households is closing over time. Very little improvement was observed in areas with high transmission. Primary contributing factors to the delays included household size, wealth quintile, PPR malaria risk, residence (rural or urban), illness severity, child’s age, gender, seasonality, maternal age, and education.

The findings suggest a need to invest in improving effective malaria case management, particularly early diagnosis and prompt treatment, especially for the most vulnerable and hard-to-reach populations and in high transmission areas. This is a vital component of malaria control and elimination strategies. The study findings could be used to assist in creating more effective strategies to combat malaria and help reduce malaria mortality among children in sub-Saharan Africa.

IMPLEMENTING GENOMIC SURVEILLANCE FOR MALARIA IN GHANA: OPPORTUNITIES AND CHALLENGES

Joyce M. Ngoi, Collins M. Moranga, Kukua A. Thompson, Gordon A. Awandare, Lucas Amenga-Etego
West African Centre for Cell Biology of Infectious Pathogens, Accra, Ghana

Malaria remains a significant public health problem in sub-Saharan Africa despite intensified malaria control efforts. Malaria control programs have historically relied on surveillance measures like parasite counts, entomological inoculation rates, incidence rates and self-reported travel history. While these metrics provide some idea of malaria transmission dynamics, they are less sensitive in areas of declining transmission intensity. They also provide limited information on parasite diversity and mechanisms of adaptation to environmental and interventional pressures. Next generation sequencing (NGS) has revolutionized genomic research by allowing scientists to understand complex biological systems at a greater depth. This has created new opportunities to study and understand the genomic landscape of malaria control and ultimately generate actionable data that could inform NMCPs to develop more effective and sustainable malaria control and elimination strategies. Unlike traditional genotyping methods, NGS provides a more refined and sensitive high throughput, cost-effective and scalable means of surveillance. Building on existing infrastructure and expertise, this project aimed to establish a genomic surveillance hub in Ghana to perform targeted deep amplicon sequencing of malaria parasites. Using this approach, we can assess the distribution of variants associated with drug resistance both at patient and population level enabling the efficient tracking of novel variants as well as known drug resistant loci. Implementing such a system in a low resource setting, however, comes with significant challenges. Some of these challenges include slow reagent supply chain, inflated reagents costs, lack of local technical support, poor retention of trained staff and insufficient core NGS and bioinformatic infrastructure. Here, we present our experience with
PRIORITIZATION OF PLASMODIUM FALCIPARUM ANTIGENS ASSOCIATED WITH REDUCED RISK OF MALARIA DURING PREGNANCY

Lucy Mwai1, Jesse Gitaka1, Hikaru Nagakawa2, Sebastian Musundi1, Takafumi Tsuboi5, Eizo Takashima2, Bernard N. Kano1
1Centre for Research in Infectious Diseases, Directorate of Research and Innovation, Mount Kenya University, Thika, Kenya, 2Division of Malaria Research, Proteo-Science Center, Ehime University, Matsuyama, Japan, 3Division of Cell-Free Sciences, Proteo-Science Center, Ehime University, Matsuyama, Japan

Plasmodium falciparum infection during pregnancy leads to substantial maternal and infant morbidity and mortality. Such infection may result to placental malaria when infected erythrocytes adhere to the placenta via parasite-derived ligands. Despite the risk of infection being similar for women of all gravidities, the risk of poor birth outcomes is increased in primigravida due to lack of protective antibodies against placental malaria parasites. Thus, understanding how specific P. falciparum proteins interact with host’s immune system during first and subsequent pregnancies provides insights on immunopathology of malaria and guide vaccine target prioritization. In this study, we assessed human antibody reactivity to 698 P. falciparum recombinant proteins from different protein groups among Kenyan primigravida and multigravida women (n=53). We observed high immunoreactivity across the protein families with the number of antigens identified increasing with gravidity. This was consistent with existing literature where repeated exposure to malaria reduces adverse pregnancy outcomes due to increased levels of antibodies. Principal component analysis revealed that the first six components accounted for 53.5% of the total variation within the dataset with antibodies against P. falciparum PIEMP1, CIDR and DBL domains contributing 83% of the total variation to the first component. In addition, PIEMP1, CIDR, DBL domains and the selected proteins which suggests co-acquisition, none significantly associated with gravidity. Although further work on the significance of the selected antigens will be required, these approaches may provide insights for targets that could be prioritized for vaccine development to reduce risks associated with malaria in pregnancy.
EVALUATION OF THE SAFETY AND IMMUNOGENICITY OF A SINGLE- VERSUS TWO-VIAL PRESENTATION OF R21/ MATRIX-MTM IN CHILDREN IN MALI

Yahia Dicko¹, Fernando Ramos Lopez², Almahamoudou Mahamaren³, Seycina O Maguiraga⁴, Abasse Diaby¹, Kalifa Diarra¹, Duncan Bellamy², Lisa Stockdale², Mehreen S. Datoo¹, Sophie Weston³, Alison Lawrie⁴, Nicola Williams¹, Samuel Provstgaard Mønys³, Yaya Sankare¹, Makanon Diallo¹, Koualy Sanogo¹, Omar M. Dicko¹, Soumyeula Diarra¹, Oumar Attaher², Djibrilla Issiaka², Amagana Dolo¹, Umesh Shaligram⁴, Prasad Kulkarni⁴, Adrian V. S. Hill³, Katie Ewer¹, Alassane Dicko¹

¹Malaria Research & Training Centre, University of Sciences Techniques and Technologies of Bamako., Bamako, Mali, ²The Jenner Institute, University of Oxford, Oxford, UK, United Kingdom, ³Department of Primary Care, University of Oxford, Oxford, UK, Oxford, United Kingdom, ⁴Serum Institute of India Private Ltd, Pune, India, Pune, India

A two-vial presentation of R21/Matrix-MTM malaria vaccine (one vial of R21 and one vial of Matrix-MTM mixed before administration) has been used in the Phase 2 and Phase 3 trials assessing this vaccine. A single-vial ready-to-use vaccine formulation has been developed to facilitate widespread deployment of this highly efficacious vaccine following approvals. Our study aimed to assess the safety and immunogenicity of the single-vial ready-to-use formulation of this vaccine compared with the two-vial formulation in children. The design was a double-blind, randomized controlled trial. Children aged 5 - 36 months were randomised 1:1 to receive the single or two-vial presentation of R21/Matrix-MTM at 0, 1 and 2 months between May and July 2022. Analysis by an independent unblinded statistician indicated that the frequency of the solicited and unsolicited adverse events was similar between the two arms. There were no serious adverse events related to vaccination. NANP specific IgG antibodies were also similar in titre between the two groups of children who received the single or two-vial formulation of the vaccine at one month post third vaccination. The new single-vial formulation of R21/Matrix-MTM malaria vaccine was safe and immunogenic in children in malaria endemic areas. Further evaluation of the single-vial formulation when given as a booster dose is ongoing.

A SYSTEMATIC REVIEW OF PLASMODIUM FALCIPARUM CIRCUMSPOROZOITE PROTEIN BASED VACCINE CANDIDATES: IMPACT OF ANTIGEN, DOSE AND ADJUVANT ON PROTECTIVE EFFICACY AGAINST CONTROLLED HUMAN MALARIA INFECTION CHALLENGE

Paul M. Robben, Evelina Angov, Sheeti Dutta
Walter Reed Army Institute of Research, Silver Spring, MD, United States

Circumsporozoite Protein (CSP), abundantly present on the surface of the pre-erythrocytic sporozoite stage of the Plasmodium falciparum malaria parasite, represents an attractive target for protective intervention. CSP forms the basis of the two most advanced malaria vaccines RTS,S/AS01 and R21/Matrix-M. In addition to these two frontline vaccines, dozens of CSP based vaccine candidates have been developed based on a range of antigen delivery platforms. Candidates have been paired with an array of adjuvants, but many candidates only tested against CHMI challenge in the context of a single adjuvant based on pre-clinical data despite the lack of a defined correlates of protection. We present data from a systematic review of available literature regarding the protective efficacy against Controlled Human Malaria Infection (CHMI) challenge induced by viral vectored, soluble peptide(s), recombinant protein(s), DNA and particle-based CSP vaccine candidates across 35+ years. This report provides a comparative analysis of protective efficacy with particular focus on the apparent impact of different categories of adjuvant and antigen delivery modality that can guide the future development and design of vaccines targeting this antigen.

GENETIC DIVERSITY & NATURAL SELECTION OF A MALARIA VACCINE CANDIDATE GENE IN THE ETHIOPIAN PLASMODIUM VIVAX POPULATION

Alem Berihu Girmay
Aklu Lemnna Institute of Pathobiology, Addis Ababa University, Addis Ababa, Ethiopia

The burden of Plasmodium vivax in Ethiopia is amongst the highest in the world. However, P. vivax diversity, particularly that associated with antigens, such as P.vivax merozoite surface protein 3α (PvMSP3α), has rarely been studied in Ethiopia. In the present study the genetic polymorphism in the defined target was assessed by examining genes encoding two blocks of this antigen locus. Finger prick blood samples spotted onto filter papers were collected from microscopically and Rapid Diagnostic Test (RDT) confirmed malaria patients attending health facilities in the study areas. DNA was extracted by ChelexSaponin extraction method, the genomic DNA was used for confirmation of P. vivax infection by targeting the 18S rRNA gene. Positive samples were subsequently evaluated by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) for identification and assessment of the genetic polymorphism of the MSP3α gene. Further single clone infections were then analyzed using Sanger sequencing. Three size variants were amplified from the 50 isolates, Type A, B and C with frequencies of 82.97%, 12.7% and 4.2% respectively. Further details of diversity were attained by Hha I RFLP, with 11 alleles and 12% multiple clone infections. The sequence analysis showed that size polymorphisms were results of insertions and deletions in the block I component of the gene, which also had higher nucleotide diversity (r) (0.10565) than the block II (0.014). The relatively conserved block II was evolving under positive selection, but a select region that encodes a predicted B cell epitope in these blocks is under balancing selection (Tajma’s D 2.54) (P<0.05), Fu and Li’s F 1.7621 (P<0.05); furthermore, a peak diversity was recorded at this site (r=0.68) with low inter-population FST estimates. The conserved nature of PvMSP3α block II makes it an ideal vaccine candidate. However, future vaccine design strategies targeting PvMSP3αa block II. In contrast the polymorphic nature of PvMSP3αb makes it more suited for use as a rapid genotyping tool.

SAFETY OF THE MALARIA VACCINE CANDIDATE R21/ MATRIX-MTM WHEN CO-ADMINISTERED WITH EPI VACCINES FOR MEASLES-RUBELLA AND YELLOW FEVER AT 9 MONTHS OF AGE IN MALIAN CHILDREN

Fernando Ramos Lopez¹, Yahia Dicko², Mehreen S. Datoo¹, Sophie Weston³, Almahamoudou Mahamaren³, Abasse Diaby¹, Kalifa Diarra¹, Mamoudou Samassekou¹, Cheick B. Sagara³, Mahamadou A. Sidibe³, Yaya Sankare¹, Ahmadou Youssouf⁴, Amadou Tapily³, Oumar Attaher², Djibrilla Issiaka², Amagana Dolo¹, Alison Lawrie⁴, Nicola Williams³, Cheryl Turner¹, Oliver Conway¹, Umesh Shaligram⁴, Prasad Kulkarni⁴, Adrian V.S. Hill³, Katie J. Ewer¹, Alassane Dicko²

¹The Jenner Institute, University of Oxford, Oxford, United Kingdom, ²Malaria Research and Training Centre, University of Bamako, Bamako, Mali, ³Department of Primary Care, University of Oxford, Oxford, United Kingdom, ⁴Serum Institute of India Private Ltd, Pune, India

Malaria remains one of the main causes of mortality in children in Sub-Saharan Africa. Progress in reducing malaria incidence has stalled in recent years, which highlights the need for a safe and highly effective vaccine to prevent malaria in this population. R21/Matrix-MTM has shown high efficacy in a phase 2b trial in Burkina Faso (77% at 1 year after the third dose) and it is currently being assessed in a phase 3 trial in four African countries. R21/Matrix-MTM has proven to be safe and well tolerated. For the successful deployment of R21/Matrix-MTM, it is necessary to assess the safety of the co-administration of R21/Matrix-M with EPI vaccines, specifically with those administered at 9 months of age in most Sub-Saharan African countries. These are measles-rubella (MR) and yellow fever (YF) vaccines. To assess
the safety of this co-administration we have recruited 350 children that were aged 6–7 months at the time of enrolment. They were randomized 3:3:1 to three different groups. Children in group 1 (n=150) received 3 doses of R21/Matrix-M(TM) one month apart, and MR and the YF vaccines at the same time as the third dose. Participants in group 2 (n=150) received only MR and YF vaccines at 9 months of age and participants in group 3 (n=50) received 3 doses of R21/Matrix-M(TM) one month apart, and the EPI vaccines one month after the 3rd dose. We collected safety data for solicited adverse events for 7 days after each vaccination and unsolicited adverse events for 28 days after each vaccination. Serious adverse events are collected for the whole duration of the trial. The co-administration of R21/Matrix-M(TM) with the MR and YF vaccines was well tolerated and the adverse events observed were similar to the adverse events observed when R21/Matrix-M(TM) is administered alone. Most of the solicited and unsolicited adverse events were mild or moderate, and short-lived. The only serious adverse event reported to date was assessed as not related to the vaccine. These data support the co-administration of R21/Matrix-M(TM) with EPI vaccines at 9 months of age, which has positive logistic and economic implications for vaccine delivery.

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MACHINE LEARNING FOR PLASMODIUM FALCIPARUM REVERSE VACCINOLOGY

Renee Ti Chou1, Amed Ouattara2, Matthew Adams2, Andrea A. Berry2, Shannon Takala-Harrison1, Michael P. Cummings1
1University of Maryland, College Park, College Park, MD, United States, 2Center for Vaccine Development and Global Health, University of Maryland School of Medicine, Baltimore, MD, United States

Malaria vaccine development has been hampered by extensive antigenic variation and complex life stages of Plasmodium species. To date, malaria vaccine development has focused on a small number of antigens identified prior to availability of the P. falciparum genome. To leverage available P. falciparum systems data, we have implemented a machine learning-based reverse vaccinology approach to predict potential new malaria vaccine candidate antigens. We assembled and analyzed P. falciparum proteomic, structural, functional, immunological, genomic, and transcriptomic data, and trained models to predict potential antigens. We addressed the issue of incomplete antigen labeling using a positive-unlabeled learning algorithm to model the properties of the known antigens and the remaining proteins. We prioritized candidate antigens using models selected for their performance on reference antigens with different genetic diversity and quantified important protein properties associated with top candidates. The top candidates were clustered into three groups based on their similarities of the properties analyzed by the models. Overall, 85% of group 1 candidates were erythrocyte membrane proteins (PEMP1a), and 36% and 26% of candidates in groups 2 and 3, respectively, were conserved proteins with unknown function. To inform future vaccine development, candidate antigens in the three groups were further characterized by gene essentiality, gene ontology, and single-cell gene expression in various life stages. A portion of candidate antigen genes in groups 2 and 3 were found to be expressed across all life stages, suggesting they may be attractive targets for potential malaria vaccines. Next steps include expression of candidate antigens and testing their ability to elicit functional immune responses in an animal model. Beyond malaria, our approach provides a framework for identifying and prioritizing vaccine antigen candidates for a broad range of disease pathogens.

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BIOPHYSICAL CHARACTERIZATION OF NOVEL HUMAN MONOCLONAL ANTIBODIES TARGETING PLASMODIUM VIVAX APICAL MEMBRANE ANTIGEN 1

Anna C. Winnicki1, Lencore L. Carlas1, Alyssa Malachin1, Karli R. Redinger1, Olivia McLaine1, Quentin D. Watson1, Yelennia Skomorovska-Prokovl1, Chiara Drago2, Lionel Feufack-Dontack3, Lea Baidor3, Lee M. Yeh3, James G. Beeson3, Jean Popovici3, Jurgen Bosch4, Christopher L. King1
1Case Western Reserve University, Cleveland, OH, United States, 2Monash University, Melbourne, Australia, 3Institut Pasteur du Cameroun, Phnom Penh, Cambodia, 4Burnet Institute, Melbourne, Australia

Plasmodium vivax (Pv) control efforts have lagged compared to Plasmodium falciparum (Pf) due to Pv's easy transmissibility and latent stage, making new therapeutics important. Receptor-iodig interactions required for parasite invasion of host reticulocytes, such as Apical Membrane Antigen 1 (AMA1), are potential targets for monoclonal antibody therapy or vaccines. Here we characterized a panel of 13 human monoclonal antibodies (humAbs) recognizing PvAMA1 isolated from B cells obtained from a Pv-exposed individual. We hypothesize humAbs biophysical properties will help identify those with the best growth/invasion inhibition potential. Using surface plasmon resonance biosensor (SPR), anti-PvAMA1 humAbs were immobilized on a chip and PvAMA1 was passed over at increasing concentrations to determine equilibrium dissociation constants (KD). HumAbs were competed against each other to identify overlapping epitopes. Avidity index (AI) to PvAMA1 was measured using a chaotropic agent 1.5M NH4SCN compared to PBS. Anti-PvAMA1 humAbs were tested for cross-reactivity with Pf, P. knowlesi, and Toxoplasma gondii using ELISAs. Sequence similarities to PVAMA1 are PKAMA1>PAMA1>TgAMA1 (85%<60%>30% identity). KDs range between 3.05e-8 to 1.07e-9 M. Als ranged between 0.0% to 52.9%. SPR competition results, analyzed with KD and AI, identify humAbs 816817, 826827, and 832833 as having the strongest interactions with PVAMA1 and recognize unique epitopes, with 826827 and 832833 have the highest Als. Remaining humAbs compete with other epitopes and present with lower affinities. Five/13 humAbs recognize PKAMA1, 1/13 to PAMA1, and 0/13 to TgAMA1. One humAb recognized 3/5 versions of parasite AMA1. Results supporting this approach identify 826827 and 816817 as showing the best invasion inhibition potential using Pk and Pv clinical isolates in vitro with an IC50 of ~ 300 nM and limited invasion inhibition of Pf. The preliminary in vitro results complement the biophysical characteristics seen using SPR and ELISA and suggest screening new humAbs by biophysical properties will help to identify the best candidates for functional studies.

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COMPARISON OF JUNCTIONAL, MINOR REPEAT, AND MAJOR REPEAT-FOCUSED CIRCUMSPOROZOITE VACCINES USING THE TOBACCO MOSAIC VIRUS EPITOPE DISPLAY PLATFORM

Sheetij Dutta1, Emma Ryan1, William Harrison1, Tyree Wilson1, Shelby Foor1, Dallas Brown1, Shikha Sharma2, Gary Matyas3, Evelina Angov2, Paul Robben2, Jason Regules2, Robin Miller2, Lorraine Soisson5, Adrian Batchelor1
1Structural Vaccinology Laboratory, Walter Reed Army Institute of Research, Silver Spring, MD, United States, 2Biologics Research and Development Branch, Walter Reed Army Institute of Research, Silver Spring, MD, United States, 3Military HIV Research Program, Walter Reed Army Institute of Research, Silver Spring, MD, United States, 4Biologics Research and Development, Walter Reed Army Institute of Research, Silver Spring, MD, United States, 5United States Agency for International Development, Washington, DC, United States

The RTS,S vaccine construct contains three distinct antigenic regions: the immunodominant major repeats of Plasmodium falciparum circumsporozoite protein (CSP), the C-terminal region of CSP, and the non-malarial Hepatitis B surface antigen (HBsAg) fusion partner. Vaccination with RTS,S/AS01 inhumans elicits high levels of anti-repeat region antibodies,
but also induces significant bystander antibodies to HBsAg. Protection studies in murine malaria challenge models have suggested that anti-C-term antibodies may not directly neutralize sporozoite invasion. Despite these facts, no variations in the core RTS,S antigen structure have been proposed in over 30 years of its development. The current availability of several highly protective anti-CSP antibody epitope structures allow for rationally guided protein engineering approaches to improve vaccine longevity, cost, and breadth of protection elicited by CSP vaccines. To explore this potential, we have proposed next-generation CSP vaccines to immune-focus humoral responses to elicit antibodies only to the most protective epitopes. Variations of the major repeat region epitope (NPNA) targeted by mAb 317 were displayed on an exposed loop of the Tobacco Mosaic Virus (TMV) capsid. The reactivities of inhibitory mAbs to different proposed TMV antigens were determined and vaccines with the major repeat epitopes were found to be highly immunogenic and efficacious against transgenic parasite challenge in mice. The junctional repeat (DPNA) epitope of CSP targeted by mAb CIS43 was also displayed on TMV, but in the mouse model these antigens were not as efficacious as the major repeat vaccines. We are currently testing the protective effect of minor repeat (NPmV) epitopes that are targeted by the highly potent mAb L9. Results of various vaccine design cycles using the TMV platform will be summarized. Vaccine down-selection studies will form the basis for cGMP manufacture and evaluation of a TMV-based vaccine in humans.

**COMPARABILITY OF THE STANDARD MEMBRANE FEEDING ASSAY ACROSS DIFFERENT VACCINE STUDIES, STUDY SITES, AND TIME**

**JEAN C. HUME**1, Jennifer Kwan2, Olga Muratova1, Heather Goodman1, Edward Owen1, Holly Torano1, Sara A. Healy1, Issaka Sagara1, Patrick E. Duffy1

1LMIV/NIAID/ NIAID, Bethesda, MD, United States, 2LCIM/NIAID/ NIAID, Bethesda, MD, United States, 3MRTC/USTTB, Bamako, Mali

The Standard Membrane Feeding Assay (SMFA) is utilized widely to assess the efficacy of malaria transmission blocking vaccines (TBV). The assay is performed by feeding cultured P. falciparum gametocyte parasites to Anopheles mosquitoes in the presence of test sera and measuring the resulting midgut oocyst infections against a naïve control. The activity of vaccine-induced antibodies to prevent mosquito infection can be expressed as both transmission reducing activity (TRA) where the percent reduction in oocyst count per mosquito against the naïve control is calculated and transmission blocking activity (TBA) where the percent reduction in infection prevalence against the naïve control is calculated. Here we assemble data from the comparator arms of several recent TBV studies (Pfs230D1-EPA and Pfs25-EPA in alhydrogel, Pfs230D1-EPA in AS01 in adults, Pfs230D1-EPA in AS01 in a community setting and Pfs230D1-EPA in Matrix M) to assess the variability in the baseline/control values of SMFAs performed on individuals residing in malaria endemic areas. TRA and TBA data for each study were assembled along with attributes of sites, demographics of study population, and month and year of study in order to examine these control data to determine what differences in the baseline data exist. A total of 574 samples from 209 participants were analyzed using Generalized Estimating Equation (GEE) models fitted with offsets for the number of samples each individual contributed to the analysis. Results of individual variability, within study and cross study variability will be contrasted for both TRA and TBA.

**BUILDING A NEXT-GENERATION PRIME-AND-TRAP PREERYTHROCYTIC MALARIA VACCINE**

**REBEKAH REYNOLDS**1, Yining Zhu2, Anya Kalata1, Felicia Watson1, Naveen Yadav1, Melanie Shears1, Hai-Quan Mao2, Sean C. Murphy1

1University of Washington, Seattle, WA, United States, 2Johns Hopkins University School of Medicine, Baltimore, MD, United States

Highly effective malaria vaccines are needed to accelerate malaria elimination. Pre-erythrocytic (PE) vaccines that eliminate Plasmodium liver stages also abolish the blood stage and completely prevent onward transmission. “Prime-and-Trap” vaccination is a potent PE vaccine strategy that achieves sterile protection against P. yoelli (Py) challenge by inducing high frequency, parasite-specific, liver resident memory CD8+ T (Trm) cells. In its first generation, prime-and-trap consisted of plasmid DNA encoding the Py circumsporozoite protein (PyCSP) delivered by gene gun followed by a trapping dose of Py radiation-attenuated sporozoites (RAS). Here, a trapping dose of PyCSP plasmid DNA delivered by hydrodynamic transfection (HDT) replaced RAS and induced PyCSP-specific CD8+ liver Trm cells that achieved sterile protection against Py sporozoite challenge. Liver Trm cell frequency was reduced in HDT dose de-escalation studies, but antigen-specific complete protection was maintained in such studies to HDT trapping doses as low as 5 ng of plasmid DNA. HDT is a useful experimental tool but cannot be translated into non-human primates or humans. Instead, liver-targeted DNA lipid nanoparticles (LNPs) were developed to provide a safe and effective trapping vaccine. Liver-specific LNPs showing high transfection efficiencies were tested as trapping vaccines and generated PyCSP-specific CD8+ liver Trms but elicited less protection against Py sporozoite challenge than HDT and RAS. LNPs were modified by inclusion of a glycolipid adjuvant and results of those studies will be presented. Methods to adapt these LNPs to needle-free administration are also being developed. This next-generation prime-and-trap vaccine strategy may offer important features that could be harnessed to advance a highly effective and durable PE vaccine for malaria.
Malaria vaccine candidate FMP014/ALFQ is a Plasmodium falciparum Circumsporozoite Protein (CSP) that displays (NANP)6 repeats and the alpha-TSR C-terminus on a self-assembling protein nanoparticle (SAPN) and is formulated with Army Liposomal Formulation containing QS-21 (ALFQ) adjuvant. SAPN nanoparticle substructure is such that the amino and carboxyl termini of the integrated amino acid sequences locate on the SAPN surface. SAPNs can be used to produce a wide range of different antigens and have been shown to successfully produce antibodies. We present the immunological findings of the first such recombinant nanoparticle vaccine from a single center, open-label Phase I clinical trial of intramuscularly administered FMP014/ALFQ for safety and immunogenicity. In this study, five subjects were administered a low dose, 20 µg FMP014 in 0.5 mL ALFQ and five were administered a high dose, 40 µg and 1mL adjuvant, at each vaccination on a 0-, 1-, and 2-month schedule. All doses were well tolerated with an acceptable safety profile. Serological findings revealed that FMP014/ALFQ recipients developed robust CSP antibody responses recognizing both structural elements, (NANP)6 repeat and the alpha-TSR C-terminal substructure displayed on the SAPN molecule surface. Exploratory analyses of fine specificities of responses measuring both antibody isotype and oposonophagocytic activity relative to potential functional immunity suggest a positive bias toward the lower dose vaccination group. These findings suggest that the SAPN vaccine approach is suitable as a particulate repetitive antigen display system.

Diarrheal diseases constitute a serious public health problem, particularly in developing countries, because of their association with high morbidity and mortality in children under five years of age. Most strains of Escherichia coli live harmlessly in the intestines and rarely cause disease in healthy individuals. Nevertheless, a number of pathogenic strains can cause diarrhea diseases. Self-medication and overuse of antibiotics due to the scarcity of complementary diagnostic systems can lead to the development of multi-resistant bacteria causing diarrhea. The objective of this work was to identify the E. coli responsible for diarrhea in children aged 0 to 3 years and to characterize their sensitivity to a panel of antibiotics used in Mali. This study involved 538 children seen in outpatient visits at the Yirimadio community health center and diagnosed with diarrhea. Yirimadio is a peripheral district area of Bamako the capital city of Mali. Stool samples were collected and analyzed by stool culture and antibiotic susceptibility was determined by the disk diffusion method on agar medium. An isolation rate of 31.6% was found. Amoxicillin and cotrimoxazole were the most resistant antibiotics, 94.1% and 92.9% respectively. For multi drug resistance, 89.4% of our strains were simultaneously resistant to two families of antibiotics, 68.2% were cephalosporinases and 40% ESBL. This study showed that E. coli is the most frequent bacteria involved in diarrhea in children under 3 years of age in Yirimadio, which are resistant to amoxicillin and co-trimoxazole, two antibiotics commonly prescribed in this setting.

Vaccines are an effective strategy to minimize antimicrobial resistance (AMR). We aimed to evaluate Shigella antimicrobial susceptibility pre and post rotavirus vaccine introduction among children <5 years with moderate-to-severe diarrhea (MSD) in western Kenya. We used data from the Kenyan site of the Global Enteric Multicenter Study (GEMS;2008-2012) and the Vaccine Impact on Diarrhea in Africa (VIDA; 2015-2018). GEMS and VIDA were prospective, health center and community-based case-control studies which enrolled children aged <5 years with moderate-to-severe diarrhea, defined as ≥3 loose stools in the previous 24 hours with ≥1 of the following: sunken eyes, poor skin turgor, dysentery, intravenous rehydration, or hospitalization within 7 days of diarrhea onset. Stools collected at enrollment were tested for Shigella using standard culture
methods. Antimicrobial susceptibility was determined by Kirby Bauer disk diffusion. We compared AMR pre (2008-2012) and post (2015-2018) rotavirus vaccine introduction using a trend test for proportions. During the pre-vaccine period, Shigella was isolated from 130 (7.3%) of 1,778 stool specimens (S. flexneri 80 [41.5%] and S. sonnei 24 [18.5%] were the most common serotypes). Post rotavirus vaccine introduction, Shigella was isolated from 133 (8.6%) of 1,554 stool specimens (S. flexneri 84 [63.2%] and S. sonnei 35 [26.3%]) were the most common serotypes). There was no significant difference in AMR between pre and post vaccine introduction among the antimicrobials tested: cotrimoxazole (125/129 [96.9%] vs 127/132 [96.2%]; p=0.76); nalidixic acid (6/129 [4.7%] vs 5/132 [3.8%]; p=0.73); ceftriaxone (1/128 [0.8%] vs 2/130 [1.5%]; p=0.58); ciprofloxacin (2/129 [1.6%] vs 0/132 [0.0%]; p=0.15); resistant to ≥2 antimicrobials (8/130 [6.2%] vs 6/133 [4.5%]; p=0.55). We observed an increase in Shigella isolation post-rotavirus vaccine introduction. Continued monitoring of Shigella AMR trends for effective clinical treatment of shigellosis is important in this setting.

PARASITIC AND ANTIBIOTIC-RESISTANT BACTERIAL CONTAMINATION OF RAW SALAD VEGETABLES SOLD IN LOCAL MARKETS OF DHAKA, BANGLADESH

Ayan Goshwami1, Priyanka Barua1, Meher Nigad Nipa2, Sharmin Musa1
1University of Dhaka, Dhaka, Bangladesh, 2Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka, Bangladesh

Salad vegetables are widely eaten raw in Bangladesh because of their high nutritional content and health benefits. But contamination with parasites and bacteria poses a significant health risk. The purpose of this study was to document the presence of parasites and antibiotic-resistant bacterial contamination of raw salad vegetables to provide insight into the parasitological and bacteriological quality. 108 samples of raw salad vegetables: carrot, cucumber, tomato, coriander, mint, and lettuce were subjected to parasitological examination. After washing in saline water, sediments were centrifuged and analyzed using a microscope. 29.6% (32/108) of the vegetables were contaminated with parasites. Ascaris lumbricoides (20.4%), Entamoeba sp. (13.0%), Hymenolepis nana (9.3%), Cystoisospora sp. (5.6%), Ancylostoma duodenale (3.7%), Trichuris trichiura (3.7%), Taenia sp. (2.8%), and Hymenolepis diminuta (1.9%) were found. Leafy vegetables (e.g., coriander 66.7%, mint 44.4%, and lettuce 38.9%) were most contaminated compared to non-leafy vegetables (carrot 11.1%, tomato 11.1%, and cucumber 5.6%). Parasitic contamination was significantly related (p<0.05) to the type of vegetable and the vendor’s hygiene practices. Thirty-six samples were subjected to bacteriological analyses and antibiotic susceptibility tests. 61.1% (22/36) of samples were contaminated with three different types of pathogenic bacteria. Escherichia coli O157 (19.4%), Vibrio cholerae (61.1%), and Escherichia coli (33.3%) were detected, but no Salmonella was detected. The highest bacterial contamination was recorded in coriander (83.3%) and lettuce (83.3%). No norovirus were detected, but no Salmonella was detected. The highest bacterial contamination was recorded in coriander (83.3%) and lettuce (83.3%). No

LOCAL MARKETS OF DHAKA, BANGLADESH

HOSPITALISATION AND MORTALITY RATES AMONG CHILDREN WITH MODERATE TO SEVERE DIARRHOEA TREATED WITH AZITHROMYCIN OR PLACEBO: A RANDOMISED CONTROLLED TRIAL

Salman Haq, ibttisam qazi, farah naz
aga khan university, Karachi, Pakistan

Children under the age of five in South Asia and Sub-Saharan Africa are at the highest risk of dying from diarrhoeal diseases. Standard of care for treating diarrhoea includes breastfeeding and oral rehydration. The Antibiotics for Children with Diarrhoea (ABCD) trial hypothesised that addition of antibiotics to the standard care for acute watery diarrhoea (AWD) may help lower hospitalisation and death in low- and middle-income countries. We analysed the site specific data from Pakistan for the ABCD trial. Children between the ages of 2 - 23 months with AWD, some or severe dehydration, and/or moderate wasting and/or severe stunting were eligible. Participants were randomised into either the oral azithromycin or placebo arms in addition to standard care as per WHO guidelines. The outcome measure was hospitalisation and all-cause mortality within 180 days of enrolment. At day 180 there was no hospitalisation attributed to diarrhoea though up to 25% of participants in both arms were malnourished. Causes of hospitalisation included anaemia (n=1), blood in stool/ dysentery (n=1), fever (n=2), and uroteliasis (n=1). Causes of mortality included drowning (n=2), cough and cardiac arrest (n=1), breathlessness (n=1), measles and pneumonia (n=1), skin infection (n=1) and skin rash (n=1). Since all children in both groups also received standard of care this could be one of the reasons for observing reduced hospitalisation and mortality. This data supports the avoidance of antibiotics except in cases of dysentery or cholera and the reaffirmation of recommended guidelines.

THE ROLE OF VILLAGE DOCTORS IN THE TREATMENT OF PEDIATRIC DIARRHEA AND POTENTIAL FOR ANTIMICROBIAL STEWARDSHIP IN RURAL BANGLADESH: A DESCRIPTIVE ANALYSIS

Olivia R. Hanson1, Isttiakul I. Khan2, Zahid H. Khan2, Mohammad A. Amin2, Mohammad T. Islam2, Melissa H. Watt3, Eric J. Nelson3, Shania M. Ahmed1, Firdausi Qadir3, Daniel Leung1, Ashraful I. Khan2
1University of Utah, Salt Lake City, UT, United States, 2International Centre for Diarrhoeal Disease Research, Dhaka, Bangladesh, 3University of Florida, Gainesville, FL, United States

Diarrheal diseases are a leading cause of death in children and a significant reservoir of antimicrobial resistance. In Bangladesh, many people seek healthcare from informally trained providers (“village doctors”) who dispense unregulated antibiotics. This study aimed to understand the role of village doctors in treating pediatric diarrhea and the potential for antimicrobial stewardship in this population. We aimed to identify all village doctors in the Sylhet subdivision of Bangladesh. Village doctors (n = 125) were consented and verbally administered a questionnaire. The mean age was 41.3 (SD = 11.1). Participants were all male and had been practicing for an average of 15.8 years (SD = 10.1). The majority practiced in a permanent roadside building (62.4%, n = 78) and made household visits (86.0%, n = 104). Per week, participants reported seeing 106 patients (SD = 79.0); of these, 6 (SD = 7.8) were children (<5 years) with diarrhea. Almost all (99.2%, n = 120) stocked medications. The most commonly stocked antibiotics were metronidazole (95.2%, n = 119), azithromycin (95.2%, n = 119), ciprofloxacin (93.6%, n = 117), and nitazoxanide (77.6%, n = 97). The most commonly prescribed antibiotics for treating diarrhea were ciprofloxacin (90.4%, n = 113), metronidazole (73.6%, n = 92), and nitazoxanide (59.2%, n = 74). Almost all (81.8%, n = 99) participants had a smartphone with internet access, and over half (63.2%, n = 79) used their phone to assist with making clinical decisions. Village doctors provide care to a large number of patients in rural Bangladesh and care for a significant number of children with diarrhea. Village doctors commonly stock and prescribe numerous antibiotics, making this population an important target for antimicrobrial stewardship interventions. Widespread smart phone use, coupled with current use of phones for clinical decision making, supports the introduction of mHealth tools to assist with antibiotic stewardship for pediatric diarrhea. Data collection is ongoing and full results will be presented at the conference.
SAFETY, IMMUNOGENICITY, AND EFFICACY OF ETVAX® VACCINE AGAINST ENTEROTOXIGENIC E. COLI-ASSOCIATED DIARRHEA IN GAMBIAN CHILDREN AGED 6 TO 18 MONTHS

M. Jahangir Hossain1, Fatou Secka1, Lady C. Sanyang1, Taiwo Raji1, Emmanuel C. Okoh1, Olubunmi A. Olubiyi1, Mbemba Drammeh2, Emmanuel U. Richard1, Mama Drammeh1, Samba Juma Jallow3, Ouasman Secka1, Anna Pocs1, Nils Nilsson2, Ann-Mari Svennerholm2, Anna Hill2, Umberto D’Alessandro3, Thomas F. Wierzba4

1Medical Research Council Unit The Gambia at the London School of Hygiene & Tropical Medicine, Banjul, Gambia, 2Scandinavian Biopharma, Stockholm, Sweden, 3Microbiology and Immunology, University of Gothenburg, Stockholm, Sweden, 4Section on Infectious Diseases, Department of Internal Medicine, Wake Forest School of Medicine, Winston Salem, NC, United States

This is a double-blind, placebo controlled (1:1) trial evaluating the safety, immunogenicity, and efficacy of three ETVA® doses (Day 1, 15, and 90) to healthy Gambian children aged 6 to 18 months. ETVA® is an oral, whole-cell, multivalent inactivated vaccine against enterotoxigenic E. coli (ETEC) associated diarrhea. The vaccine, adjuvanted with dmtLV, includes four E. coli strains over-expressing colonization factors (CFs) CFAI, CS3, CS6, and CS6 with a CTB/LTB hybrid toxin (LCTBA). ETVA® was safe and induced strong intestinal-mucosal antibody responses to CFs and LTB in Swedish adults, Bangladeshi adults and children, and Zambian children. A trial in Finnish travelers to Benin showed broad protection against moderate-to-severe ETEC diarrhea when allowing for co-pathogens. This trial was implemented in the North Bank and parts of the Central River and Lower River Regions of The Gambia. The enrolment started in February 2021 and recruited 4,936 children over 16 months. Passive surveillance for acute moderate-to-severe diarrhea (four or more loose/liquid stools in 24 hours) will end in October 2023. For safety, 350 children were actively visited at home within seven days of any dose. A vaccine-preventable outcome (VPO) is moderate or severe culture-confirmed ETEC diarrhea producing LT with or without ST toxin and/or strains expressing vaccine homologous colonization factors. Immunogenicity against LT and CFs is measured in 150 children. Active surveillance among the 350 children detected 88 adverse events, two of them severe and considered product related (1 vomiting, 1 fever). Among all other children (n = 4586), there were 420 adverse events; four events were severe and not product related. There were 47 serious adverse events, none of them considered product related. By March 2023, 453 moderate-to-severe diarrhea cases had been detected; testing for ETEC phenotypes and co-pathogens is ongoing. Although data is blinded, ETVA® appears safe with mainly mild and moderate adverse events. Efficacy results are expected by March 2024.

ANTIMICROBIAL RESISTANCE TO ANTIBIOTICS AT A COMMUNITY-LEVEL HEALTH FACILITY IN MALI: A NANOPORE SEQUENCING AS A TOOL FOR AMR SURVEILLANCE

Antoine Dara1, Boi Kone1, Bintou Diarra1, Mamadou Tekete1, Lassina Timbire1, Hinda Doucoure2, Abdoulaye A. Djimde3

1Pathogens genomics Network Africa (PDNA), Bamako, Mali, 2Centre Charles d’Infectiologie Charles Mérieux, Bamako, Mali

Antimicrobial resistance remains a real threat to health throughout the world and resource limited countries such as Mali are not spared from this threat. The AMR detection is not a routine in most of health facilities. Antibiotic treatments are administered presumptively without laboratory tests. The lack of information of AMR could comprise treatment outcomes. To better gain insights on the extent of AMR at the community health center level, we have conducted a prospective study to phenotype bacteria involved in diarrhea in children in Bamako. Our results indicate that multi-drug resistant bacteria are circulating at the community level. More importantly, Enterobacteriaceae producing carbapenemase were detected. We are undertaking a long-read nanopore sequencing to better capture the microbial composition of stools from children with diarrhea. In addition, we will identify AMR genes involved in the resistance phenotype and fully characterize the pattern of transmission of these genes. Our results will inform healthcare workers and policy makers of rational antibiotic prescription.

A LONGITUDINAL COMMUNITY ASSESSMENT OF PLASMA CITRULLINE IN TWO COHORTS OF BANGLADESHI INFANTS

Zhanmo Ni1, Abdullah Siddique2, Mamun Kabir2, Masud Alam2, Rifaat Ara3, Tahsin Ferdous4, Rebecca M. Munday1, Rashidul Haque2, Tahsin Ferdous2, Rebecca M. Munday1

1Johns Hopkins School of Medicine, Department of Oncology, Baltimore, MD, United States, 2International Centre for Diarrheal Disease Research, Dhaka, Bangladesh, 3Johns Hopkins School of Medicine, Department of Genetic Medicine, Baltimore, MD, United States, 4Johns Hopkins Bloomberg School of Public Health, Department of Epidemiology, Baltimore, MD, United States

Plasma citrulline (CIT) is an emerging biomarker for environmental enteric dysfunction (EED). In Peruvian children, CIT was inversely related to markers of systemic inflammation. We sought to describe CIT longitudinally, and to investigate the association between CIT and socioeconomic factors and diarrheal episodes in community-dwelling infants. Two hundred children between age 6-8 months in Mirpur, Bangladesh were enrolled in two cohorts, the first beginning in December 2020 and the second, August 2021. Each cohort was followed with biweekly visits for 8 months. Baseline and end of study serum was collected and tested for plasma citrulline. At baseline, the mean CIT levels for the two cohorts were significantly different (24.0 μmol/L, SD= 9.2 μmol/L versus 17.7 μmol/L, SD = 6.0 μmol/L) (t-test, p <0.001). The end of follow-up mean CIT levels were lower than baseline in both cohorts, 22.4 μmol/L (SD = 7.8 μmol/L) and 15.2 μmol/L (SD = 6.1 μmol/L). There was no significant difference (p = 0.05) in demographic or socioeconomic factors between the two cohorts, including age, sex, household overcrowding, income, water treatment, water source, maternal education, baseline height-for-age adjusted z-score (HAZ) and weight-for-age adjusted z-score (WAZ). The number of diarrheal episodes per child was not associated with the change in CIT over time. The direction of individual CIT change was also not correlated with either (HAZ) or (WAZ) trend. We found that CIT decreased after 8 months of follow up among most of the children in this study, which suggests that most children had an increasing level of systemic inflammation as they aged. This change in CIT was not associated with number of diarrheal episodes. We propose that this increase in systemic inflammation could be related to subclinical insults to the gut due to non-diarrheal enteric infection. Future studies should investigate whether common enteric pathogens are associated with CIT, to further define the relationship between enteric pathogens and chronic inflammation in infants living in impoverished settings.

CHOLERA DEATHS DURING OUTBREAKS IN UVIRA, EASTERN DEMOCRATIC REPUBLIC OF CONGO, SEPTEMBER 2021-JANUARY 2023

Patrick Musole Bugeneri1, Hanmeng Xu1, Chloe Hutchins2, Juan Dent Huise2, Jaime Saidi2, Barin Bashire Rumejeka2, Moise Itongwa3, Justin Bengheya2, Amanda Dobbs1, Iza Ciglenecki4, Esperance Tshwed4, Faïza Kitoga5, Daniel Mukadi-Bamuleka4, Jackie Knee6, Placide Oktayemba Welo7, Andy Adazm8, Espoir Bwenge Malembaka1

1Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, 2Department of Disease Control, London School of Hygiene & Tropical Medicine, London, United Kingdom, 3Zone de Santé d’Uvira, Ministère de la Santé Publique, Uvira, Congo, Democratic Republic of the, 4Ministère de la Santé Publique, Division Provinciale de la Santé Publique du Sud-Kivu, Bukavu, Congo, Democratic
Estimates of the true burden of cholera are uncertain due to poor surveillance systems that do not routinely identify or test suspected cholera cases, and the limited documentation of community cases and deaths. We describe cholera deaths across 2 seasonal outbreaks after cholera vaccination campaigns in Uvira, DR Congo in 2020. We recruited all suspected cholera cases presenting to 2 cholera treatment facilities in Uvira between September 2021 and January 2023. Suspected cases were eligible for enrollment if they were ≥ 1 year old and reported ≥ 3 watery, non-bloody diarrheal stools 24 hours before admission. Cholera was confirmed by enriched rapid diagnostic test (RDT) and culture or PCR. 1237 suspected cholera cases were admitted to health facilities in Uvira, including 777 (62.6%) confirmed by culture/PCR. We recorded 18 suspected cholera deaths, of which 15 (83%) occurred within health facilities and three in the community. The confirmed facility-based CFR was 1.2% when using culture or PCR (n=9/777) and 1.5% when using RDT (n=12/777); one of the health facility deaths was not tested for cholera. Cholera deaths were older (median: 62 years) than survivors (median: 18 years, p<0.001), with no difference by sex. While only 1 death occurred among children < 5 years, over half (55%) of all deaths were ≥ 60 years old, and 60% of health facility death occurred after ≥ 1 day of hospitalization. Cases who died were twice as likely to have been admitted with severe dehydration compared to those who survived (93.3 vs 45.7%, p<0.001), and appeared to present for care later than those who survived, but this difference was not statistically significant. Two of the cases who died, both culture positive, reported having received 1 dose of the oral cholera vaccine in the 2020 vaccination campaign. We found that most of the deaths occurring in cholera treatment facilities in Uvira tested positive for cholera. Documenting both community and confirmed facility deaths is critical to identify drivers of cholera deaths, better target preventative measures-like vaccination-and treatment to those most at risk, improve patient care and estimates of cholera burden worldwide.

A PATHWAY TO A MORE DURABLE CHOLERA RAPID DIAGNOSTIC TEST

Eric Nelson
University of Florida, Gainesville, FL, United States

High throughput molecular and mass spectrometry technologies have provided insight on the impact of virulent bacteriophage and antibiotics on cholera diagnostic performance, including rapid diagnostic tests (RDTs). The effects of a cholera RDT testing positive decrease by more than 80% when virulent phage and antibiotics (azithromycin) are present. These factors contribute to variable performance and limit the use of RDTs. When the common Vibrio cholerae-virulent bacteriophage ICP1 is present, we hypothesize adding a monoclonal antibody (mAb) that targets ICP1 to the RDT will increase sensitivity without compromising specificity. To test this hypothesis, we developed an alpha RDT prototype that used an anti ICP1 capsid protein (ORF122) mAb for both the mobile and fixed antibody components in the lateral flow device. The alpha prototype failed because the fixed mAb did not capture the phage bound to the mobile colloidal gold labeled mAb. To address this problem, we proposed a beta prototype with distinct mobile and fixed mAbs. We performed bioinformatic analyses on ICP1 from the Democratic Republic of Congo and Kenya compared to Bangladesh. We found that the capsid decoration protein (ORF123) and two tail fiber proteins (ORF69 and ORF93) of ICP1 were sufficiently conserved; the percent conservation at the amino acid level was 99.4-100% for ORF123, 99.4-100% for ORF123, 99.5-100% and 98.5-100% for ORF69, and 90.4-100% and 94-100% for ORF93, respectively. Production of mAbs to these new targets was subsequently initiated. These candidate mAbs will be analyzed using competitive ELISA assays. One of the two leading mAbs will be gold labeled and used in the mobile component and the second will be used as the fixed component in the mAb::ICP1::mAb ‘sandwich’ of the RDT. If successful, the beta prototype will be validated in a prospective clinical study in Bangladesh. We hope this research will address the negative impact the common virulent bacteriophage ICP1 has on cholera RDT performance. Future steps include a pivot to address the negative impact antibiotic exposure has on diagnostic performance.

GENOMIC EPIDEMIOLOGY OF CAMPYLOBACTER SPP. ISOLATED FROM CHILDREN WITH MEDICALLY ATTENDED DIARRHEA IN IQUITOS, PERU

Katia Manzanares Villanueva1, Francesca Schiaffino2, Lucero Romaina Cachique1, Tackeshy Pinedo Vasquez3, Maribel Paredes Olortegui1, Paul F. Garcia Bardales1, Steven Huynh4, Pablo Peñatario Yori1, Evangelos Mourkas5, Ben Pascoe6, Kerry K. Cooper7, Craig T. Parker3, Margaret N. Kosek8
1Asociacion Benefica Prisma, Iquitos, Peru, 2Faculty of Veterinary Medicine, Universidad Peruana Cayetano Heredia, Lima, Peru, 3Agricultural Research Service, U.S. Department of Agriculture, Produce Safety and Microbiology Research Unit, Albany, CA, United States, 4Division of Infectious Diseases, University of Virginia, Charlottesville, VA, United States, 5Ineos Oxford Institute for Antimicrobial Research, Department of Biology, University of Oxford, Oxford, United Kingdom, 6Centre for Genomic Pathogen Surveillance, Big Data Institute, University of Oxford, Oxford, United Kingdom, 7School of Animal and Comparative Biomedical Sciences, Tucson, AZ, United States

Campylobacter is one of the leading causes of bacterial diarrheal disease worldwide. Within resource poor countries, Campylobacter burden is concentrated in young children, with most children exposed to this pathogen by the age of two. Campylobacteriosis is clinically characterized by symptoms including diarrhea, abdominal pain, and fever, all of which are usually self-limiting. However, antimicrobial treatment is recommended in severe clinical cases. This study aimed to describe the genomic characteristics of Campylobacter isolated from children 0 to 36 months of age seeking medical care for diarrheal illness in primary health posts in the city of Iquitos, Peru. A fecal sample from whole fecal samples or a rectal swab were placed in Cary Blair and transported within less 6 hours for processing. Samples were cultured in Columbia blood agar base supplemented with 5% lysed horse blood and incubated in microaerophilic conditions at 37°C for 48 hours. Colonies morphologically and biochemically compatible with Campylobacter spp. were replicated and confirmed by qPCR. Genomic libraries were prepared using the Illumina DNA Prep Tagmentation kit. Libraries were sequenced using a 2 x 250 bp paired end v2 reagent kit on a MiSeq reagent kit (illumina). Genomes were assembled using the Spades assembler. Multi Locus Sequence Types were determined through the PubMLST allelic database. Clinical isolates from other high resource and low resource settings were downloaded from PubMLST for phylogenetic comparisons. Between November 2019 and February 2023, a total of 153 Campylobacter genomes were isolated and sequenced from 46 different children seeking care. Phylogenetic comparison between Loreto isolates and isolates from other regions is presented. Antimicrobial resistance genes and point mutations are presented. Ongoing analysis intends to determine genomic differences between Campylobacter isolates obtained from samples from children with different diarrhea severity scores. This is one of the first analysis describing genomic characteristics of Campylobacter isolates from pediatric cases of gastroenteritis in the Peruvian Amazon.

SEROCOIDENCE OF ENTERIC FEVER IN RURAL AND URBAN POPULATIONS OF SIERRA LEONE

Polina Kamenskaya1, Ibrahim Swaray2, Ahmed Osman2, Karla Fischer4, Rashid Ansumana2, Hellen Gelband3, Kristen Aiemjoy2, Prabhat Jha4, Jason R. Andrews9, Isaac I. Bogoch4, Richelle C. Charles1
1Massachusetts General Hospital, Boston, MA, United States, 2Centre for Global Health Research, Unity Health Toronto and Dalla Lana School of Public Health, Toronto, ON, Canada, 3Unity Health Toronto, Toronto, ON,

astmh.org
Enteric fever remains a major global health problem. The recent availability of typhoid conjugate vaccine (TCV) is a promising control strategy to reduce disease burden. However, lack of an optimal diagnostic and limitations of blood-culture surveillance (e.g. cost, requirement of laboratory capacity, low sensitivity) have left many low- and middle-income countries (LMICs), including Sierra Leone, without any estimates of burden data. To address this gap, we have developed a serum surveillance tool based on detection of HlyE IgG and IgA, that overcomes the limitation of blood culture surveillance. In this analysis, we leveraged archived dried-blood spots collected in 2022 from a cross-sectional population-based surveillance in Bo, Sierra Leone. We included 656 representative samples from individuals 5-15 years of age and stratified our analysis by rural/urban setting. We found that the enteric fever seroconversion rate was highest in children from rural areas (57 cases per 100 person-years) compared to urban areas (44 cases per 100 person-years). In conclusion, Sierra Leone has a high force of infection for enteric fever. These findings demonstrate the need for further surveillance and study capacity for enteric fever in Sierra Leone, for which seroconversion can be a great tool.

A SPOTTED RASH HELPS SPOT A RARE PATHOGEN: CAPNOCYTOPHAGA CANIMORSUS ENDOCARDITIS WITH GLOMERULONEPHRITIS

Divya Chandramohan, Moyosore Awobajo, Ermasi Sori, Sean O’Nei, Gregory M. Anstead
UT Health, San Antonio, San Antonio, TX, United States

Capnocytophaga canimorsus is a gram-negative bacterium commonly found in the salvia of dogs. Despite the frequency of dog bites, infection with this organism is rare, and severe infections are usually associated with risk factors. Infective endocarditis is a rare presentation of C. canimorsus infection, and post-infectious glomerulonephritis has never since been reported. We present a case of a 67-year-old male with alcohol use, and intravenous methamphetamine use who presented with a lower extremity rash. Blood cultures revealed Capnocytophaga canimorsus. A trans-esophageal echocardiogram (TEE) revealed tricuspid valve endocarditis with vegetations and the tricuspid valve was replaced. 16s ribosomal RNA amplicon sequencing performed on the isolate that was categorized as ExPEC compared with 24 (60.0%) of 40 decedents for whom E. coli was not adjudicated as in the causal pathway to death in 15 (27.3%). Among isolates, the three most common sequence types (ST) were ST131 (n=14, 18.7%), ST38 (n=9, 12.0%), and ST648 (n=9, 12.0%). For decedents with E. coli adjudicated as in the causal pathway to death, 13 (86.7%) had at least one isolate that was categorized as ExPEC compared with 24 (60.0%) of 40 decedents for whom E. coli was not adjudicated as in the causal pathway to death. The odds of isolating ExPEC when E. coli was adjudicated as in the causal pathway to death was 4.33 (95% CI 0.86-21.84, P=0.076). Our results suggest that evaluating virulence gene profiles may help clarify the role of E. coli in the causal pathway to death. The role of ExPECs may also have a higher propensity to translocate post-mortem. When E. coli and other Enterobacteriales are isolated. Here we evaluate if virulence gene profiles distinguishing extraintestinal E. coli (ExPEC) from non-ExPEC discriminated E. coli in the causal pathway to death from those translocating post-mortem. We enrolled pediatric and adult febrile deaths in a prospective autopsy study at two hospitals in Moshi, Tanzania. At autopsy, we collected specimens for culture from blood, lung, and, liver, and, when possible, cerebrospinal, pericardial, pleural, and peritoneal fluid. E. coli isolates underwent whole genome sequencing and classification as ExPEC or non-ExPEC based on a validated set of virulence genes. Adjudication of the role of E. coli in the causal pathway was done by reviewing of clinical history, microbiologic results, and pathologic findings and was blinded to virulence gene data. Data were analyzed using frequencies, proportions, and bivariate logistic regression. From October 2016 through May 2019, we enrolled 218 decedents yielding 75 E. coli isolates from 55 (25.2%) decedents. Of decedents with E. coli isolated, median (IQ) age was 40 (22-63) years, 14 (25%) were female, and E. coli was adjudicated as in the causal pathway to death in 15 (27.3%). Among isolates, the three most common sequence types (ST) were ST131 (n=14, 18.7%), ST38 (n=9, 12.0%), and ST648 (n=9, 12.0%). For decedents with E. coli adjudicated as in the causal pathway to death, 13 (86.7%) had at least one isolate that was categorized as ExPEC compared with 24 (60.0%) of 40 decedents for whom E. coli was not adjudicated as in the causal pathway to death. The odds of isolating ExPEC when E. coli was adjudicated as in the causal pathway to death was 4.33 (95% CI 0.86-21.84, P=0.076).

FACTORS ASSOCIATED WITH IN-HOSPITAL MORTALITY IN ADULT PATIENTS WITH SEPSIS AT TWO RURAL BORDER PROVINCES OF THAILAND

Woradee Lurchachaiwong, Peeriya Watakulsin, Emily Bloss, Philip Mock, Pitiphon Promduangsi, Anupong Sujaryakul, Supphachoke Khemla, Tanapat Lertwityaketorn, Nuttakarn Chuenchom, Thant Rattanathumaksakul, Patrunuch Sapchookkul, Beth Skagg, Saithip Bhengsi, Ornuma Sangwichian, James D. Heffelfinger, John R. MacArthur, Somsak Thamthitiwat
1Division of Global Health Protection (DGHP), Thailand MOPH-US CDC Collaboration, Nonthaburi, Thailand, 2Department of Disease Control (DDC), Ministry of Public Health (MOPH), Nonthaburi, Thailand, 3Nakhon Phanom Hospital, Nakhon Phanom, Thailand, 4Mae Sot General Hospital, Tak, Thailand

Sepsis is a leading cause of morbidity and mortality among hospitalized patients in Thailand. We conducted a retrospective medical record review at 12 hospitals in northern provinces in Thailand during October to December
2017 to evaluate demographic, clinical management characteristics and outcomes among adult patients with sepsis, severe sepsis, or septic shock as defined in the 2012 consensus guidelines (Sepsis-2). Bivariate and multivariate regression analyses measured factors associated with in-hospital mortality within 28 days of admission. Among 719 patients who met a Sepsis-2 case definition, 55% were > 60 years old, and 49% were male. Of the 153 (21.3%) patients who met the criteria for sepsis, 1 (0.7%) died within 28 days of admission; of 442 (61.5%) for severe sepsis, 45 (10.7%) died; of 124 (17.2%) for septic shock, 51 (41.8%) died. Of the 552 (77%) sepsis patients with available hemoculture results, 270 patients (49%) had positive bacterial cultures; the three most detected bacteria were Escherichia coli (n=86, 32%), Klebsiella species (n=41, 15%), and Pseudomonas species (n=28, 10%). A greater proportion of septic shock patients were referred from district hospitals to provincial hospitals with critical care capability (p<0.001). Multivariate regression analysis found risk factors independently associated with in-hospital mortality: admission through an emergency room (adjusted relative risk [aRR] 2.98; 95% confidence interval [95%CI] 1.00, 8.93); respiratory infection (aRR 1.76; 95%CI 1.22, 2.53); malignancy (aRR 2.29; 95%CI 1.36, 3.85); skin, soft tissue or bone infection (aRR 5.08; 95%CI 2.58, 10.02); and, compared to sepsis, severe sepsis (aRR 5.69; 95%CI 1.18, 63.38) and septic shock (aRR 28.51; 95%CI 3.91, 207.92). In-hospital mortality for septic shock was higher at general hospitals than at district hospitals (aRR 2.37; 95%CI 1.32, 4.26). Our findings could inform development of, locally driven, early sepsis detection and management algorithms to reduce sepsis mortality in both district and general hospitals.

**DETECTING AND TREATING SEPTIC SHOCK IN DIARRHEAL PATIENT WITH POINT OF CARE [POC] LACTATE TESTING: A LIFE-SAVING STRATEGY BEYOND ICU**

Lubaba Shahrin, Monira Sarmin, Irin Parvin, Mohammad Jobayer Chisti
International Centre for Diarrheal Disease Research, Bangladesh, Dhaka, Bangladesh

Increased lactate level corresponds with deteriorated metabolic status in critical illnesses such as severe sepsis and septic shock. Progression to septic shock from severe sepsis was 69% in adults with diarrhea, and mortality was 30-50%. Identifying a bedside test to guide clinicians to make a timely decision is crucial in managing severe sepsis and septic shock. In this prospective observational study, we enrolled diarrheal adults ≥18 years from November 2021 - March 2023 in Dhaka hospital, icdc.bdu. POC lactate test is done at hours 1st, 2nd, and 6th by StatStrip Lactate meters from Nova Biomedical, US. Patients fulfilling surviving sepsis-3 septic shock criteria comprise a case, and hypovolemic shock includes as control. For comparison of POC Lactate levels, we used paired t-test. The odds ratio (OR) and their 95% confidence intervals (CI) were used to demonstrate the strength of the association. The study was registered in Clinicaltrials.gov (NCT05108467) and received institutional ethical approval (PR-21097). Of 360 patients, 100 had septic shock, and 100 had a hypovolemic shock. The death rate was 18% among septic shock group. The Patients with septic shock residing outside Dhaka city than the hypovolemic shock patients (55% vs. 28%; p <0.001). Comparison of median POC Lactate in hours 1st, 2nd, and 6th between the two groups are statistically significant (p=0.004; p<0.001; p<0.001), respectively. POC Lactate test can detect septic shock in a diarrheal patient with variable levels of dehydration. This test can help clinicians quickly diagnose and treat time-sensitive conditions like septic shock by providing quick, reliable, and accurate results before arriving in ICU.

**CHARACTERIZATION OF ACINETOBACTER SPP. ISOLATED FROM BLOOD AND URINE OF PATIENTS IN RURAL GHANA**

Charity Wiafe Akenton¹, John Amuasu¹, Jürgen May², Eva Lorenz², Denise Dekker²
¹Kumasi Center for Center for Collaborative Research in Tropical Medicine, KUMASI, Ghana, ²Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany

Acinetobacter spp. is an important cause of nosocomial infections, mainly affecting patients in the intensive care unit and in the immunocompromised. Furthermore, its spread in hospital wards and long-term care facilities is of particular concern as infections can be life-threatening, such as bacteremia in immunocompromised patients. In recent years, Acinetobacter spp., associated with multidrug resistance (MDR) has been on the rise worldwide. Despite being among the most common causes of hospital outbreaks often resulting in difficult-to-treat infections, studies on Acinetobacter spp. in Ghana are rare. In this study, we investigated clinical isolates of Acinetobacter spp., and their antibiotic profiles from Ghanaian patients.
Blood (456) or urine (258) samples were collected from patients visiting the St. Francis Xavier Hospital in Asan Fuso, Ghana. The samples were collected from January 2020 to February 2022 for identification and antibiotic profiles of Acinetobacter spp., isolated from the samples were performed using the VITEK 2 compact system. Isolates resistant to at least three classes of antimicrobial agents were classified as MDR. Twenty-nine (29) Acinetobacter spp. were isolated during the 2-year period, among which, 22 were isolated in the duration of 7 months (August 2020 to April 2021). The majority of the isolates were from urine (55.17%, n/N = 16/29) and the remaining (44.83%, n/N = 13/29) were from blood cultures samples. 58.6% (n/N = 17/29) of the isolates were resistant to tetracycline, 51.7% (n/N = 15/29) to cotrimoxazole and 27.6% (8/29) to cefazidime. Resistance to carbapenems was 20.7% (n/N = 6/29). Of most concern was the presence of MDR, which was detected in 24.13% (n/N = 7/29) of the isolates. We report a high frequency of Acinetobacter spp likely indicating an outbreak which will be confirmed through a further investigation into the molecular epidemiology. Antibiotic resistance observed for the locally available antibiotic is a concerning finding that necessitates the implementation of effective prevention measures to control the spread of the bacteria in the hospital environment.

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LEPTOSPIROSIS AMONG HOSPITALIZED PATIENTS WITH ACUTE FEBRILE ILLNESS IN BANGLADESH

Tanzir Ahmed Shuvo1, Anik Palit1, Mohammed Ziaur Rahman1, Zubair Akhtar1, Probir Kumar Ghosh1, Muntasir Alam1, Md. Mahfuzur Rahman1, Mahmudur Rahman2, Pawan Angra3, Matthew Mikoletz4, Daniel Martin5, Fahmida Chowdhury1
1icddr,b, Dhaka, Bangladesh, 2Global Health Development, EMPHNET, Dhaka, Bangladesh, 3Center for Disease Control and Prevention (CDC), Atlanta, GA, United States

The burden of leptospirosis is underestimated in developing countries such as Bangladesh. In the current study, we aimed to evaluate the presence of leptospirosis through sentinel surveillance among hospitalized patients with acute febrile illness (AFI) in Bangladesh. We conducted the study at five tertiary care hospitals from September 2021 to February 2023 to identify the circulating pathogens causing AFI. Eligible patients were aged ≥2 months with measured fever or history of fever (≥ 100.4°F) within the past 14 days, without focal-infection symptoms and not already in treatment for a fever-causing condition. We randomly enrolled 1,544 patients, consisting of 778 adolescents and adults above 12 years and 766 children ranging from 2 to 12 years. Blood samples were collected within 24 hours of admission. Leptospira species were detected in 67 (4%) patients of which 59 (88%) were adults. Among the positive patients 51 (76%) were male. Of the Leptospira positive cases 59 (88%) were detected through Leptochek rapid diagnostic test (RDT) kit alone (Zephyr Biomedicals), and eight were detected exclusively by RT-PCR. For the RT-PCR confirmed cases, the onset of fever ranged from 1-4 days (median: 2 days), while for RDT, the onset of fever ranged from 2-14 days (median: 7 days). None of the cases were positive for both PCR and RDT. Out of the five study sites the highest number of positive cases (21) were detected in Khulna Division (southwestern region), and the lowest number (3) were from Rangpur Division (Northwestern region). Ceftriaxone was the most frequently (58%) used antibiotic whereas the recommended drug doxycycline was used in only 10% of the patients. The finding of Leptospira species among AFI patients suggests that, physicians should more consider Leptospira infection as a differential diagnosis of AFI. In addition, appropriate diagnostic tools for a low-resource setting should also be made available to aid in the proper management of leptospirosis.

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NANOPORE SEQUENCING USING THE FULL LENGTH 16S RRNA GENE IS A PROMISING VETERINARY DIAGNOSTIC TOOL FOR THE DETECTION OF BLOOD-BORNE BACTERIAL PATHOGENS

Lucas Huggins, Vito Colella, Rebecca Traub
University of Melbourne, Melbourne, Australia

Animals of veterinary importance are afflicted by diverse blood- and vector-borne bacteria (VBB), of which many cause severe disease and can be fatal. Diagnosis of VBB infections can be challenging due to the low concentration of bacteria in the blood, the frequent occurrence of coinfections and the wide range of known, emerging, and potentially novel VBB species encountered. Therefore, there is a need for diagnostics that address these challenges by being both sensitive and capable of detecting all VBB simultaneously. We detail the first employment of a nanopore-based sequencing methodology conducted on Oxford Nanopore Technologies’ (ONT) MinIONTM device to accurately elucidate the ‘haemobacteriome’ from canine blood through sequencing of the full-length 16S rRNA gene. We detected a diverse range of important canine VBB, including Ehrlichia canis, Anaplasma platys, Mycoplasma haemocanis, Bartonella clarridgeiae, Candidatus Mycoplasma haemotarvaparum, a novel species of haemotropic mycoplasma and Wolbachia endosymbionts of filarial worms, indicative of florisis. Our nanopore-based protocol was equivalent in sensitivity to both qPCR and Illumina sequencing when benchmarked against these methods, achieving high agreement as defined by the Kappa statistics (k > 0.81) for three key VBB. Utilising ONT’s ability to sequence long read lengths provides an excellent alternative diagnostic method through which the ‘haemobacteriome’ can be accurately characterised to species-level in a way previously unachievable using short-reads. We envision our method to be translatable to multiple contexts such as the detection of VBB in other vertebrate hosts, including humans, whilst the small size of the MinIONTM is highly amenable to field use.

6983
BACTERIOLOGICAL RESISTANCE PROFILES TO ANTIBIOTICS AS REPORTED AT THE MEDICAL SERVICE, CENTRE HOSPITALIER UNIVERSITAIRE DE KATI, KATI, MALI

Hamsatou CISSE1, Youba Sangaré1, Abdramane Traoré1, Nagou Tolo1, Amadou Kassogué1, Djeneba Diagne1, Cheick Oumar Sanogo2, Bourreima Kodic1, Abdoulaye Traoré1, Garan Dabo1, Yacouba Cissoko1, Ghislain Podé1, Daouda k Mintah1
1Centre hospitalier universitaire de Kati, Kati, Mali, 2Centre de rhumatologie Primun, Bamako, Mali

The emergence and spread of antibiotic resistance remain major public health threat. Globally, the main cause of this emergence of resistance is an unreasonable consumption of antibiotics. Few data available at BSS Teaching Hospital of Kati. To study bacterial infections and the resistance profile of antibiotics at Kati Teaching Hospital. Cross-sectional analytical study using prospective data on a period of 20 months from January 2019 to August 2020. The study involved 102 patients out of 840 hospitalizations. The mean age was 55.5 dominated by patients aged 60 years (47.1%). Most of participants are male (72.5%) and the sex ratio is M/F of 2.6. Urine samples (78.4%), pus (16%), blood samples (12.7%), stool (2%) were tested. The main germs isolated were Escherichia coli (52.9%); Klebsiella pneumonia (14.5%); Staphylococcus aureus (9.9%); Acinetobacter baumannii and Enterococcus faecium (4.9%). The level of resistance of Escherichia coli and Klebsiella pneumonia was very high to ampicillin, amoxi-clavulanic acid; relatively high in C3G, and fluoroquinolones; but it retains a relative sensitivity to aminoglycosides. However, the Imipenem, Amikacine, Ertapenème remain the most active. Staphylococci were resistant to penicillin, ciprofloxacain, and oxacillin. Thus Acinetobacter baumannii had a high level of resistance to C3G, Ticarcillin,
and Piperacillin-tazobactam. Enterococcus faecium had strong resistance to Cotrimoxazole and Ciprofloxacin. 51% multi-resistant bacteria. HIV infection; antibiotic therapy; a long hospital stay had a relatively significant risk of acquiring BMRs (p=0.000; p=0.000; p=0.004). In conclusions, these results show a variable proportion of resistance and should be guided by practitioners during probabilistic antibiotic therapy in this context.

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**ANTIMICROBIAL SUSCEPTIBILITY PATTERNS OF GRAM NEGATIVE BACTERIA GRAM POSITIVE BACTERIA AND FUNGI SPECIES ISOLATED FROM BLOOD CULTURE BOTTLES IN YAOUNDE CAMEROON**

Laure Ngando¹, Leopold Mbous Nguimbus², Elaize Akenji², Albert Legrand Same Ekobo¹

¹University of Yaounde 1, Faculty of Medecine, Yaounde, Cameroon, ²University of Yaounde 1, Faculte des sciences, Yaounde, Cameroon

Bloodstream infections (BSIs) are the leading cause of mortality and morbidity worldwide. The aim of this study was to determine the antimicrobial susceptibility patterns of bacteria and fungi species isolated from blood culture bottles. This was a retrospective study. It carried out in Yaounde, at the Centre Pasteur of Cameroon from January 2010 to December 2019. Samples from patients with a clinical picture of a BSI were contained in the Bact/Alert FA, FN, and PF Plus bottles and incubated in the Bact/Alert 3D automaton. Antimicrobial susceptibility testing was performed immediately for positive cultures using the diffusion method and the Vitek 2-Compact device. A total of 5667 samples were analyzed during the study period for a prevalence of contaminated samples of 96.4%. Among the germs isolated from blood culture bottles, the most bacteria represented were: Staphylococcus sp. (13.3%), Klebsiella pneumoniae (11.9%), Micrococcus sp. (6.3%), Staphylococcus epidermidis (6.2%), Staphylococcus aureus (5.3%), Staphylococcus haemolyticus (4.7%), Escherichia coli (4.6%), Enterobacter cloacae (4.2%), Acinetobacter baumannii (4.0%) and Staphylococcus hominis (3.6%). For fungi species, Candida sp. and Candida parapsilosis were most represented (0.9% and 0.5% respectively). Antimicrobial susceptibility patterns showed that, the most represented germs were resistant to the antibiotics of penicillin family (>80%) and the cephalosporin family (>70%). However, most antifungal agents tested during the study period showed high sensitivity results. The high prevalence of a bloodstream infections and the high rates of antibiotic resistance show the need to expand the surveillance of multidrug resistance in all regions of the country.

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**NEONATAL PAENIBACILLIOSIS: A NOVEL INFECTION LEADING TO INFECTIOUS HYDROCEPHALUS IN INFANTS**

Sarah U. Morton¹, Jessica E. Ericson², Steven J. Schiff³

¹Boston Children’s Hospital, Boston, MA, United States, ²Penn State College of Medicine, Hershey, PA, United States, ³Yale University, New Haven, CT, United States

Worldwide, the majority of pediatric hydrocephalus is acquired after an early life infection. In most cases we do not know the causative agents. In Uganda, in 2020, we found that a novel strain of Paenibacillus thiaminolyticus was the most common organism associated with postinfectious hydrocephalus. We performed two prospective case-control studies in 100 maternal-newborn pairs enrolled at birth and 400 infants undergoing surgery for hydrocephalus at less than 90 days of age; as well as a cohort study of 800 neonates with sepsis. In these 1400 patients, we employed 16S gene sequencing to identify bacterial species, accompanied by targeted qPCR for P thiaminolyticus. P thiaminolyticus was the dominant organism and was identified in 44% of postinfectious hydrocephalic patients. We found no evidence of P thiaminolyticus in specimens from vaginal, placental, or maternal/cord blood samples obtained at birth. From 800 neonates with sepsis, we identified P thiaminolyticus in the blood and/or cerebrospinal fluid (CSF) of 6% of patients. Among 37 neonates with P thiaminolyticus sepsis, 14% died and 14% of survivors developed postinfectious hydrocephalus with persistence of P thiaminolyticus in the CSF. We characterized a new disease syndrome - Neonatal Paenibacillosis - underlying infant hydrocephalus in the East African country of Uganda.

**6986**

**ASSESSMENT OF THE ANTIGEN DYNAMICS DURING THE CALCIFICATION PROCESS IN NATURALLY INFECTED PIGS**

Luz M. Toribio¹, Javier Bustos¹, Laura Baquedano¹, Gianfranco Arroyo¹, Yesenia Castillo¹, Hector H. Garcia²

¹Center for Global Health, Universidad Cayetano Heredia, Lima, Peru, ²Instituto Nacional de Ciencias Neurologicas, Lima, Peru

Cysticercosis is a neglected disease caused by the infection of Taenia solium larvae and takes special attention when invading the central nervous systems (CNS) causing Neurocysticercosis (NCC), the principal cause of epilepsy around the world. Natural degeneration process concludes in calcified lesions, which are associated with inflammation and relapsing seizures. Although it is known that calcified lesions are usually associated with negative antigen detection by enzyme-linked immunosorbent assay (Ag-ELISA), the dynamics of circulant antigens during the calcification process are poorly known. We assessed the antigen dynamics of three experimental groups of pigs infected with NCC that were sacrificed after 4 (n=5), 8 (n=5) and 12 (n=6) months after antiparasitic treatment. Califications were evident on CT since month 4. We compared the capacity of two Ag-ELISA based on monoclonal antibodies (MAbs) against T solium (TsW8/TsW5) and the reference test B158/B60 Ag-ELISA. Additionally, we evaluated antibody response using ETTB assay, and histopathological stainings to describe the calcification process. Calcified lesions were found in 4/5 pigs from 4 and 8 months (60.9% and 79.3% of calcifications in brain cysts, respectively); and all 5 pigs of 12 months group (48.5% of calcifications in brain). Califications from the 8 months group presented calcium deposits within the cysts and higher of inflammation on histopathology. Circulating antigen levels followed a similar trend that was described for both assays, presenting a notable decay after 5 or 6 months of treatment. Unlike antigen drop, the antibody response decreased slowly and continued being maintained positive even after an entire year of follow-up. Calcification of brain NCC cysts is a dynamic process in which antigen is still present for a long time after treatment onset. Circulating parasitic antigen is a consistent biomarker which can be of help to monitor this process.

**6987**

**CONCORDANCE BETWEEN TWO ANTIGEN-DETECTION ENZYME-LINKED IMMUNOSORBENT ASSAYS IN DETECTING URINARY ANTIGENS AND MONITORING ANTIGEN DECAY IN PATIENTS WITH NEUROCYSTICERCOSIS**

Luz M. Toribio¹, Carolina Guzman¹, Yesenia Castillo¹, Cindy Espinoza¹, Gianfranco Arroyo¹, Herbert Saavedra¹, Javier Bustos¹, Pierre Dorny³, Seth O’Neal¹, Hector H. Garcia²

¹Center for Global Health, Universidad Cayetano Heredia, Lima, Peru, ²Instituto Nacional de Ciencias Neurologicas, Lima, Peru, ³Department of Biomedical Sciences, Institute of Tropical Medicine, Antwerp, Belgium

Neurocysticercosis (NCC) is caused by the invasion of the Taenia solium metacestode into the central nervous system. NCC is one of the most important zoonotic diseases and contributes to neurological morbidity worldwide. Imaging diagnosis (CT or MRI) is supported by with serology techniques that detect antibodies or antigens (Ag) into the host circulation. Monoclonal antibody (mAb)-based enzyme-linked immunosorbent assay (ELISA) represents a useful immunological tool to confirm an active infection, correlates well with the parasitic load, and can be performed in urine, a non-invasive sample. We evaluated the concordance between the most widely used B158/B60 Ag-ELISA and our in-house TsW8/TsW5 Ag-ELISA based on T solium mAbs for the detection of parasite antigens in urine.
the urine of patients with NCC. A total of 172 urine samples were collected and classified as in subarachnoid (n=51), parenchymal (n=18) and calcified (n=103) NCC. Concordance analysis was performed by determining the agreement and the Lin's concordance coefficient (LCC) in all the samples, as well as stratified by NCC type. Agreement between assays in all samples and stratified by NCC cases was over 90%. Equally, an overall concordance of 0.89 was obtained, and subarachnoid NCC patients presented the highest correlation (LCC=0.93), followed by calcified and parenchymal NCC (LCC= 0.77 and 0.66, respectively). Despite this high correlation, variations around a cutoff point resulted in TsW8/TsW5 Ag-ELISA categorizing 105/176 NCC cases as positive, compared to 78/176 in, the reference test B158/B60 Ag-ELISA. So far, our results indicate that urine testing with the novel TsW8/TsW5 Ag-ELISA provides comparable results to B158/B60 Ag-ELISA, providing an alternative assay for the diagnosis of NCC in a non-invasive sample, which can be applied in community settings where neuroimaging is limited.

**6988**

**PHENOTYPIC DETERMINATION OF REGULATORY T AND B REGULATORY CELLS IN NEUROCYSTICERCOSIS PATIENTS**

*Iskra Tuero*, 1 Luz Toribio2, Yazmin Marin3, Isidro Gonzalez4, Javier Bustos5, Hector H. Garcia6

1Department of Molecular and Cellular Science, Universidad Cayetano Heredia, Lima, Peru, 2Center for Global Health, Universidad Cayetano Heredia, Lima, Peru, 3Instituto Nacional de Ciencias Neurologicas, Lima, Peru

Taenia solium causes neurocysticercosis (NCC), a neglected disease that is the leading cause of acquired epilepsy worldwide. Although NCC is a heterogeneous disease, the most determinant factor for clinical manifestations is the location of parasites into the brain parenchyma or in subarachnoid spaces and the host immune responses contribute with inflammatory component as parasite apply several strategies to diminish the host inflammatory response and preserve their permanence. In this context, regulatory T cells (Tregs) play a crucial role for the parasite survival, permanence, and longevity. Although regulatory B cells (Bregs) have been associated with their participation in neurological disorders, their role in NCC infection has not been addressed before. This study aims to characterize the phenotype of Tregs and Bregs in patients with parenchymal and subarachnoid NCC. For this purpose, we evaluated peripheral blood mononuclear cells (PBMC) from untreated NCC patients with parenchymal (n=4) or subarachnoid (n=15) infection and compared it with control patients (n=6) matched by age and gender. PBMC were phenotyped by flow cytometry in the FACS Canto II using antibody panels to determine T (CD45RO+CD4+CD25+FoxP3+) and B (CD19+CD5+) regulatory cells, as well as cytokines such as IL-10 and were analyzed using FlowJo (v10.8). Statistical analyses were made using GraphPrism V6. So far, our results described a marked difference between NCC cases and controls showing high levels of regulatory cells and upregulated production of IL-10. However, the stratified analysis by NCC type has not evidenced notably differences yet.

**6989**

**CALCIFIED PORCINE NEUROCYSTICERCOSIS: IMMUNOHISTOCHEMISTRY BASED ON MONOCLONAL ANTIBODIES**

*Lizziee Tello*, 1 Luz M. Toribio1, Gianfranco Arroyo2, Manuela Verastegui2, Kayla Togneni2, Javier Bustos3, Robert Gilman3, Hector H. Garcia1

1Center for Global Health, Universidad Cayetano Heredia, Lima, Peru, 2Infectious Diseases Laboratory Research-LID, Universidad Cayetano Heredia, Lima, Peru, 3Department of International Health, Bloomberg School for Public Health, Johns Hopkins University, Baltimore, MD, United States

Cysticercosis is a parasitic disease caused by Taenia solium larvae and is a major problem of public health when established in the central nervous system (CNS) producing neurocysticercosis (NCC). NCC is the principal cause of acquired epilepsy worldwide. Numerous reports have described calcified lesions as a common cause of focal epilepsy, and pericystic edema is consistently found by the time of a seizure, suggesting intermittent release of residual parasite antigens. To assess whether parasite antigens can be demonstrated in calcified NCC lesions, we developed an immunohistochemistry (IHC) assay based in our in-house T solium mAbs, directed against total cysts (TsW2, TsW5, TsW8 and TsW12), vesicular fluid (TsV3 and TsV4) and secretory/excretory products (TsE1) in degenerate cysts (granulomas n=7) and calcifications (n=22) from 17 pigs slaughtered at 2.4, 8 and 12 months after antiparasitic treatment. IHC was standardized, processed and images were digitized. Quantification of immunoreactive areas and intensity was performed using Image J software. TsW8 and TsV3 reacted, in different intensities, in all the samples evaluated from 2, 4, 8 and 12 months; followed by TsW12 (96%) and others in a lower percentage (79%–76%). Reaction was absent in control samples. On visual examination intensity and immunoreactive areas decreased gradually along time, being less reactive in 12 months calcifications (p-trend<0.05). We also identified two immunoreactivity patterns, immunoreaction within the cyst (TsW2, 5,8,12 and TsV4) and in the pericystic tissue only (TsV3 and TsE1). This finding could hint to antigen diffusion into the tissue surrounding the lesions. Therefore, we developed a novel IHC based in the best performing mAb (TsW8) that specifically identify cystercerosis lesions and confirmed the presence of residual antigen in calcified lesions, which could contribute to an inflammatory response and consequently focal epileptic seizures in calcified NCC.

**6990**

**RESIDUAL CALCIFICATION IN NEUROCYSTICERCOSIS: NOT ONLY IN PARENCHYMAL CYSTS**

*Daniel Bustamante*, 1 Christina Coyler2, Daniel Muñoz3, Carolina Guzman4, Erick Castillo5, Javier Bustos6, Hector Garcia1

1Center for Global Health, Universidad Peruana Cayetano Heredia, Lima, Peru, 2Department of Medicine, Division of Infectious Disease, Albert Einstein College of Medicine, New York, NY, United States, 3Universidad Peruana Cayetano Heredia, Lima, Peru, 4Centro de diagnóstico por imágenes de la Clínica internacional, Lima, Peru

Considered as the most severe presentation of neurocysticercosis (NCC), subarachnoid NCC can cause inflammation and mass effects in adjacent tissues, due to the unorganized and massive growth of the parasitic membrane. Whereas abundant literature exists about the presentation and clinical impact of infraparenchymal NCC calcifications, there is basically no mention to whether subarachnoid cysts will result in residual calcification. We present 4 four adult patients from an endemic country, with a history of subarachnoid NCC lesions. All patients were very strongly positive for circulating antigen at baseline and became negative during follow up. CT was performed at 2, 4, 8 and 12 months after resolution of subarachnoid NCC cysts and calcifications in the area adjacent to the primary lesion were observed in two patients. In two cases, cystic lesions in the Sylvian fissure resulted in a punctate calcification as those seen in parenchymal NCC lesions. In the other two, larger, irregular areas of calcification were seen. Subarachnoid NCC lesions may occasionally result in residual calcification. Further studies must determine the frequency, possible causes and consequences of the presence of calcification in subarachnoid spaces and/or adjacent tissues, as a possible cause of neurological symptoms after cysts resolution.
Neurocysticercosis is the most common parasitic disease of the central nervous system. Its location on the brain can be parenchymal or extraparenchymal, the latter being more aggressive and associated with a poor prognosis. The clinical presentation of NCC depends on many variables including the location, number, and size of lesions, and the immunologic response of the host. Diagnosis criteria prioritize the use of neuroimaging techniques such as MRI or CT depending on the stage of life of the parasite, as well as location. MRI spectroscopy determines the tissue concentrations of specific metabolites and may be used to diagnose that has been used in recent years in various neurological disorders such as abscesses, tumors, and cystic lesions. We assessed MRI spectroscopy findings and performed this technique in 44 subarachnoid cysts and two parenchymal cysts. MRI spectroscopy showed peaks of lactate (1.31 ppm), lipids (0.92-1.41 ppm), isoencephalic-leucine-valine (0.87 ppm), N-acetylaspartate (2 ppm), creatinine (3 ppm) and choline (3.2 ppm) in all NCC cases. When separated, we obtained a bigger Subarachnoid lesions that had higher peaks for in all metabolites in comparison to parenchymal cysts, for subarachnoid NCC, except for Glutamine-Glutamate beta region which was higher in parenchymal NCC. These preliminary results demonstrate the ability of MRI spectroscopy to identify NCC lesions and suggest a differential pattern between subarachnoid and parenchymal lesions.

**MR spectroscopy of patients with subarachnoid and parenchymal neurocysticercosis racemosa and parenchymal neurocysticercosis of the national institute of neurologic sciences, Lima, 2021-2022**

**Concordance between two monoclonal antibody-based (mAB) enzyme-linked immunosorbent assays (ELISAs) for measuring cysticercus antigen levels insera from pigs experimentally infected with taenia solium and T. hydatigena**

**Transcranial doppler ultrasonography to detect vasculitis in neurocysticercosis**

**The early stage of Taenia solium cysticercus secrete transforming growth factor-beta mimic proteins**

**Antigen detection in cysticercosis confirms viable infection in the intermediate host(either pig or human). The reference B158/B60 Ag-ELISA for antigen detection has acceptable levels of sensitivity and specificity in NCC cases with two or more cestocerci (85% and 87% respectively), although sensitivity is lower in NCC cases with single infections (60%), whereas in rural pigs the specificity is very low (50%) due to its frequent cross-reaction with Taenia hydatigena. Our group has produced 21 anti-Taenia solium monoclonal antibodies (mAbs) reacting against antigens of the whole cyst, vesicular fluid, and secretory/excretory products, identifying TsW8/TsW5 as the most promising pair of mAbs for a sandwich Ag-ELISA format. This study reported the use of our in-house TsW8/TsW5 Ag-ELISA for measuring antigen levels (OD values) in two panels of sera from pigs experimentally infected with T. solium (n = 26) and T. hydatigena (n = 12) and assessed the concordance of this assay with the reference B158/B60 Ag-ELISA using Bland-Altman (BA) plots and Lin’s concordance coefficients (LCC). In pigs infected with T. solium, almost all paired log-OD values between assays were within the limits of agreement (LoA) in the BA plot at days 0, 28, and 90 post-infection (92.3%, 100%, and 100%), and increased concordance between assays was also found (LCC: 0.69, 0.92, and 0.96, respectively, all P<0.001). In pigs infected with T. hydatigena, almost all paired log-ODs were within the LoA, whereas increased discordance between assays was low or moderate at days 0, 28, and 90 PI (LCC: 0.24 and 0.88). This was higher at day 90 PI (LCC: 0.98, P<0.001). Our TsW8/TsW5 Ag-ELISA recognizes antigens in pigs with T. solium cysticercosis but its diagnostic use is hampered by cross-reactions with T. hydatigena, as in other mAb-based antigen detection ELISA assays.
grow and survive to complete its development to cysticercus; however, the mechanism that this parasite uses to evade the host immune response at this stage is not yet known. Taenia solium has been reported to express Transforming Growth Factor-beta (TGF-β) receptors, which exert an essential role in the parasite's growth and survival, suggesting that this parasite could secrete a TGF-β mimetic protein. This study aimed to evaluate if the different developmental times of T. solium postoncospheral form secrete a TGF-β mimetic protein. For this, T. solium activated oncospheres were grown on HCT-8 monolayers for 15, 30, and 60 days in vitro. The postoncospheral forms obtained at different times of culture were collected and co-culture for 24 h with the MFB-F11 cells (transfected mouse fibroblast cells) using a Transwell permeable supports (the MFB-F11 can detect biologically activated TGF-β in a cell co-culture system). Our results showed that postoncospheral form can activate the TGF-β signaling pathway in MFB-11 cells, suggesting that the parasite has a protein that mimics TGF-β. Further studies are needed to identify this protein and evaluate its role in modulating the host's immune response.

EVALUATION OF AXONAL TRANSPORT DAMAGE IN AN ANIMAL MODEL OF NEUROCYSTICERCOSIS

Ayme Yadine Huaman-Navarro1, Lizbeth Clemen Fustamante-Fernández2, Maria Milagros Dueñas-Mendoza1, Danitza Griselda Dávila-Villacorta1, Fabio Cesar Torres-Bocanegra1, Alejandra Jimena Bustamante-Portocarrero1, Valeria Alejandra Chancáfe-Rubio1, Edson G. Bernal-Teran1, César M. Gavidia1, Robert H. Gilman1, Manuela R. Verástegui1, Cysticercosis Working Group in Peru1

1Infectious Diseases Laboratory Research-LID and Faculty of Science and Philosophy, Universidad Peruana Cayetano Heredia, Lima, Peru, 2School of Veterinary Medicine, Universidad Nacional Mayor de San Marcos, Lima, Peru, 3Bloomberg School of Hygiene and Public Health, Johns Hopkins University, Baltimore, MD, United States

Neurocysticercosis (NCC) is a parasitic disease of the central nervous system caused by Taenia solium larvae, which causes acquired epilepsy and seizures in people from endemic countries. In a previous study with the rat infection model, axonal damage is evidenced in the tissue surrounding the parasite characterized by the formation of spheroids, which are areas of axonal swelling with accumulation of proteins of the axonal cytoskeleton as neurofilament (NFP) and that could be associated to a probable alteration in axonal transport. For this reason, in this study we evaluated by immunohistochemistry the reactivity of the motor protein kinesin and NFP in brain tissue from 3 experimental groups (treated, untreated and control rats with NCC). Rats 12 days old were inoculated intracranially with activated T. solium oncospheres and at 6 months post-infection one group received anti-parasitic treatment (oxfendazole and praziquantel) orally on 5 days and the untreated group received a vehicle and were sacrificed at different post-treatment times (48 hr, 5 days, 2 months, 8-10 months and 12 months). When evaluating NFP reactivity in the treated and untreated groups, the number of spheroids reactive to NFP was higher in gray matter than in white matter and was maintained over time, in healthy rats no reactivity to NFP in the form of spheroids was found. When evaluating kinesin reactivity around the cyst in the treated and untreated groups, reactive areas in the form of clusters called kinesin-reactive varicosities were found, with a smaller reactive area size than that observed in NFP. The number of kinesin-reactive varicosities around the cyst was similar in the groups of treated and untreated rats and was maintained over time, being higher in gray matter than in white matter; in healthy rats no kinesin-reactive varicosities were found. These results show the existence of axonal transport damage in rats with NCC, where a pathological accumulation of kinesin protein and NFP is observed over time, which would contribute to neurodegeneration and symptomatology in NCC.

EVALUATION OF OLIGODENDROCYTE DENSITY AND APOPTOSIS DAMAGE AT DIFFERENT POST-INFECTION TIMES IN A RAT MODEL WITH NEUROCYSTICERCOSIS

Alejandra Jimena Bustamante Portocarrero1, Danitza Griselda Dávila Villacorta1, Rensson Homero Célio Zgnacio1, Fabio César Torres Bocanegra1, Valeria Alejandra Chancáfe Rubio1, Maria Milagros Dueñas Mendoza1, Ayme Yadine Huaman Navarro1, Edson G. Bernal Teran1, César M. Gavidia1, Robert H. Gilman1, Manuela R. Verástegui1, Cysticercosis Working Group in Peru1

1Infectious Diseases Laboratory Research-LID and Faculty of Science and Philosophy, Universidad Peruana Cayetano Heredia, Lima, Peru, 2School of Veterinary Medicine, Universidad Nacional Mayor de San Marcos, Lima, Peru, 3Bloomberg School of Hygiene and Public Health, Johns Hopkins University, Baltimore, MD, United States

Neurocysticercosis (NCC) is a disease caused by the infection of the larval stage of Taenia solium in the central nervous system (CNS) and is the main cause of late epilepsy in endemic regions such as Latin America, Asia and Africa. It is well known that NCC causes axonal damage, however, it is undetermined whether the death of oligodendrocytes (OL) increases axonal damage. In addition, it is unknown if the presence of the parasite in the CNS is affecting the density of oligodendrocytes throughout the post-infection process. Using rats as an animal model of NCC, they were intracranially infected with activated T. solium oncospheres in the brain tissue. The animals were sacrificed at ten post-infection times and classified into five groups: group A (one and one and a half months), group B (two and two and half months), group C (three months), group D (four, six and eight months) and group E (ten and twelve months). Olig2 marker was used to evaluate the oligodendrocyte lineage using immunohistochemistry. The presence of oligodendrocytes was observed heterogeneously distributed throughout the brain, with the white matter being the area of the brain with the highest number of OL, especially in the corpus callosum. It was also observed that infected brains showed a statistically significant decrease in OL over time compared to the control group. Subsequently, oligodendrocytes that undergo apoptosis were evaluated using Olig2 and caspase-3 markers. A higher number of apoptotic OL were found in infected brains compared to healthy brains. From the obtained results, it can be concluded that OL decrease and may be associated with apoptosis.

GASDERMIN D IMMUNOREACTIVITY IN RAT BRAIN TISSUES WITH NEUROCYSTICERCOSIS

Maria Milagros Dueñas-Mendoza1, Lizbeth Clemen Fustamante-Fernández1, Ayme Yadine Huaman-Navarro1, Danitza Griselda Dávila-Villacorta1, Alejandra Jimena Bustamante-Portocarrero1, Valeria Alejandra Chancáfe-Rubio1, Fabio César Torres-Bocanegra1, Gino Castillo-Vilca1, Edson G. Bernal-Teran1, César M. Gavidia1, Robert H. Gilman1, Manuela R. Verástegui1, Cysticercosis Working Group in Peru1

1Infectious Diseases Laboratory Research-LID and Faculty of Science and Philosophy, Universidad Peruana Cayetano Heredia, Lima, Peru, 2School of Veterinary Medicine, Universidad Nacional Mayor de San Marcos, Lima, Peru, 3Bloomberg School of Hygiene and Public Health, Johns Hopkins University, Baltimore, MD, United States

Neurocysticercosis (NCC) is a parasitic disease affecting the brain caused by the larval form of the parasite Taenia solium; seizures are the predominant clinical feature when the cyst is located at the level of the brain parenchyma, but asymptomatic cases also occur. During the development of the parasite in the central nervous system (CNS) tissue, a mild to moderate inflammatory response is observed, which becomes chronic over time and after three months post-infection, fibrotic tissue formation around the cyst is evident. Treatment of NCC with anthelmintic drugs has been shown to exacerbate the clinical symptoms, which are associated with parasite damage and increased inflammatory response in the tissue around the parasite. Many CNS diseases are closely related to pyroptosis cell death which is a type of programmed cell death mediated by...
**A Rat Model for Neurocysticercosis Presenting Nitro-Oxidative Stress and Neuronal Damage**

Edson G. Bernal-Teran¹, Ana D. Delgado-Kamiche¹, Laura E. Baquedano¹, Roger P. Carmen-Orozco¹, Oksana O. Huerta Reyes¹, Karla Villalobos-Camizán¹, Ayne (Yadine) Huaman Navarro¹, Oscar Nizama Salazar¹, Nancy Chile¹, Edith S. Málaga-Machaca¹, Cesar M. Gavidia-Chucan³, Manuela R. Verastegui-Pimentel¹, Javier A. Bustos¹, Robert H. Gilman³, Cysticercosis Working Group in Perú¹

¹Infectious Diseases Laboratory Research-LID, Faculty of Science and Philosophy, Universidad Peruana Cayetana Heredia, Lima, Peru. ²School of Veterinary Medicine, Universidad Nacional Mayor de San Marcos, Lima, Perú, Lima, Peru. ³The Department of International Health, Bloomberg School of Hygiene and Public Health, The Johns Hopkins University, Baltimore, Maryland, Baltimore, MD, United States

Neurocysticercosis (NCC) is an infection of the Central Nervous System (CNS) by the larval stage of the flatworm parasite Taenia solium; epilepsy is the most common clinical manifestation of this infection and is associated with inflammatory processes such as angiogenesis, gliosis, and neuronal damage; however, whether nitro-oxidative stress plays a role in the epileptogenic process in NCC has not yet been evaluated. In this study, an indicator of nitro-oxidative stress (iNOS) and its relationship with the presence of recurrent seizures (Epilepsy) in rats were evaluated by immunohistochemistry, as well as whether the presence of nitro-oxidative stress is associated with the presence of spheroids or axonal swelling, and gliosis (CD68) or astrogliosis (GFAP) in the NCC animal model comparing NCC rats with epilepsy, NCC rats without epilepsy and healthy rats. iNOS expression was found to be higher in NCC rats presenting with epilepsy (p<0.05); the presence of macrophages and astrocytes around the cyst is also increased in rats with NCC presenting with epilepsy (p<0.05) and is associated with iNOS immunoreactivity (rho=0.75); Finally, the presence of spheroids reactive to NFP and SOD1 was higher in rats with NCC that developed Epilepsy with p<0.05 and p=0.06 respectively; In addition, there is an association between the number of spheroids and iNOS immunoreactivity (Spearman rho=0.81 for NFP spheroids and Spearman rho=0.75 for SOD1 spheroids). These results indicate that there is nitro-oxidative stress in the brain tissue of rats with NCC that present epilepsy and is associated with the severity of gliosis and astrogliosis as well as the presence of neuronal damage in the form of axonal swelling, so it is necessary to deepen the study of this event in order to find therapeutic targets in epilepsy due to NCC.

**An Update on the Clinical-Epidemiology of Neglected Sexually Transmitted Infections in Impoverished Southern USA Adolescent Populations**

Lauren Turner, Melissa Nolan

University of South Carolina, Columbia, SC, United States

While sexually transmitted infections are not traditionally considered a neglected tropical disease, the health disparities aspects of these infections makes a strong case for their neglected nature among vulnerable populations—as highlighted in an AJTMH neglected parasitic infections editorial. South Carolina is home to some of the highest national poverty rates and sexually transmitted infection rates. Here, 60% of at-risk adolescents are positive for at least one infection: half being pregnant girls. Further, 18% are triply infected with concomitant gonorrhea, trichomoniasis and chlamydia. The clinical and immunologic impact of co-infection is unknown, especially among adolescents, yet hypothesized to contribute to growing drug resistance and treatment failure. The South Carolina STICK study was started in October 2022 to evaluate the long-term clinical, epidemiological, and immunological impact of trichomoniasis, chlamydia and gonorrhea concomitant infection in adolescents and young adults. This presentation will describe the risk factors for treatment failure, prospective vaginal microbiome changes by disease group, and host immunologic response to concomitant infection from the first enrolled cohort. This study sheds light on an emerging neglected group of diseases, whose clinical-epidemiologic profile is changing in real-time.
**SPOT SEPSIS: PREDICTION OF DISEASE SEVERITY IN YOUNG CHILDREN WITH ACUTE FEBRILE ILLNESSES IN RESOURCE LIMITED SETTINGS**

Arjun Chandra1, Raman Mahajan2, Riris Ahmad3, Eggi Arguni3, Elizabeth A. Ashley4, Quoc Dat5, Nicholas PJ Day4, Arjen Dondorp6, Carolina Jimenez7, Kevin Kain8, Rungrupa Khamboocha9, Constantine Koshiaris10, Estrella Lazry11, Mayfong Mayxay11, Chonticha Menggred6, Dinesh Monal12, Lazzaro Mwandigha8, Rafael Perera-Salazar12, Phan Huu Phuc12, Tiengkham Pongyongsa11, Sayaphet Rattanavong12, Michael Rekart2, Melissa Richard-Greenblatt12, Mohammad Shomik13, Pouthalavanh Souvannasang13, Keang Suy1, Claudia Turner1, Paul Turner1, Dinh Van Anh10, Naomi Waithira13, James A. Watson6, Michael Yosia14, Yoel Lubell15, Sakib Burza1

1Cambodia Oxford Medical Research Unit, Siem Reap, Cambodia, 2Medecins Sans Frontieres, New Delhi, India, 3Universitas Gadjah Mada, Yogyakarta, Indonesia, 4Lao-Oxford-Mahosot Wellcome Trust Research Unit, Vientiane, Lao People’s Democratic Republic, 5Hanoi Medical University, Hanoi, Viet Nam, 6Mahidol-Oxford Tropical Medicine Research Unit, Bangkok, Thailand, 7University Health Network, Toronto, ON, Canada, 8Department of Primary Care Health Sciences, Oxford, United Kingdom, 9Centre for Nutrition and Food Security, Dhaka, Bangladesh, 10Vietnam National Children’s Hospital, Hanoi, Viet Nam, 11Savannahak Provincial Health Office, Savannakhet, Lao People’s Democratic Republic, 12University of Toronto, Toronto, ON, Canada, 13Salavan Provincial Hospital, Salavan, Lao People’s Democratic Republic

Infections remain the leading cause of preventable childhood mortality in low- and middle-income countries. Reliable guidelines to help community healthcare providers identify children whom may benefit from referral to higher-level care are lacking. This talk will present findings from a multicountry prospective observational cohort study (NCT04289502) which recruited children under the age of five presenting with acute febrile illness to seven hospitals in Bangladesh, Cambodia, Lao PDR, Indonesia, and Vietnam. The primary objective was to develop and externally validate a clinical prediction model containing simple clinical parameters and host biomarkers amenable to measurement using point-of-care tests, to support risk stratification of children presenting in resource-limited primary care settings and decentralised models of care. The primary outcome was severe febrile illness, defined as death, vital organ support, or admission to any health facility for > 2 days in the 28 days following enrolment. Between March 2020 and October 2022, 3,450 children were recruited, of whom 6.1% met the primary outcome. Penalised logistic regression was used to develop the model using data from Bangladesh, Lao PDR, Indonesia, and Vietnam, which will be externally validated in the dataset from Cambodia. Discrimination, calibration, and classification at clinically plausible referral thresholds will be reported. Generalisability of the model will be evaluated using decision curve analyses to account for heterogeneous contexts commonplace in community care settings in many LMICs. Targeted aetiological investigations will allow exploration of the performance of the model in confirmed bacterial and viral infections. The results of the study will inform planning of an interventional trial to evaluate the clinical impact of the model on improving health outcomes for children presenting with acute febrile illnesses in resource-limited community settings.

**EVALUATION OF THE IMPACT ON CHILDHOOD MORTALITY OF AZITHROMYCIN PLUS INTERMITTENT PREVENTIVE TREATMENT ADMINISTERED THROUGH THE EXPANDED PROGRAM OF IMMUNIZATION IN SIERRA LEONE**

Haily Chen1, Kwabena Owusu-kyei1, Mireia Llach1, Mohamed Samari2, Clara Menéndez1

1Barcelona Institute for Global Health, Barcelona, Spain, 2College of Medicine and Allied Health Sciences, Freetown, Sierra Leone

Sub-Saharan Africa (SSA) continues to concentrate the highest child mortality rate with half of the 5.3 million deaths of children under 5 years of age (U5) in 2018 worldwide. Infectious diseases, such as respiratory and gastrointestinal infections are common mortality causes preventable by treatments and prevention. Sierra Leone is one of the countries with the highest malaria and U5 mortality rates worldwide with malaria prevalence among U5 ranging from 20.9 to 40.4% and an infant mortality rate of 75 infants per 1,000 live births. It is also the only country that nationally implemented the Intermittent Preventive Treatment of malaria in infants with sulfadoxine-pyrimethamine (IPTi-SP) as recommended by the World Health Organization (WHO), which is now renamed Perennial Malaria Chemoprevention (PMC). The ICARIA (Improving Care through Azithromycin Research for Infants in African clinical trial is carried out in Sierra Leone to provide the evidence needed to inform policy and accelerate the implementation of this intervention. The trial is designed as an individually randomized doubled blind two-arm placebo-controlled superiority trial, where AZi will be administered three times alongside routine preventive health interventions of the EPI, such as immunizations and IPTi-SP, as recommended by the WHO for malaria prevention in this age group. Additionally, IPTi-SP administration will be increased to six doses and expanded to the second year of life up to 15 months of age. Implemented in March 2021, the trial aims to recruit 20,560 children visiting health facilities for the first EPI pentavalent vaccine and follow up until they reach 18 months of age. The potential development of antibiotic resistance, SP resistance, interactions with routine immunizations, safety, and the impact on the health system of AZi and IPTi-SP administration will be all assessed in this trial.

**SPOT SEPSIS: PREDICTION OF DISEASE SEVERITY IN YOUNG CHILDREN WITH ACUTE FEBRILE ILLNESSES IN RESOURCE LIMITED SETTINGS**

Robert Duncan1, Carolyn Fisher1, Scott Espich1, Moussa Kourout1, Sean Smith1, Luis Santane-Quintero1, Anjan Purkayastha2, Morgan Chandler1, Maria Rios1, Hong Zheng1, Sanjai Kumar1, Raman Mahajan2, Riris Ahmad3, Eggi Arguni3, Elizabeth A. Ashley4, Quoc Dat5, Nicholas PJ Day4, Arjen Dondorp6, Carolina Jimenez7, Kevin Kain8, Rungrupa Khamboocha9, Constantine Koshiaris10, Estrella Lazry11, Mayfong Mayxay11, Chonticha Menggred6, Dinesh Monal12, Lazzaro Mwandigha8, Rafael Perera-Salazar12, Phan Huu Phuc12, Tiengkham Pongyongsa11, Sayaphet Rattanavong12, Michael Rekart2, Melissa Richard-Greenblatt12, Mohammad Shomik13, Pouthalavanh Souvannasang13, Keang Suy1, Claudia Turner1, Paul Turner1, Dinh Van Anh10, Naomi Waithira13, James A. Watson6, Michael Yosia14, Yoel Lubell15, Sakib Burza1

1FDA/CBER, Silver Spring, MD, United States, 2OpenBox Bio, LLC, Vienna, VA, United States

The large number of infectious agents in blood and their continued emergence are constant challenges for diagnostic and blood donor screening devices. To increase multiplicity while maintaining sensitivity and specificity, new technologies must be designed and tested. Effective platforms must also achieve rapid processing and provide results that are easy to interpret. For example, dozens of patients arrive at a clinic in Uganda with a fever of unknown origin each week. To diagnose under such conditions, a single, affordable, multiplex molecular test could replace a multitude of costly assays. We have developed and published a resequencing microarray and are now completing work on an expanded blood borne pathogen microarray (BBPv2) that could satisfy that need. To determine whether the portable DNA sequencing technology can replace the microarray for pathogen identification, we are conducting a comparative analysis of the Oxford Nanopore MinION sequencing device and the BBPv2. Our study involves testing both devices to determine the infectious agents in various samples, such as blood and plasma samples, spiked with lab cultured pathogens, infectious agent reference panels and blood donor specimens. The accuracy, speed and complexity of each device will be compared and evaluated to determine the method most suited for use in endemic areas. My comments/My contributions are an informal communication and represent my own best judgement. These comments do not bind or obligate FDA
A RANDOMIZED CLINICAL TRIAL INVESTIGATING THE EFFECT OF BCG REVACCINATION ON THE RESPONSE TO UNRELATED VACCINES IN UGANDAN ADOLESCENTS: THE POPVAC C TRIAL

Jacet Nassyuna, Agnes Natukunda, Gyaviira Nkurunungi, Ludoviko Zirimenya, Emily L. Webb, Alison M. Elliott
1MRC/UVRI & LSHTM Uganda Research Unit, Kampala, Uganda, 2London School of Hygiene & Tropical Medicine, London, United Kingdom

There is evidence that BCG immunisation may protect against unrelated infectious illnesses. We hypothesized that administering BCG before unrelated vaccines modifies responses to subsequent vaccines.

We designed a randomised controlled trial of BCG versus no BCG immunisation to determine the effect of BCG on subsequent unrelated vaccines, among adolescents (aged 13–17 years) from a Ugandan, urban birth cohort. Our schedule comprised three main immunisation days (week 0, week 4 and week 28): BCG (or no BCG) revaccination at week 0; yellow fever (YF17D), oral typhoid (Ty21a) and human papillomavirus (HPV) prime at week 4; and HPV boost and tetanus/diphtheria (Td) boost at week 28. The primary outcomes were anti-YF-17D neutralising antibody titre, Salmonella typhi lipopolysaccharide-specific IgG concentration, IgG titre for L1-proteins of HPV-16/HPV-18, all assessed at 4 weeks after immunization and tetanus and diphtheria toxoid-specific IgG concentration assessed at week 28. We enrolled 300 participants of which 151 were in the BCG revaccination arm and 149 in the no BCG revaccination arm. 178 (58%) of the participants were male. 142 (94%) of the participants in the BCG group and 136 (91%) in the no BCG group completed the trial. Comparing trial arms, no difference in response was observed for any vaccine: Geometric Mean Ratios (GMR) (95% CI) were, for yellow fever 0.97 (0.77-1.22), Ty21a 0.981 (0.82-1.18) at week 8 and 1.17 (0.91-1.50) and 0.982 (0.85-1.13) respectively, at week 52. The GMR for TT and DT IgG responses at week 52 was 1.04 (0.81-1.33) and 0.95 (0.84-1.08) at week 52 respectively. The outcomes for HPV are still being processed.

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DIFFERENTIATION OF ACUTE EXACERBATIONS OF CHRONIC HEPATIS B AND ACUTE HEPATITIS B IN ANTIHBc IGM POSITIVE PATIENTS AT HOSPITAL FOR TROPICAL DISEASESIN VIETNAM

Thao Tu, Hung Le, Hien Tran
1Hospital for Tropical Disease, Ho Chi Minh, Viet Nam, 2Pham Ngoc Thach University of Medicine, Ho Chi Minh, Viet Nam

Acute exacerbations of chronic hepatitis B (CHBE) are common in endemic areas and difficult to distinguish from acute hepatitis B (AHB) in anti-HBc IgM positive patients with prior HBV history infection. We performed a retrospective and prospective observational study for adult patients who presented with clinical features of acute hepatitis along with antiHBc IgM antibody to hepatitis B core antigen (antiHBc IgM) positive at Hospital for Tropical Diseases (HTD), Hochiminh City, Vietnam from January 2018 to September 2022. Diagnostic confirmation was based on HBsAg loss after 6 months. Multivariables logistic regression was done to identify the factors that differentiated AHB from CHBE in anti-HBc IgM positive patients. A total of 610 patients were enrolled and divided into two groups: AHB (n = 491) and CHB-AE (n = 119). AntiHBc IgM cutoff of 8 S/CO provided the best sensitivity and specificity for predicting patients with CHB-AE (91.2% and 90.8% respectively). The multivariate analysis demonstrated that the level of platelet, AFP and the S/C ratio of antiHBc IgM were significant factors. AFP and the S/C ratio of IgM antiHBc were significantly higher in CHBE group (OR 0.995, (95% CI 0.991 - 0.999) and OR 1.356, (95% CI 1.124 - 1.635), respectively), while the platelet level was significantly higher in the AHB group (OR 1.031, (95% CI 1.008 - 1.056). In conclusion, antiHBc IgM should be included concurrently with liver fibrosis biomarkers, particularly the levels of platelet and AFP to discriminate CHBE and AHB.

STOOL CALPROTECTIN AND ASSOCIATED GUT-PATHOGENS IN A COHORT OF PATIENTS WITH GIT DISORDERS WITH AND WITHOUT IMMUNE-MEDIATED INFLAMMATORY INTESTINAL DISEASES IN SAUDI ARABIA

Ayman A. Elbadry, Nehal M.M. Hosin
Imam Abdulrahman Bin Faisal University, College of Medicine, Microbiology department, Dammam, Saudi Arabia

Stool calprotectin is a neutrophil-specific zinc- and calcium-binding protein. It releases in the intestinal lumen in acute intestinal inflammation and is used as a biomarker for gut inflammation. This study’s goal is to determine the distribution pattern of calprotectin in a cohort of patients with GIT disorders, including inflammatory bowel disease (IBD), irritable bowel syndrome (IBS) and other non-IBD/IBS GIT disorders. Also, to assess the association between the presence of stool calprotectin and patients’ data, including stool test results for gut pathogens. Stool specimens and related data were collected from 408 patients attending the King Fahad Hospital of the University (KFHU), Eastern Province, Saudi Arabia whose stool examination was done in the microbiology lab as part of the management of the patient’s clinical condition. Each stool specimen was examined microscopically for the pathogen, cultured on Jones for Blastocystis, and stool calprotectin was measured using ELISA assay. All related patients’ sociodemographic and clinical data were recorded and analysed as associated factors/variables. Except for structure colonic diseases (Anal fissure, fistula, abscess, colon polyp) and food allergies, calprotectin was detected in all GIT disorders mainly inflammatory bowel diseases. Results of calprotectin showed a pattern with age distribution, it was detected in all age groups with a decrease in positivity with increasing age. Males had more positive cases with calprotectin in their stool than females. Stool leukocytes, occult blood in stool, the presence of the gut parasite in stool and the presence of H. pylori antigen in stool were more common in calprotectin-positive patients. The presence of Clostridium difficile and negative stool culture was more common in calprotectin-negative patients. Stool calprotectin is present in stool and in patients with IBD.
to a relatively healthy gut. Further studies to assess the distribution of calprotectin in health and disease are needed, in order to provide a better understanding of the role of calprotectin as a biomarker in GIT disorders.

7008

CLINICAL CHARACTERIZATION AND ANALYSIS OF INFLAMMATION AND COAGULATION OF LASSA FEVER PATIENTS TREATED AT THE IRRUA SPECIALIST TEACHING HOSPITAL IN EDO STATE, NIGERIA IN 2022 AND 2023

Cyril Erameh1, Osahogie Edeawe1, Joseph Okegbele1, Jerome Christian1, Iboi Matthew1, Hannah Müller2, Matin Kochar3, Christian Erohbue1, Beneveloche Ohomoi2, Rita Esun4, Charity Oseghale1, Alirebhe Ujiagbe1, Charlotte Kriebel5, Ludmila Unrau2, Anke Thielebein2, Colette Sih3, Stephan Günther2, Michael Ramharter2, Sylvanus Okogbenin1, Lisa Oestereich2, Till Omansen1

1Irrua Specialist Teaching Hospital, Irrua, Nigeria, 2Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany

Lassa fever (LF) is a viral hemorrhagic fever occurring in West Africa with a high case fatality rate (CFR) in hospitalized patients. To date there is no approved treatment or vaccine and the diseases’ pathophysiology is poorly understood. Hemodynamically relevant hemorrhagic presentations are rare; severe cases are complicated by renal failure and neurological manifestations. Here, we longitudinally characterized an observational cohort of currently 339 acute RT-PCR confirmed LF patients (March 23rd, 2023; recruitment ongoing) during two LF outbreaks in Edo State, Nigeria in 2022 and 2023. By combining detailed clinical examinations and testing for molecular markers of inflammation and coagulopathy, as well as transcriptomics we aim to identify correlates of severe disease. Our study visits consisted of physical examinations, brief neurological status, vital parameter measurement and point-of-care testing for PT and aPTT. In selected cases rotational thromboelastometry and ultrasound scanning is done. Besides standard laboratory analysis (hematology, clinical chemistry, electrolytes, blood gas analysis) we measure consecutive viral load and biomarkers of inflammation and coagulopathy such as plasminogen activator inhibitor-1 (PAI-1) and soluble thrombomodulin which have previously been shown to be associated with severe LF. In our currently analyzed subset of patients (n=278; mean age 32 years ± 13.2; 41 % female, CFR 11,2 %) we classified 67 (24%) as severe. Severe cases were hallmarkmed by elevated acute phase markers such as CRP and ferritin, acute kidney injury (AKI), septic shock and respiratory failure with massive pleural effusion. Bleeding was rare though some fatal cases had highly abnormal coagulation parameters and fulfilled DIC criteria. In summary we characterized relevant organ complications of LF and identified a hyperinflammatory state to be associated with severe disease which both have important implications for the development of appropriate medical countermeasures. Recruitment is currently ongoing until July 2023 and the study’s final results will be presented at the meeting in November 2023.

7009

CHAGAS DISEASE CLINICS IN ENDEMIC LOCALITIES FROM ARGENTINA: RESULTS FROM 2015 TO 2022

Karina Cardone1, Mariela Contreras2, Mariela del Pilar Cegna1, Cinthia Deigado1, Marcelo Wirtz1, Mariana Fernández1, Maria Victoria Periago1, Favio Crucido1

1Fundación Mundo Sano, Buenos Aires, Argentina, 2CONICET/Fundación Mundo Sano, Buenos Aires, Argentina

Chagas Disease (ChD), caused by Trypanosoma cruzi, is historically a vector-borne disease mostly present in Latin America with other minor forms of transmission. In Argentina, vector presence (Triatoma infestans) has been reported in 19 of the 24 provinces. Recently, 10 provinces were certified free of vector-borne transmission of ChD (Corrientes, Entre Ríos, Jujuy, La Pampa, Misiones, Neuquén, Río Negro, San Luis, Santa Fe, and Tucumán). In Chaco and Santiago del Estero, traditionally endemic, Mundo Sano (MS) implemented entomological surveillance and control (S&C) actions since 2008 in Pampa del Indio and 2002 in Añatuya, respectively. Given a decrease in vector populations due to sustained S&C and environmental/land changes, MS has been recently and actively promoting diagnosis and treatment (D&T) of ChD together with the local municipalities. For this purpose, specialized ChD clinic days were set up in municipal or provincial health posts of all three localities since June 2015 in collaboration with local professionals coordinated by MS. Up to December 2022, 3,433 individuals received medical attention specifically for ChD, of which 873 (25.4%) tested positive for T. cruzi. Prevalence varied depending on the locality; 39.3% for Pampa del Indio, 43.1% for Colonia Dora (Santiago) and 19.4% for Añatuya. Treatment was offered to positive individuals with indication for treatment based on national guidelines and medical consensus. From 873 positive individuals, 666 (76.3%) fit the treatment criteria and more than half of these (59.2%) started treatment with Benznidazole (Laboratorios Elexa Phoenix S.A.). Some reasons for lack of treatment in eligible individuals included: fear, lack of trust, interest, or urgency, or unwillingness to adhere to the necessary dieting restrictions during treatment. This initial approach through specific clinics has shown the need for D&T in older population to avoid progression of the disease to more severe forms and generates evidence for integration of this model of attention into the regular public health system.

7010

ISOLATED HYPERPARASITEMIA IN IMPORTED SEVERE PLASMODIUM FALCIPARUM MALARIA: NO PREDICTOR FOR COMPLICATIONS

Tilman Lingscheid, Pinkus Tober-Lau, Juliane Dörfler, Leif-Erik Sander, Florian Kurth

Charité - Universitätsmedizin Berlin, Berlin, Germany

Severe malaria remains a health threat for travelers to endemic areas. In addition to the criteria for severe malaria, WHO has included an entity termed uncomplicated hyperparasitemia (HP) (range: 4-10%) in its latest treatment recommendations. Such parasite density, however, usually defines severe disease in patients outside endemic areas where HP is not specified with recommendations ranging from ≥2 to ≥5%. We retrospectively analyzed all adult in-patients with HP defined as ≥4% and/or severe Plasmodium falciparum malaria according to WHO criteria, treated from 2013 to 2023 at Charité university hospital. The primary objective was to identify and stratify the risk for a critical disease trajectory based on the WHO criteria for severe malaria; in addition, HP was evaluated as an independent factor. The sum of positive criteria was calculated and analyzed for the primary endpoint “need for intensive care”. Overall, 75 patients were included in this study. Median age was 48 (IQR 36-56) with 50 male patients (67%). Patients were tourists (n=36, 48%), VFRs (n=35, 47%) and visitors from endemic regions (n=4, 5%). 51 patients (68%) received arsunate, 17 (23%) an ACT and 7 (9%) quinine as primary treatment. The likelihood of requiring intensive care increased steadily with the number of criteria for severe malaria met by an individual, from 20% in patients with only one criterion to 58% for 2 criteria and 100% for ≥ 3 criteria. The exception were patients with severe clinical presentation (cerebral malaria, shock), who all required intensive care, whereas all other patients with only one criterion did not. This was especially true for the 39 patients (52%) with isolated HP, who formed the majority in the group of patients with only one criterion (39/44, 89%), all of whom did not require intensive care. Among these patients, median parasitemia was 7% (IQR 5-11%); 15 patients (38%) presented with a parasitemia of ≥10%. Patients with HP - even above 10% - in the absence of clinical or laboratory criteria for complicated malaria did not require ICU treatment in our patient population whereas severe clinical presentation regularly predicted need for ICU treatment.
THE BURDEN AND IMPACT OF TORCH INFECTIONS AMONG PERINATAL WOMEN AND THEIR NEONATES IN EL SALVADOR

Mary K. Lynn1, Marvin Stanley Rodriguez Aquino2, Stella C. W. Self1, Mufaro Kanyangarara1, Berry A. Campbell1, Melissa S. Nolan1
1University of South Carolina, Arnold School of Public Health, Columbia, SC, United States, 2University of the Witwatersrand, Johannesburg, Gauteng, South Africa

TORCH pathogens are a group of congenitally transmitted infectious agents that can cause severe health outcomes in mothers and their fetus or neonate. TORCH pathogen burdens are higher in low-and-middle-income countries; however, they receive little public health attention. Therefore, the goal of this study was to investigate the prevalence of and maternal-fetal impact of four key TORCH pathogens (Toxoplasma gondii, toxoplasmosis, Trypanosoma cruzi (Chagas disease), Zika virus, and dengue virus) among women presenting for labor and delivery to a large referral hospital in western El Salvador. This study was approved by the Salvadoran National Health Research Ethics Committee, and 201 women ≥15 years of age were enrolled at parturition. From this cohort, 36% of women had diagnostic evidence of ≥1 TORCH pathogen, and 5% had evidence of ≥2 TORCH pathogens at the time of childbirth or within the last trimester of their pregnancy. Pathogen specific infection incidence were: 25% Toxoplasma gondii, 3% Trypanosoma cruzi, 9% Zika virus, and 6% dengue virus.

Toxoplasma gondii positive mothers were significantly more likely to have had Ministry of Health household fumigation or mosquito abatement within the past year. Maternal T. gondii infection was significantly associated with keeping animals in the home, and lower maternal education. Lastly, maternal T. cruzi positivity was significantly associated with older maternal age, lower maternal education level, and neonatal admission to the NICU. Clinicians and public health officials working in Central America should prioritize TORCH pathogen surveillance among pregnant women and women of childbearing age, as intervention efforts in this population have benefit to both mother and fetus. This presentation will review our findings and TORCH pathogen basics for practitioners needing a refresher on congenitally-transmitted neglected tropical diseases.
treated in the United States. Contributing factors to these low estimates are multiple but include low physician awareness and knowledge. This study measured physician knowledge before and after training in New Orleans, Louisiana (2022-2023). Hybrid training was given at grand rounds, resident didactics, and conferences. Demographics data was collected. Questions were on disease epidemiology, diagnosis, and treatment. McNemar's test assessed the effect of the training on knowledge. A total of 248 physicians consented from two hospitals and four medical centers. Thirty-six percent were from internal medicine, 29% pediatrics, 15% obstetrics and gynecology, 11% family medicine and 9% cardiology. Forty-three percent were residents, 24% attendings, 19% medical students, 10% fellows, 2% nurses and 2% others. Most (95%) had heard of Chagas disease; 62% had not taken a course or lecture on Chagas disease. At baseline, 80% answered correctly on causative agent, endemic regions, disease course but only 56% answered correctly on various transmission routes. A quarter knew diagnostic methods, 42% knew diagnostics for infants, and 38% knew treatment. Following training, knowledge increased and >90% answered questions correctly on the causative agent, endemic regions, disease course and transmission routes (p<0.05), 78% on diagnostic methods (p<0.05), 85% on diagnostics for infants, and 90% on treatment. In this sample, there was greater knowledge on disease basics compared to other reports, but inadequate knowledge of diagnostics and treatment prior to training physicians. In the short term, the training improved knowledge gaps in all areas tested, like a previous study. Sustained training of physicians on Chagas disease is needed to increase awareness and improve patient access to diagnostic and treatment.

7015
UNVEILING CONGENITAL SY Philip in MINIMALLY INVASIVE TISSUE SAMPLING: A CASE REPORT FROM CHILD HEALTH AND MORTA TREATMENT SURVEILLANCE, BANGLADESH SITE
Mohammad Sabbir Ahmed1, Mohammad Mosiur Rahman2, Ta Mis Wilson1, Muntasir Alam2, Kazi Munisul Islam1, Afrun Rahman1, Rajib Biswas1, Md. Fakhruddin1, Afsana Afrin1, Afsana Rashed1, Sanwarul Bar1, Shams El ANtife1, Mustafizur Rahman1, Mohammad Zahid Hossain1
1, david, Dhaka, Bangladesh, BSMMU, Dhaka, Bangladesh, 2Emory University, Atlanta, GA, United States

Despite the availability of preventive strategies such as antenatal screening for syphilis in the first trimester of gestation, congenital syphilis remains a serious public health problem worldwide, particularly in developing countries. Worldwide 12 million people are infected with syphilis annually. The Child Health and Mortality Prevention Surveillance (CHAMPS) network aims to determine the causes of stillbirth and under-5 years of child death as the underlying cause of stillbirth. Here the samples were tissues from the brain, lungs, and liver through minimally invasive tissue sampling (MITS) and blood. The mother was hospitalized at 29 weeks of gestational age with complaints of less fetal movement, lower abdominal pain and per vaginal bleeding. The mother delivered a dead female baby by normal vaginal delivery after 2 days of admission. She had four ante-natal check-ups (ANC) from government facilities and the first ANC was taken at 4 months of pregnancy. There is no document available of any test for syphilis. The baby exhibited autolysis, consistent with macerated stillbirth. The samples from the brain, lungs, and liver were analyzed for congenital syphilis using the CHAMPS network.

7016
RAISING STANDARDS IN DIAGNOSTIC PARASITOLOGY - PERSPECTIVES FROM AN INTERNATIONAL EXTERNAL QUALITY ASSESSMENT SCHEME
Peter L. Chiiodini
UK NEQAS Parasitology, London, United Kingdom
Diagnostic Parasitology is approaching an identity crisis. Traditionally dependent on classical light microscopy, it now faces a scarcity of expert staff with the necessary morphological skills. At the same time, molecular techniques have entered the field and machine learning is being applied to the microscopical diagnosis of malaria. Whatever technique is deployed, individual laboratories must provide a quality-assured service. Data from the UK National External Quality Assessment Schemes for Parasitology highlight common errors such as failure accurately to report malarial parasitaemias, confusion of pollen grains with Taenia ova and confusion of plant fibres with nematode larvae. The possible role of machine learning and electronic EQA schemes in raising and maintaining standards is considered in this presentation.

7017
PURIFICATION OF EXTRACELLULAR VESICLES PRODUCED BY FILARIAL PARASITES FOR PROTEOMIC DETECTION OF BIOMARKER CANDIDATES IN HUMAN PLASMA
Devyn Yates1, Lucia S. Di Maggio1, Reid Townsend1, Robert Sprung1, Petra Erdmann-Gilmore1, Bruce Rosa1, Philip J. Budge1, Joseph Kamgno2, Makedonka Mitrev1, Gary Weil1, Peter Fischer1
1Washington University School of Medicine, St. Louis, MO, United States, 2Centre for Research on Filarisis and other Tropical Diseases, Yaoundé, Cameroon

Lymphatic filariasis (LF) is a neglected tropical disease caused by the nematodes Wuchereria bancrofti, Brugia malayi and Brugia timori. Tests to detect circulating filarial antigen as biomarkers for adult W. bancrofti are available, but there is no antigen test for Brugian filariasis or a biomarker for microfilariae (MF). Additionally, infection with Loa loa can render false positive results as well as causing severe adverse effects, both of which complicate LF elimination programs. Extracellular vesicles released by filarial parasites may carry antigen cargo suitable as a biomarker, but reliable protocols for isolation and proteomic analysis need to be evaluated. The main objective of these studies was to identify novel biomarkers in vesicles isolated from host plasma. Using the ME kit, vesicles were isolated from a gerbil and one cat, both infected with B. malayi, and sera from humans infected with loiasis. Patients with loiasis were selected due to their high Mf densities (between 29,120 and 81,120 Mf/ml) which are not typical of other filarial species. A more in depth bioinformatics analysis was completed to remove the host proteins that were identified with LC-MS to find proteins unique to B. malayi or L. loa. Gerbil and cat plasma contained 110 and 55 B. malayi proteins, respectively. 21 L. loa proteins were identified in 10 human plasma samples; 7 of these were present in 3 or more samples and spectral counts greater than 10. A L. loa protein with BmR1 homology was detected in 10 of 10 loiasis samples and a 14-3-3 zeta protein was found in 9 of 10 samples. Two 14-3-3 B. malayi homologs were detected in the gerbil (Bm4259 and Bm10299). This finding supports the idea that results obtained with L. loa can help to identify biomarkers for other filarial species. From these experiments, it is shown that vesicles can be detected in plasma from patients with high infection of parasite. With this, new biomarker candidates for both LF and loiasis have been identified and will be explored in future work.
TOWARDS IMPROVED ONCHOCERCIASIS DIAGNOSTICS: CHARACTERIZATION OF A MAJOR ANTIGEN OF ONCHOCERCA VOLVULUS IDENTIFIED FROM THE PLASMA OF INFECTED INDIVIDUALS

Adebiyi A. Adeniran, Kurt C. Kurpis, Lucia Sanchez Di Maggio, Kerstin Fischer, Gary Weil, Peter U. Fischer
Washington University School of Medicine, St Louis, MO, United States

Onchocerciasis is a neglected tropical disease (NTD) caused by the filarial worm Onchocerca volvulus that infects people living in tropical regions with an estimated 220 million people at risk of infection. The World Health Organization targets onchocerciasis for elimination in the NTD roadmap and aims to stop mass drug administration (MDA) and/or interrupt transmission in most endemic areas by 2030. Several countries have eliminated the disease with repeated rounds of ivermectin MDA, prompting increased confidence in the feasibility of global elimination. Improved diagnostics tools that aid surveillance are critical to achieving this objective. Using direct proteomics and mass spectrometry, we previously reported 19 O. volvulus proteins detected in human serum and/or urine as potential biomarkers. We report results from the characterization of OVOC11613, the top biomarker candidate as a serodiagnostic target. The protein is an orthologue of the kinetochore associated IF-1 protein of Caenorhabditis elegans and 2021 AA in length. Two fragments of OVOC11613 were expressed in pET100-D and pTrHis-A expression systems in Escherichia coli. The protein's potential for antibody detection was evaluated using western blot with sera of infected and uninfected subjects. Purified recombinant protein was used for production of antibodies in mice and rabbits to design antigen capture assays and for immunolocalization. 60% of subjects from Uganda infected with O. volvulus had IgG4 antibodies reactive with the recombinant antigen and the specificity with sera from subjects with no travel history to endemic regions was 100%. However, there was cross-reactivity with Loa loa, Wuchereria bancrofti or Brugia malayi patient-infected sera. Immunohistochemical studies with sections of onchocerca nodules containing adult worms and microfilariae showed strong labeling of all worm tissues except the cuticle, supporting the essential function of the protein in all cell types. These results support the abundance of OVOC11613 and potential as a biomarker for onchocerciasis that could be valuable for surveillance and monitoring in elimination programs.

THE POLY-LYSINE PEPTIDE AS A BROAD-SPECTRUM ANTIPARASITIC, AND EFFECTIVE AGAINST FILARIAL AND MALARIA PARASITES

Arun Ashutosh1, Sergej Djuranovic2, Makedonka Mitreva1, Slavica Pavlovic Djuranovic1
1Washington University School of Medicine, Saint Louis, MO, United States, 2Washington University School of Medicine, Saint Louis, MO, United States

Tremendous efforts have been made to control and eliminate neglected tropical and diseases. Filarial worms cause multiple neglected tropical diseases that have a debilitating effect on the worldwide population. One of them is Onchocerca volvulus which causes river blindness. Currently, there are no effective drugs against filarial worms that cause river blindness. There is a therapy against killing larvae (microfilariae), but there is no effective drug against the adult parasite (macrofilaricides) which survive and reproduce in the host for a long time. Similarly, Plasmodium falciparum, the causative malaria parasite, is globally the primary source of parasite infection-related deaths. The current treatment of choice is artemisinin-combination therapy, but due to the development of the parasite's resistance to existing drugs, there is an increased need for new therapies. Here we show that a feature of the P. falciparum proteome - runs of poly-lysine residues found primarily in adhesion and pathogenicity-related proteins - can be used as a successful peptide treatment against multiple human parasites. Our data indicate that a single dose of poly-basal peptides can successfully reduce P. falciparum parasitemia in human erythrocytes in vitro with a favorable low or no toxicity. The treatment efficiency of 30 lysine residues, in L and D form (PKL30 and PDL30, respectively), is sufficient for the complete clearance of the parasites in erythrocytes at nanomolar concentrations. In a similar experimental set-up, we used poly-lysine peptides to treat Brugia pahangi, similar species to O. volvulus and filarial worm model for animal infection. We used Brugia pahangi as a primary screen species and animal infection model to do poly-lysine peptide drug screens against Brugia adults. Assaying motility inhibition, we show 100% inhibition of worm motility in a single day with a dose value of 317nM for both peptides PKL30 and PDL30. These values for poly-lysine peptide-based compounds show promising results against filarial worms and potential for new drugs with the lowest effective dose tested until now.

ASSOCIATION BETWEEN BLOOD LOA LOA MICROFILARIAL DENSITY AND PROTEINURIA LEVELS: A POPULATION-BASED CROSS-SECTIONAL STUDY IN A RURAL AREA OF THE REPUBLIC OF CONGO

Jérémy T. Campillo1, Marthand C. Hemilembolo2, Sébastien D. S. Pion1, Elodie Lebrédonchel3, Valentin Dupasquier1, Charlotte Bouillé2, Ludovic G. Rancé1, Michel Boussinesq4, François Missamou5, Cédric B. Chesnais3
1Institut de Recherche pour le Développement, Montpellier, France, 2PNLO, Brazzaville, Congo, Republic of the, 3AP-HP, Paris, France, 4CHU de Montpellier, Montpellier, France

Case-reports hypothesized that proteinuria, sometimes with glomerulopathy or nephrotic syndromes, may be associated with loiasis. No specific study has ever been conducted to assess this association. We conducted a cross-sectional study to assess the relationship between Loa loa microfilarial densities and level of proteinuria in a rural area of the Republic of Congo. For each microfilaricidal individual, 2 individuals without L. loa microfilaria were matched on age, sex and place of residence. Among the 990 participants (62.5% were male, 35.6% were L loa microfilaremic), the prevalence of traces of proteinuria (< 300 mg/24h), light proteinuria (300 - 1 g/24h) and high proteinuria (> 1 g/24h) was, respectively, 37.5%, 50.6% and 71.4% among microfilaremic individuals and 62.5%, 49.4% and 28.6% among amicrofilaremic individuals. Individuals with high proteinuria had significantly higher L. loa MFDs (p < 0.001); mean ± SD mfd/mf = 1595 ± 4960, 2691 ± 7862, 3833 ± 9878 and 13541 ± 20118 among individuals with no, traces, light and high proteinuria, respectively. Individuals with 5000 - 14999 mfd/mf and individuals with ≥ 15 000 mfd/mf were respectively 5·39 and 20·49 more likely to have a high proteinuria than individuals with no microfilaria. The risk of proteinuria increases with L. loa microfilaria. Further studies are needed to identify renal disorders (tubulopathies, glomerulopathies or nephrotic syndromes) responsible for loiasis-related proteinuria.

ASSOCIATION BETWEEN BLOOD ARTERIAL STIFFNESS AND LOA LOA MICROFILAREMIA: A POPULATION BASED CROSS-SECTIONAL STUDY IN A RURAL AREA OF THE REPUBLIC OF CONGO

Jérémy T. Campillo1, Valentin Dupasquier2, Elodie Lebrédonchel3, Ludovic G. Rancé1, Marthand C. Hemilembolo2, Sébastien D. S. Pion1, Michel Boussinesq4, François Missamou5, Antonia Perez Martin, Cédric B. Chesnais3
1Institut de Recherche pour le Développement, Montpellier, France, 2CHU de Montpellier, Montpellier, France, AP-HP, Paris, France, PNLO, Brazzaville, Congo, Republic of the, 3CHU de Nimes, Nimes, France

Loa loa filariasis (loiasis) is considered a benign disease. However, recent epidemiological data suggest an increased mortality in L. loa infected individuals, underscoring the importance of studies on the possible morbidity associated with loiasis. Chronic inflammation due to the host response to L. loa microfilaria and the chronic exposure of vessel walls to the microfilariae (which may involve dysregulation of the structure of the vessel walls) may result in arterial stiffness. We conducted a cross-
sectional study in May–June 202 of 991 individuals living in rural areas of the Republic of the Congo and matched for age, sex, place of residence and microfilaremia to assess if L. loa microfilariae (mf) density in the blood is associated with arterial stiffness. Arterial stiffness and peripheral arterial disease (PAD) were assessed using a point-of-care device (OpOptimetr®) allowing the measurements of the pulse wave velocity (PWV), the ankle-brachial index (ABI) and the pulse pressure (PP). Among 982 and 976 individuals included in the PWV and ABI analysis, 159 (16.2%) had a PWV considered off-limit and 137 (14.0%) had an ABI considered off-limit, respectively. Factors significantly associated with off-limit PWV measurements were: male sex, younger age, high mean arterial pressure, high pulse rate, high creatininemia, and L. loa microfilaremia (especially for the densities ≥ 10 000 mf/mL of blood). Factors significantly associated with PAD presence were: older age, high pulse rate, high body mass index, smoking, and L. loa microfilaremia. Compared to subjects without mf in the blood, those with more than 10,000 mf/mL were 2.99 times more likely to have an off-limit PWV (P < .001). There appears to be a strong association between L. loa microfilaremia and arterial stiffness, which can lead to cardiovascular diseases and mortality. It is essential to carry out further studies on this subject in order to investigate this association.

**Efficacy and Feasibility of Short-Stretch Compression Garments Enabled by Three-Dimensional Infrared Imaging for Stage 3 Filarial Lymphedema in Sri Lanka**

Jaimee M. Hall, Sandani S. Thilakaratne, Nirmitha L. De Silva, Janaka Ruben, Thishan C. Yahathugoda, Philip J. Budge

1Washington University in St. Louis, St. Louis, MO, United States,
2University of Ruhuna, Galle, Sri Lanka

Standard of care (SOC) for filarial limb lymphedema includes a WHO-recommended hygiene regimen of daily limb washing, exercises, and elevation. Further benefits have been observed with complete decongestive therapy (CDT), which includes compression bandaging applied by trained therapists. Unfortunately, CDT is not available for most individuals in low- and middle-income countries (LMIC) due to a lack of access to durable compression garments and trained therapists. Given the recent availability of inelastic, self-adjustable, short-stretch compression garments (SSCG), employable with minimal patient training, we sought to determine whether SSCG, enabled by portable, three-dimensional, infrared imaging (3DII) in addition to SOC is effective and feasible in LMIC settings. We conducted a six-week, interventional, single group, open-label, pilot study in nine adults (five female) with Dreyer stage 3 lymphedema. A four-week lead in period of SOC hygiene was followed by a two-week intervention SSCG period. Subjects were a median age of 74 years with affected mean leg volumes of 3052 mL (SD 454). Adherence to SOC measures alone resulted in a small reduction (mean reduction 296 mL, SD 241, p=0.006). Only 2/9 participants achieved goal usage of SSCG for ≥23 hours/day, while 9/9 reported ≥21 hours/day of use. There was a non-significant reduction in WHO-Disability Assessment Schedule scores between enrolment and study end (18.7, SD=9.5 vs. 17.5, SD=8.1, p=0.30). Garment acceptability was high throughout the study and qualitative interviews demonstrated satisfaction, ease, and willingness to use SSCG during the study and thereafter. In this study, CDT using SSCG was effective in reducing lower limb volume as well as being reported as comfortable and well accepted by participants. These results provide proof of concept for 3DII-enabled SSCG in LMIC where trained therapists for filarial lymphedema may not be available.

**Identification of Novel Potential Biomarkers for Bancroftian Filariasis**

Nikhilesh Joardar, Kurt C. Kurtis, Kerstin Fischer, Amy Rush, Bruce A. Rosa, Petra Erdmann Gilmore, Robert Sprung, R. Reid Townsend, Gary J. Weil, Peter U. Fischer, Philip J. Budge

Washington University School of Medicine in Saint Louis, Saint Louis, MO, United States

Wuchereria bancrofti is responsible for 90% of the world's burden of lymphatic filariasis (LF). The Global Programme to Eliminate LF relies on rapid diagnostic tests that detect a circulating filarial antigen (CFA) to monitor for active filarial infections, and to monitor progress towards elimination. Unfortunately, clearance of CFA can persist up to five years following clearance of microfilaremia, making CFA a suboptimal way of monitoring for transmission potential. Development of antigen or serologic assays for W. bancrofti that more accurately reflect active transmission is a priority for LF elimination programs. To identify novel antigen biomarkers for W. bancrofti, we used a proteogenomic approach to examine 17 plasma samples from W. bancrofti-infected individuals from Cote d'Ivoire (4 samples) Papua New Guinea (5 samples), and Sri Lanka (8 samples) and 7 non-endemic controls (from St. Louis, USA) for the presence of W. bancrofti antigens. Filarial antigens were immunoprecipitated by one or more of following methods: immune complex (IC) precipitation with polyethylene glycol, monoclonal antibody DH6.5, which recognizes an abundant glycan moiety on many filarial glycoproteins, or polyclonal antibodies against Dirofilaria immitis or Brugia malayi. For 3 of the IC samples, deep scale fractionation was performed, and the generated hits were validated by comparison with stable isotope-labeled heavy peptides run under the same conditions. Seventy-six W. bancrofti proteins were detected in one or more infected samples, including 16 proteins with minimal human homology. We selected 4 for further development based on their relative abundance of detection. To date we have generated polyclonal sera to the leading candidate, a homologue of Brugia major allergen and validated its specificity by immunohistochemistry on female and male B. malayi worm sections. Ongoing work will determine the validity of these markers in detecting active filarial infections.

**Plasma Pentraxin 3 and Angiopoietins Are Elevated in Lymphatic Filariasis Lymphedema Patients with Essential Hypertension**

Charles Gyasi, Linda Batsa Debrah, Jubin Osei-Mensah, Derrick Adu Mensah, Ute Klarmann-Schulz, Patricia Korir, Achim Hoerauf, Alexander Yaw Debrah

1Kwame Nkrumah University Of Science And Technology, Kumasi, Ghana,
2Institute For Medical Microbiology, Immunology and Parasitology (IMMIP), Bonn, Germany

Essential hypertension is one of the comorbidity features in lymphatic filariasis lymphedema (LE). Close connections between inflammation and development of hypertension have been described. But whether inflammation induced by filarial worms impairs blood pressure (BP) homeostasis, and consequently enhance clinical severity among LE patients is not established. This study aimed to examine the distribution of plasma pentraxin (PTX)-3, angiopoietin (Ang)-1 and angiopoietin (Ang)-2 and their relationship with blood pressure homeostasis in LE participants with essential hypertension. Two hundred and twenty-two LE patients and 56 non-LE participants were enrolled in the study. LE patients were divided into hypertensive (n=111) and normotensive (n=111) based on clinical diagnosis of hypertension. Non-LE participants were normotensive and served as controls. Blood samples were taken and levels of inflammatory and angiogenic markers were determined by standard ELISA techniques. The results showed that plasma PTX3 and Ang2 and not Ang1 are significantly increased in LE patients with essential hypertension compared to controls (p=0.002, 0.015, respectively). However, we did not observe any significant difference in PTX3, Ang1 and Ang2 between LE patient with
essential hypertension and those without essential hypertension (p>0.05). Interestingly, LE patients with hypertension had significantly higher alanine transaminase (ALT) and gamma-glutaryl transferase (GGT) levels compared to normotensive LE patients (p=0.038, 0.001, respectively). PTX-3, Ang1 and Ang2 did not show significant correlation with each other and BP homeostasis within each group of comparison. In conclusion, elevated plasma PTX3 and Ang2 are significantly associated with LE. Increased levels of ALT and GGT are characteristics of LE with hypertension.

FIELD EVALUATION OF STANDARD™ Q FILARIASIS ANTIGEN TEST FOR LYMPHATIC FILARIASIS DURING A PRE-TRANSMISSION ASSESSMENT SURVEY IN SIERRA LEONE, 2022

Benoit Dembele1, Mohamed Saleu Bah2, Abdulai Conteh3, Habib Kamara4, Ibrahim Kargbo-Labour5, Ashley Souza6, Patricia Houck7, Steven D. Reid7, Joseph P. Shott8, Yaobi Zhang5, Kimberly Y. Won7, Jonathan King9, Angela Weaver5

As part of a multi-country evaluation, the SD Bicsensor STANDARD™ Q Filariasis Ag Test (QFAT) was compared with (the currently used test) the Abbott Bioline™ Filariasis Test Strip (FTS) for classifying lymphatic filariasis (LF) prevalence at a population level and for ease of use in field conditions in Sierra Leone. The evaluation was done in two districts, Bombali and Karene, where repeat pre-transmission assessment surveys (pre-TAS) were planned. Two sites with high LF antigen prevalence in 2020 (4.1% in the village of Kagbo and 7.7% in the village of Makorba Yelimi) were chosen. Convenience sampling was used to recruit 350 community members ≥5 years in each site. Blood was collected by fingerstick (20µl for QFAT and 75 µl for FTS). The reading time of the result for both tests was 10 minutes. For all positive or invalid results, a repeat test was performed for both tests. In total, 728 participants (5 - 91 years) were tested by QFAT and FTS. The positive rate was 4.8% (17/357) and 3.5% (13/367) for FTS and 3.4% (12/357) and 4.1% (15/367) for QFAT in Kagbo and Makorba Yelimi, respectively. All participants testing positive for FTS or QFAT underwent further testing of mid-night blood smear to detect microfilariae using microscopy. None of the positive participants had circulating microfilariae. Nearly half (14/30) of those who tested positive with FTS during this survey also tested positive with FTS in re-pre-TAS in 2020. Four FTS and three QFAT samples were indeterminate (meaning a positive result followed by a negative result). In field conditions, QFAT was easy to handle and recorded zero invalid tests compared to FTS (six invalids). Using the FTS results as a reference standard, the sensitivity and specificity of the QFAT was 78.6% and 99.4% respectively. The concordance between FTS and QFAT was 0.81 (Cohen’s Kappa). The discrepancy found between the two tests in terms of positive tests was not statistically significant (P value=0.78). The results suggest that the QFAT is a credible LF diagnostic test when compared to the routinely used FTS; use of either test would result in the same program decision.

EFFECT OF SEASONALITY AND HYGIENE ON THE INCIDENCE OF ACUTE FILARIAL ATTACKS IN PATIENTS WITH LYMPHEDEMA IN MALI

Moussa Sangare1, Yaya Ibrahim Coulibaly1, Aboul Fatao Diabate1, Houssenni Dolo2, Sekou Oumarou Thera1, Lamine Diarra1, Michel E. Coulibaly1, Lamine Soumaoro1, Siaka Y. Coulibaly1, Safit Dounibia2, Abdallah A. Diallo2, Ayouba Diarra1, Amy D. Kion2, Charles Mackenzie3, Mariana Stephens3, John Horton2, Eric Ottessen3, Sarah Sullivan2, Thomas B. Nutman2

Lymphatic Filariasis can cause disfiguring swelling of the limbs (lymphedema or elephantiasis for advanced stages) and/or damage to other organ systems including the genital-urinary system (e.g., hydrocele or vulvar disease). Current research has indicated that filarial lymphedema patients not only face severe social stigma and prejudice but also frequently undergo acute filarial attacks marked by limb swelling, fever, skin blistering and excoriation. Furthermore, there is little empirical data on the extent to which seasonal variations affect filarial attacks in lymphedema patients. To this end, we used data from a double blinded placebo-controlled study on the utility of doxycycline/placebo to improve lymphedema in Wuchereria bancrofti infection (in Mali) to investigate the incidence and effect of seasonal variation (rainy/wet and dry seasons) on acute filarial attacks. Using data from the 200 patients (87% female) all of whom received limb hygiene (with lymphedema followed for 24 months) We found that study participants experienced filarial attacks equally frequently during both the dry and wet seasons. In contrast, the incidence of filarial attacks differed depending on the season (11.11 per person-month in the dry season) compared to 54.67 per person-month in the wet season) The difference in attack means between rainy/wet and dry seasons was statistically significant (P=0.0068). Doxycycline administration was not associated with alterations in the frequency of acute attacks (no statistical difference between doxycycline/placebo). Over the 24-month course of the study, there was a statistically significant decrease in the frequency of acute attacks for each arm of the study (chi-square trend for the doxycycline group was 7.20; p=0.007) and 17.76 p<0.0001 for placebo group), but the 2 groups did not differ from each other. Our data suggest that the frequency and the incidence of filarial attacks is significantly increased during the rainy season compared to the dry season and that hygiene (rather than doxycycline) was the major driver of progressive decease in the frequency of filarial attacks over the 24 months of the study.

THE CLINICAL SPECTRUM OF LYMPHATIC FILARIASIS. A CASE OF PROTEIN-LOSING ENTEROPATHY

Roque Diaz Diaz1, Vanessa Jarne Beltrán2, Paul Nguewa3

1Laboratorio Hospital García Orcoyen, Estella, Estella, Navarra, Spain, 2Servicio Medicina Interna, Hospital García Orcoyen, Estella, Estella, Navarra, Spain, 3Institute of Tropical Health University of Navarra (ISTUN), Pamplona, Spain

Populations from endemic areas of Lymphatic Filariasis (LF) usually remain asymptomatic or develop chronic manifestations, while the acute form typically occurs in travelers to filariasis-endemic regions. Therefore, cases of imported chronic filariasis are rare. Our goals were to illustrate the complexity of the diagnosis of a chronic form of LF in a patient with a remote history of travel to an endemic area; and to know some significant clinical manifestations. The patient, 76-year-old, of the Garcia Orcoyen Hospital (Spain, Europe) was a retired military man. His personal history included arterial hypertension, former smoker, atrial fibrillation anticoagulated with edoxaban, and duodenal lymphangiectasias described in several upper gastrointestinal endoscopies performed in 2019 and 2020 for study of iron deficiency anemia. He was admitted to the Internal Medicine unit for study,
with edema in the lower limbs and at the scrotal level of approximately 6 months of evolution, together with dyspnea on moderate exertion. The patient also exhibited lymphopenia with a normal CD4/CD8 ratio, serositis, and hypoproteinemia, all compatible with protein-losing enteropathy (PLE). This was confirmed by performing a scintigraphy with labeled albumin. Considering PLE as the main cause of the patient’s clinical picture, a broad differential diagnosis was carried out. Colic disease, Whipple’s disease, solid tumors, hematologic or paraneoplastic syndrome were ruled out. Since the patient spent part of his youth, as a military man, in the Sahara area, a study of imported diseases such as LF was performed, obtaining a positive serology (IgG and IgG4) for Wuchereria bancrofti, with absence of microfilariae in the examination of the peripheral blood smear. After ruling out the presence of other filariae (Loa loa and Onchocerca volvulus) before starting specific treatment, due to the high risk of microfilaricidal activity, Albendazole 400mg/12h/21 days and Doxycycline 100mg/12h/6 weeks were administered.

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SHIFT IN THE SKIN MICROBIOME AMONG INDIVIDUALS PRESENTING WITH FILARIAL LYMPHEDEMA COMPARED TO NON-FILARIAL HEALTHY INDIVIDUALS IN GHANA
Alexander Kwarteng1, Solomon Wireko2, Samuel Opoku Asiedu1
1Kumasi Centre for Collaborative Research in Tropical Medicine, Kumasi, Ghana, 2Department of Biochemistry and Biotechnology, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

Lymphatic filariasis (LF) is a debilitating neglected tropical disease that remains a major public health challenge in endemic countries. In addition to providing mass drug administration (ivermectin, albendazole, and diethylcarbamazine) to reduce parasite burden, there is also a need to mitigate the challenges associated with lymphedema progression. Filarial lymphedema is known to be complicated by secondary bacterial infections; however, this has yet to receive considerable attention in LF-endemic communities in rural Ghana. Thus, the focus of this study was to understand the skin microbiome of individuals presenting with filarial lymphedema over time. This longitudinal study employs culture and Matrix-Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS) to characterize the microbiota of filarial lymphedema lesions over 24 months of follow-up and how it differs non-filarial individuals outlined in literature. The results reveal four marked phyla with varying distributions in the filarial lymphedema lesions relative to healthy skin: Firmicutes (69.7%), Proteobacteria (16.6%), Actinobacteria (13.3%), and Bacteroidetes (0.3%). Proponobacterium and Corynebacterium, which are usually resident and abundant in healthy skin, are underrepresented in the skin from lymphedema lesions. Most of the taxa found in the skin from lymphedema lesions are not the typical organisms found on human skin; instead, they are potentially pathogenic, with the Streptococcus, Acinetobacter, Klebsiella, Pseudomonas, Bacillus, Corynebacterium, Micrococcus, Enterococcus, Proteus, and Staphylococcus genera being the most common isolates. Our data reveals a significant shift in the bacterial population with the introduction of potentially pathogenic bacteria that compete with the healthy skin resident microbiota during LF infection.

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DETECTION AND DISCRIMINATION OF ONCHOCERCA VOLVULUS AND O. OCHENGII FROM BLACKFLY POOL DNA USING A NOVEL POLYMERASE CHAIN REACTION - RESTRICTION FRAGMENT LENGTH POLYMORPHISM (PCR-RFLP) TECHNIQUE
Isaac Owusu-Frimpong, Edward Jenner Tettevi, Queenstar Dedei Quarshire, Naa Adjeley Kuma, Nfayem Imoro, Yusuf Al-Mahroof, Reuben Enchill, Mawutor K. Ahiaibu, Mike Yaw Osei-Atweneboana
CSIR - Water Research Institute, Accra, Ghana

The WHO has approved the O150 PCR-ELISA in blackflies, a molecular xenomonitoring technique, as part of the Onchocerciasis Elimination guideline. However, the high cost, non-availability, and delay of the ELISA component make its application difficult in resource-limited settings. Therefore, this study focused on developing a PCR-RFLP assay to detect and discriminate O. volvulus and O. ochengii in blackflies. The speciation assay was designed based on the genetic difference between the COX1 mitochondrial gene of O. volvulus and O. ochengii, which identified Haell as a unique restriction enzyme for O. volvulus only. The assay validation was performed with archived blackflies (S. damnosum sensu lat) collected in 2011 from Agbelekeme, an endemic onchocerciasis community. Triplicates of 50 and 100 Blackfly pools were performed separately for heads and bodies. Blackfly pool DNA was extracted, and the Onchocerca-DNA was captured with the Onchocerca-COX1 probe and magnetic beads. The PCR-RFLP assay was applied to the Onchocerca-captured samples, after which the PCR products were sequenced and analyzed. Of the three 50 blackfly head pools, only 1 (1/3) carried infective O. ochengii L3. All three 100 blackfly head pools (3/3) carried infective O. volvulus L3. However, two of the three 100 blackfly body pools (2/3) were infected with O. ochengii. Only one of the two infected 50 blackfly body pools carried both O. volvulus and O. ochengii. After the DNA sequence analysis, the enzyme-restricted samples showed high homology with O. volvulus, whereas the unrestricted ones were highly homologous to O. ochengii. The novel PCR-RFLP assay has demonstrated its effectiveness in detecting and discriminating between O. volvulus and O. ochengii in blackflies. With zoonotic onchocerciasis in sight in some parts of the world, this tool will be useful for the early detection of potential zoonotic transmission of the bovine onchocerciasis in the human population.

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ANTIBIOTIC RESISTANCE AND MEGA CHARACTERIZATION OF STAPHYLOCOCCUS HOMINIS FROM FILARIAL LYMPHEDEMA PATIENTS IN THE AHANTA WEST DISTRICT, GHANA: A CROSS-SECTIONAL STUDY
Priscilla Osei-Poku1, Priscilla Kini1, Solomon Wireko1, Emmanuel Amewu2, Caleb Mensah3, Samuel Opoku Asiedu2, Ebenezer Asiedu1, Ernest Amanor1, Mary Wilson1, Amma Larbi1, Kennedy Gyau Boahen1, Augustina Angelina Sylversten1, Katherine Ryan Armato1, Alexander Kwarteng1
1Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, 2Kumasi Center for Collaborative Research (KCCR), Kumasi, Ghana, 3University of Cape Coast, Cape Coast, Ghana, 4Department of Anthropology, Northwestern University, Evanston, IL, United States

Filarial infections affect over 150 million people in the tropics. One of the major forms of filarial pathologies is lymphedema; a condition where the immune response is significantly altered, resulting in changes in the normal flora. Staphylococcus hominis, a human skin commensal, can also be pathogenic in immunocompromised individuals. Therefore, there is the possibility that S. hominis could assume a different behavior in filarial lymphedema patients. To this end, we investigated the levels of antibiotic resistance and extent of mecA gene carriage in S. hominis among individuals presenting with filarial lymphedema in rural Ghana. We recruited 160 individuals with stages I-VII lymphedema, in a cross-sectional study in the Ahanta West District of the Western Region of Ghana. Swabs from lymphedematous limb ulcers, pus, and cutaneous surfaces were cultured using standard culture-based techniques. The culture isolates were subjected to Matrix-Assisted Laser Desorption/Ionization Time of Flight (MALDI-TOF) mass spectrometry for bacterial identification. Antimicrobial susceptibility testing (AST) was performed using the Kirby-Bauer method. mecA genes were targeted by PCR for strains that were cefoxitin resistant. In all, 112 S. hominis were isolated. The AST results showed resistance to chloramphenicol (87.5%), tetracycline (83.3%), penicillin (79.2%), and trimethoprim/sulfamethoxazole (45.8%). Of the 112 strains of S. hominis, 51 (45.5%) were resistant to cefoxitin, and 37 (32.5%) of the cefoxitin-resistant S. hominis harbored the mecA gene. This study indicates a heightened level of methicillin-resistant S. hominis isolated among filarial lymphedema patients. As a result, opportunistic infections of S. hominis among the already burdened filarial lymphedema patients in rural Ghana may have reduced treatment success with antibiotics.
GUIDELINES FOR SELECTION OF FIRST- AND THIRD-LINE COMMUNITIES FOR MONITORING AND EVALUATION DURING ONCHOCERCIASIS ELIMINATION MAPPING

Joseph Harold Nyarko Osei, Sellassie Pi-Bansa, Kwadwo Kyereme Frempong, Millicent Opoku, Franklin Ayisi, Millicent Daniels Selassie Afatodzi, Sarah Mawunyo Dogboe, Abena Akyeamia Nyarko, Sampson Otco, Wilma Stok, Sake J. de Vlas, Aissatou Diawara, Daniel Adjei Boakye

Noguchi Memorial Institute for Medical Research, Accra, Ghana

Since the era of OCP and APOC, control of onchocerciasis was achieved by treating individuals in 1st line (communities 5km from breeding sites - high-risk), and 2nd line (communities 10km away - medium-risk) excluding 3rd line (communities >=15km away - low-risk) to reduce microfilariae load and prevent blindness via infective blackfly bites. Currently, there is a paradigm shift from control to elimination with focus on onchocerciasis elimination mapping based on evaluating infections levels in the 1st line, 2nd line, and 3rd line communities. There are no structured protocols for accurately selecting these communities since a 3rd line community to a given breeding site may be a 1st line community to another breeding site. The purpose of this study is to develop guidelines for monitoring and evaluation of selected communities using blackfly breeding sites prospection and mapping. This study was piloted using the proposed guidelines in selecting six communities (three each from 1st and 3rd line). This involved initial mapping of proposed study area and prospecting for blackfly larvae. Three positive larval breeding sites about 15km apart were selected and blackfly collection points were set up. Maps were updated with data on breeding sites. This was followed with selecting three 1st and three 3rd line corresponding communities based on positive larval breeding sites such that no natural barrier existed between breeding sites and communities. Three concentric circles of radius 5km, 10km, and 15km from each selected breeding sites was drawn to show communities found within the transmission focus. Blackfly densities at 3rd line communities was significantly lower than in 1st line communities (p < 0.01) with no difference in forest and savannah blackfly species (p = 0.42). The results indicate that the guidelines used in selecting the 1st and 3rd line communities were appropriate and hence flies captured from the 3rd line communities migrated from the breeding sites within the first line communities. These guidelines tested will be useful in the current onchocerciasis elimination mapping in selecting communities for monitoring and evaluation.

DEVELOPING A TRAP FOR AFRICAN CHRYSOPS SPECIES TO ACCELERATE ONCHOCERCIASIS ELIMINATION: PROOF OF CONCEPT IN CAMEROON


1Department of Entomology, Center for Infectious Disease Dynamics, Pennsylvania State University, State College, PA, United States, 2Centre for Research on Filaria and other Tropical Diseases (CRFilMT), Yaoundé, Cameroon, 3Sightsavers, Yaoundé, Cameroon, 4RICT, Yaoundé, Cameroon, 5University of Alabama, Birmingham, Birmingham, AL, United States

Circulating Loa loa antigens that cross-react with lymphatic filariasis (LF) rapid diagnostic tests (RDT) are often detected in individuals with high L. loa microfilariae (Mf) density, which poses a major challenge to LF mapping/elimination in co-endemic areas. To test the hypothesis that L. loa cross-reactive antigens (CRLAgs) are released by dying Mf, we enrolled two cohorts of loiasis infected individuals. Cohort 1 (spontaneous antigenemia group) included 86 heavily infected patients (median Mf count: 34,450 Mf/mL; IQR 22,860-54,720), 73 of whom had CRLAgs at enrollment. Cohort 2 (induced antigenemia group) included 39 moderately infected loiasis patients (median Mf count: 8,600 Mf/mL; IQR 5,500-11,980) who were negative for CRLAgs at enrollment, treated with a single dose of ivermectin (150 µg/kg), and monitored over 7 days for release of CRLAgs. In cohort 1, there was no significant change in Mf counts over time (Chi-square=3.261; df=4; p-value=0.52152) and 17% experienced a status conversion (i.e. from positive to negative or vice versa). In cohort 2, Mf counts decreased ~75% by day 3 post-treatment in all participants, and 11 participants (28.2%) developed post-treatment CRLAgs detectable by RDT. Mass spectrometry (MS) detected many peptides belonging to the NAS-14 metallopeptidase family in all 50 tested samples in cohort 1, including the 13 samples where CRLAgs were not detected by RDT. However, MS detected NAS-14-derived peptides in only 7 (64%) of the 11 CRLAgs positive samples. In cohort 2, the presence of post-treatment antigenemia was not significantly associated with baseline Mf count or Mf reduction (OR=1.0413; 95%CI=[0.9805-1.10590; p-value=0.1873). Thus, although CRLAgs can be induced by ivermectin treatment, the antigen profiles of induced and spontaneous antigenemia are not identical, suggesting additional mechanisms contribute to the release of CRLAgs in heavily infected individuals.
EFFORT TOWARDS ELIMINATION OF LYMPHATIC FILARIASIS IN CAMEROON: RESULTS OF THE LAST TRANSMISSION ASSESSMENT SURVEY IN TWENTY HEALTH DISTRICTS OF ADAMAOUA, CENTER AND FAR-NORTH REGION

Biholong Benjamin1, Ebene Clarisse1, Georges Nko Ayissi1, Carine Fokam1, Benoit Dembele1, Patricia Houck2, Ernest Mensah3, Yaobzi Zhang3, Steven D. Reid4, Angela Weaver5, Ismael Teta1

1Ministry of Public Health, PNLO, Yaoundé, Cameroon, 2Ministry of Public Health, NTD Coordination Unit, Yaoundé, Cameroon, 3Helen Keller International, Yaoundé, Cameroon, 4Helen Keller International, Dakar, Senegal, 5Helen Keller International, New York, NY, United States, 6FHI 360, Accra, Ghana

Cameroon is endemic for lymphatic filariasis (LF) in 144 of 200 health districts (HDs) in the country. The goal of Cameroon is to eliminate LF as a public health problem by 2026. Mass drug administration (MDA) using ivermectin and albendazole were implemented between 2008 and 2017 in all endemic HDs. The first transmission assessment survey (TAS-1) was conducted between 2014 and 2019 in 143 HDs and in 2021 in Akwaba HD, and as all HDs passed, MDA was stopped in all 144 endemic HDs. To date, 143 out of 144 districts have successfully passed TAS2, except Akwaba (due in 2023). Twenty HDs from Adamaoua, Far North and Central regions underwent TASS in 2022, in compliance with WHO guidelines. These HDs were grouped into 8 evaluation units (EUs) according to their epidemiological profiles and geographical locations. The Survey Sample Builder (SSB) was used to calculate the sample size and select the clusters. According to school enrolment rate, the survey was conducted in communities for the Adamaoua and Far North regions and in schools for the Centre region. The Filariasis Test Strip (FTS) was used to detect LF antigen and electronic data collection was used for this survey. Children testing positive were all confirmed by a second FTS test. Out of the 13,168 children in 249 clusters tested, 7 children tested positive by FTS. The number of positive children in each EU ranged from 0 to 4 and was below the critical cut-off value of 18-20 per EU. The results of the TAS3 confirmed the sustained interruption of LF transmission in these 20 HDs x to y years after stopping MDA, bringing the number of districts that have passed the second surveillance survey (TAS3) to 59 in Cameroon. The national program will establish a post validation surveillance system in HDs that have completed TAS3. Cameroon is well placed to submit the elimination dossier for validation by WHO in 2026.

IMPLEMENTING THE LYMPHATIC FILARIASIS REPEAT PRE-TRANSMISSION SURVEY IN A CONTEXT OF INSECURITY IN TWO HEALTH DISTRICTS IN BURKINA FASO

Mamadou Serré1, Ouedraogo Mathias1, Christophe Nassa1, Clarisse Bougouna1, Zoromé Harouna1, Ilboudo Adama1, Ogaobiga Fernand1, Issa Guiri2, Soubeiga Joseph3, Dieudonné Naré3, Georges Diminthe3, Regina Khassanova4, Micheline Ouedraogo4, Lucien Mano4, Elisabeth Chop4, Angel Weng5, Patricia Houck5, Ernest Mensah5, Yaobzi Zhang5, Benoit Dembele5, Steven D. Reid5, Angela Weaver5


Lymphatic Filariasis (LF) was endemic in all 70 health districts (HDs) of Burkina Faso. Since 2019, LF transmission assessment surveys (TAS) have been postponed in some areas of the country due to insecurity. In December 2022, the National Neglected Tropical Disease Program (NTDP) conducted a repeat pre-transmission assessment survey (re-pre-TAS) in two insecure HDs: Fada N’gourma and Tenkodogo. These were the fifth pre-TAS in Fada and fourth pre-TAS in Tenkodogo; both most recently failed re-pre-TAS in 2020. The objectives were to assess the prevalence of LF and to test the feasibility of a new approach to implement surveys in a context of insecurity. The new strategy used local health center staff who were trained at the district level on survey methodology, use of filariasis test strips (FTS) and electronic data collection via ESPEN Collect. Mitigation measures were taken to ensure the security of interviewers and respondents. The study was conducted in 7 and 6 sentinel and spot check sites in Fada and Tenkodogo, respectively, from December 2022, to January 2023. Age, sex, treatment status, and blood samples for FTS and microfilaraemia (mf) tests were collected from individuals >5 years. The NTDP conducted daily remote supervision to ensure quality data collection. Night blood samples from FTS-positive individuals were sent to the district laboratory for mf testing. Of the 4,403 people tested, over 92% reported having swallowed ivermectin and albendazole at least once. Former positives from the 2020 survey who were still FTS positive in 2022 and for whom treatment had been provided were excluded from the sample and current analysis. The filarial antigen prevalence was below 2% in 10 sites and between 2% and 4% in three sites. The program sought and obtained WHO advice to continue to TAS1 because most positives (49%) were over 15 years old and mf was negative in all positives in both HDs. The use of local health workers allowed for the successful completion of the re-pre-TAS surveys. Lessons learned will be used to develop similar approaches for conducting surveys in other insecure areas and where mf testing was not feasible.

EFFORTS TOWARDS ELIMINATION OF LYMPHATIC FILARIAISIS IN GUINEA: RESULTS OF TRANSMISSION ASSESSMENT SURVEYS IN ELEVEN HEALTH DISTRICTS

Nouhou Konkouro Dia1, Lamine Lamah1, Mamadou Siradiou Baldé1, Assatou Diaby2, Christophe Zougrana3, Mamadou Miadiou Bah3, Abdoul A. Diallo4, Abdoul Karim Camara5, Ernest Mensah5, Steven D. Reid5, Benoit Dembele5, Angela Weaver5

1Ministry of Health, Conakry, Guinea, 2Helen Keller International, Conakry, Guinea, 3Helen Keller International, New York, NY, United States, 4Helen Keller International, Dakar, Senegal

Lymphatic filariasis (LF) is a major neglected tropical disease in Guinea. Mapping carried out from 2005 to 2013 identified 24 endemic health districts (HD) out of 36 requiring annual mass drug administration (MDA). MDA for LF started in 2014. By 2021, 11 HDs (Beyla, Boké, Dalaba, Dinguiraye, Forécariah, Guéckédou, Kérouané, Kindia, Mali, Mandiana and Telimélé) with baseline prevalence between 1.5% - 14.4% completed five rounds of MDA with epidemiological coverage > 65%. These HDs conducted pre-transmission assessment survey in 2021-2022. The results showed prevalence below the 2% threshold in all sentinel and control sites, making all 11 HDs eligible to conduct the first transmission assessment survey (TAS1) in 2022. For TAS1, a cross-sectional survey, was conducted in 11 HDs corresponding to 11 evaluation units (EU). The survey sample builder was used to determine the sample size and to select clusters. In 11 EUs, the sample size per EU ranged from 1,500 to 1,695. A school-based survey strategy was used in four EUs (Boké, Dalaba, Guéckédou and Kindia) while a community-based strategy was used in seven EUs (Beyla, Dinguiraye, Forécariah, Kérouané, Mali, Mandiana and Telimélé) due to low school enrollment (<75%). Among the 343 clusters surveyed, the school-based survey strategy was used in 133 clusters in four EUs to test 6,709 children in first and second grade. A total of 17,719 children were tested in 343 clusters with 43% (7,554/17,719) of girls and 57% (10,162/17,719) of children in first and second grade. A total of 17,719 children were tested in 343 clusters with 43% (7,554/17,719) of girls and 57% (10,162/17,719) of boys. Seven EUs recorded between 1 - 9 FTS positives, all well below the critical cut-off value for each EU. Four EUs did not record any positive FTS cases. All the EUs met the minimum sample size. These results indicate that after five rounds of annual LF MDA with sufficient epidemiological coverage, these 11 HDs have successfully reached the criteria to stop MDA and 4,031,909 people in Guinea are now in need of MDA. The 11 HDs
Microfilaria (Mf) load is an essential indicator of the severity and transmissibility of filarial infections. Real-time Loa Ia Mf load prediction using the LoaScope enables exclusion of those with high Mf counts from ivermectin treatment during mass drug administration permitting re-implementation of MDA for onchocerciasis and LF in loiasis-endemic areas. However, the LoaScope is not commercially available, and alternative methods to quantify Mf load may lead to more efficient estimation. Rapid assays of Mf loads would also aid LF elimination by providing a direct measure of transmissibility, as opposed to current antigen detection tests that remain positive for months to years after clearance of microfilaria. We sought to identify blood metabolite biomarkers predictive of microfilariaemia using loiasis, which achieves very high Mf loads, as a model. For discovery, we used banked plasma samples from 62 microfilaric and 40 amicrofilaric individuals from an endemic area, as well as 15 non-endemic controls. Samples were analyzed by liquid chromatography-mass spectrometry using a Thermo ID-X high-resolution accurate mass instrument. Using a compositional data approach (DICOVar) based on pairwise metabolite ratios, we identified multiple features correlated with microfilariaemia. Targeted measurement of these features by LC-MS/MS identified two which were individually significantly associated with Mf positivity: a mass of 363 Da (p=2.22e-16), and a mass of 179 Da (p=3.2e-13). Each of these features exhibited a significant linear association with L. loa Mf load. The 179 Da feature was confirmed to be N-acetyl-tyramine by comparison with a reference standard. The 363 Da feature (predicted formula C13H21N3O9 has not yet been determined. Model optimization and validation against an independent set of patient samples is ongoing. Further work will determine whether these molecules can be developed as viable quantitative biomarkers for microfilarial load.

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**EXAMINING THE OVERLAP IN LYMPHATIC FILARIASIS PREVALENCE AND MALARIA INSECTICIDE-TREATED NET ACCESS AND USE IN AFRICA**

John Robinson, Amy Rush, Nikhilse Joardar, Jeffrey Henderson, Philip Budge
Washington University School of Medicine, St. Louis, MO, United States

Eradication and elimination strategies for lymphatic filariasis (LF) primarily rely on multiple rounds of annual mass drug administration (MDA), but also may benefit from vector control interventions conducted by malaria vector control programs. We aim to examine the overlap in LF prevalence and malaria vector control to identify potential gaps in program coverage. We used previously published geospatial estimates of LF prevalence as well as publicly available insecticide-treated net (ITN) access and use estimates among the total population, and malaria Plasmodium falciparum parasite rates (PPR) from the Malaria Atlas Project (MAP). We overlaid the LF prevalence estimates with ITN estimates and malaria PPR at the 5km² level for 38 LF and malaria-endemic locations in Africa. In this analysis, almost half of the locations (47.1%; 82/174 of IUs) with the highest LF prevalence (>5%) had at least 50% coverage with ITN access and use. Among high prevalence LF areas low ITN use largely corresponded to areas with low access, with 84.8% (78/92) of these IUs having both access and use estimates under 50%. Additionally, when classified using malaria PPR, some (27.2%; 25/92) of these low ITN coverage, high LF prevalence locations were also considered high burden for malaria. Among areas with lower LF prevalence (<5%), the majority (44.6%; 2313/5182) had low ITN access, while only 1.78% (92/5182) had low use, and 1.51% (78/5182) had both low access and use. These results illustrate the degree that malaria control programs have achieved access to and use of ITNs in LF-endemic areas. As LF can be transmitted by multiple vector species, the impact of ITNs on LF prevalence may vary depending on the predominant vector species and vector biting patterns. Furthermore, location differences in MDA coverage, ITN implementation, and other factors will also affect ITN impact. Spatial analyses like these can be combined with other context-specific knowledge to help inform future elimination and control strategies.
Edinburgh, United Kingdom, 1Ministry of Health Liberia, Monrovia, Liberia, 2Actions Transforming Lives, Monrovia, Liberia, 3Queen Margaret University, Edinburgh, United Kingdom, 4American Leprosy Mission, Monrovia, Liberia, 5American Leprosy Mission, Bethesda, MD, United States, 6Effect Hope, Markham, ON, Canada, 7Carter Center, Monrovia, Liberia

Progress toward eliminating NTDs continues, but many persons will experience lifelong impacts including stigma, and mental health consequences. Health system gaps, and a historic focus on disease control have meant affected persons often lack access to effective services resulting in physical and psycho-social consequences, complex treatment journeys, and catastrophic socio-economic impacts. Yet effective care for people with NTDs is critical in reaching elimination targets. Integrated health system approaches to managing skin NTDs are a key solution to addressing equity and effectiveness challenges. Liberia is one of the first countries globally to develop a national integrated approach to managing skin NTDs, but evidence on optimal approaches for quality service delivery integrating mental health at scale is limited. To address this, we use person-centered approaches to co-develop and adapt health systems interventions, integrating mental health, for the management of people affected by skin NTDs in Liberia. We worked with Ministry of Health officials, health workers, informal health providers, and affected persons drawing on a diverse range of participatory research methodologies to identify strategies to detect, refer, treat and support people living with skin NTDs. We developed and are piloting a comprehensive intervention manual to support the integrated management of skin NTDs, including mental health. We found that mid-level health workers appreciated training approaches and that new knowledge facilitated better patient care, including improved understanding of causes of NTDs, combatting myths and stigma. They commit to implement new knowledge and have plans to raise awareness about NTDs and to counsel patients based on new skills gained. After training, persons affected have increased awareness of the physical cause for their condition and expressed plans to seek care from facility, instead of informal providers. They described new knowledge of how to care for themselves, feeling braver and proud to join their friends again. Integrating mental health and NTDs requires a collaborative cross-sectoral approach.

THE IMPACT OF INDEPENDENT MONITORING (COVERAGE SURVEY) AGAINST THE 2019 MASS DRUG ADMINISTRATION DATA: THE USE OF IVERMECTIN AND ALBENDAZOLE IN LIBERIA

Alexlyn Secunda Monluo1, Vivian Ekie Monluo2
1Ministry of Health, Monrovia, Liberia, 2Ministry of Health, Liberia

Liberia is endemic for Lymphatic Filariasis in 13 of the 15 counties. The country began implementing the annual Lymphatic Filariasis mass drug administration since 2012 with Ivermectin and Albendazole but had to suspend the program in 2014/15 due to the Ebola Virus outbreak. In October-November of 2018, the Neglected Tropical Diseases program in Liberia implemented Mass Drugs administration (MDA) in all endemic counties. To verify the 2018 mass drugs administration data, a coverage survey was conducted in nine counties (Bong, Grand Bassa, Grand Cape Mount, Grand Kru, Lofa, Margibi, Maryland, Montserrado, and Nimba) to verify the reported coverage of Ivermectin and Albendazole from the target population using a community lot quality assurance sample (LQAS) methodology. This allows the program to determine if a county (supervision area) has achieved the goal of 80% coverage. There were fourteen (14) supervision areas with two hundred and sixty-six (266) samples interviewed. The survey targeted all population older than 5 years during the campaign who lived in the selected counties. Communities were selected using proportional probability of size using the 2018 mass drug administration database as a reference. From the 14 supervision areas, 4 supervision areas had unacceptable coverage according to LQAS analysis (93% certain they are below 80%). The analysis further indicated the following: Bong with a coverage of 79%, Grand Bassa 57%, Grand Cape Mount 80%, Grand Kru 78%, Lofa 80%, Margibi 83%, Maryland 80%, Montserrado 35% and Nimba 82% for 2019. Coverage for Ivermectin and Albendazole has met the target for the 9-county area surveyed (weighted coverage 81%). The results from 2018 coverage shows Grand Bassa with a coverage of 54%, Lofa 75% and Maryland 42%. This result shows Grand Bassa failed both rounds of coverage scoring below 65% followed by Montserrado and the program should investigate sentinel sites techniques and also considered changing strategies towards MDA in those counties.

INTEGRATED SEROLOGICAL SURVEILLANCE FOR MULTIPLE INFECTIOUS DISEASES IN VANUATU

Md Saiful Islam1, Elizabeth Nguyen1, Fasihah Talec2, Fernando Santiago1, Clare Dyer1, Arunasingam Abayasingam1, David Kennedy1, Macklyne Katenga1, Stephanie Tabet1, Prudence Rymill1, Anastasia Pantelas1, Julie Jacobson1, Nicodemus Tedia1, John Kaldor1, Susana Vaz Nery1
1University of New South Wales, Sydney, Australia, 2World Health Organization, Port Vila, Vanuatu, 3Sydney Local Health District, Sydney, Australia, 4Ministry of Health, Government of Vanuatu, Port Vila, Vanuatu, 5Bridges to Development, Washington, WA, United States

Vanuatu’s population is at risk of infection with neglected tropical diseases (NTDs), vaccine-preventable diseases (VPDs) and other infectious diseases due to low vaccine coverage, climate change, remote locations, and poor access to water and sanitation. Serological surveys that measure the prevalence of antibodies (seroprevalence) are a strategy for monitoring current or past exposure to infectious pathogens. Integrated serosurveillance using novel multi-bead assays that can detect ~100 different disease-specific antibodies from a single dried blood spot (DBS) has the potential to establish nationally representative programs. We conducted an integrated serological survey to assess the seroprevalence of antibodies against multiple NTDs, VPDs and other infectious diseases. Between 2021 and 2023, we conducted cross-sectional serosurveys in 92 villages in Tafea, Sanma, and Shefa provinces, Vanuatu. After seeking informed consent, approximately 2000 participants aged >1 year of age provided a finger prick blood sample to prepare a DBS that was analysed using the Luminex technology. At the time of writing, we are finalising the collection and analysis of samples. We will estimate the prevalence of antibodies against the following diseases/agents: NTDs (including trachoma, yaws, lymphatic filariasis, strongyloidiasis and dengue), VPDs (measles, rubella, diphtheria, pertussis, tetanus, mumps and variella-zoster virus), additional arboviruses (zika and chikungunya), as well as malaria, COVID-19, cryptosporidiosis, giardiasis, amoebiasis, taeniasis and cysticercosis. Our results will provide a measure of effective population-level immunity or exposure to multiple infectious diseases, with the added advantage of being cost-effective, scalable, acceptable, and able to target hard-to-reach and high-risk populations. These data can complement other surveillance mechanisms, including case-based reporting and vaccine coverage estimates obtained from electronic or paper-based records and household surveys for VPDs and parasitological surveys for NTDs and other infectious diseases.

LESSONS LEARNED FROM DEPLOYING ELECTRONIC DATA COLLECTION AS PART OF MASS DRUG ADMINISTRATION CAMPAIGNS IN SOUTH SUDAN

Stephen Ohidoro1, Angelia M. Sanders2, Yak Yak Bo1, Lochebe Boniface1, James Niquette1, E. Kelly Callahan2, Scott D. Nash2
1The Carter Center, Juba, South Sudan, 2The Carter Center, Atlanta, GA, United States, 3Ministry of Health, Juba, South Sudan

Public health programs require the collection and analysis of data from remote locations. In many countries, programs continue to conduct a large percentage of their programmatic data collection using paper. This is especially true among neglected tropical disease (NTD) programs given the remote nature of the work. Increasingly NTD programs are exploring the use of electronic data collection. The Enhancing the ‘A’ in SAFE (ETAS) study, a randomized control trial (NCT05634759), was designed to understand the cost, feasibility, and acceptability of enhanced mass drug administration (MDA) interventions for trachoma. As part of this trial, 2 different electronic data collection systems were incorporated into MDA activities between
ONCHOCERCIASIS ELIMINATION IN CAMEROON

Emilienne Epee1, Aïssatou Diawara2, Julienne Louise Ngo Likeng3, Shona Wynd4, Clarisse Ebene5, Hugues C. Nana Djeunga6, Georges Nko Ayissi5, Bertrand Ndzana7

1University Of Yaounde 1, Yaounde, Cameroon, 2Glide, Abu Dhabi, United Arab Emirates, 3Higher Institute Of Scientific And Medical Research (Ism), Yaounde, Cameroon, 4Global Institute For Disease Elimination (Glide), Abu Dhabi, United Arab Emirates, 5, 6, 7044

Onchocerciasis is a vector-borne parasitic disease that is endemic in Cameroon, affecting all ten regions of the country. An estimated 60% of the population is at risk of the infection, and around six million people are currently infected. The Community-Directed Treatment with Ivermectin (CDTI) strategy, which promotes the empowerment of communities by involving them in mass drug administration (MDA) organization and delivery was implemented in Cameroon in 1998 by the Ministry of Public Health. After more than two decades of uninterrupted annual delivery of ivermectin, the prevalence of microfilaria has significantly declined in most of the foci throughout the country, while the infection was found to persist in some hotspots foci, thus hindering the progress towards elimination. The persistence of the infection may be due to inadequate therapeutic coverage and low participation. To address these challenges, it is essential to identify barriers to the implementation of CDTI activities at the community level. We therefore aim to investigate community views and perspectives towards MDA with ivermectin. To this end, a cross-sectional mixed study was conducted in 32 communities in four regions of Cameroon, selected based on their treatment coverage levels. All individuals, aged 21 years and above, and living in the targeted communities were eligible to this study. Enrollees were interviewed about onchocerciasis and CDTI using a structured questionnaire and focus group discussions. A total of 140 individuals were interviewed, and 32 focus group discussions were conducted. Overall, population interviewed was exhibited higher level of knowledge in areas with the highest treatment coverage 50%. Regarding CDTI, the consumption of alcohol after ivermectin intake was reported in all the regions, and was reported as a potential reason of non-participation to MDA. These findings bring additional insights and open new avenues to increase awareness about onchocerciasis and better engage communities to improve participation acceptance and adherence to CDTI.
for descriptive, univariate and multivariate levels. A total of 634 participants were recruited in the study. 74.5%(N=472) reported having been tested for CD during pregnancy or at the time of delivery of their recent child. This KAP study clearly concluded that higher levels of mothers’ knowledge, attitude and practice factors were strongly associated with mothers who claimed to experience Chagas test. And 6 significant factors influenced the experience of Chagas test were educational history, Maternal and child handbook, Knowledge of newborn test, Knowledge of National Chagas program, necessity of family member’s test, and Experience of treatment. Conclusion is the study estimated that 74% of the participants performed serologic test. Those who have high knowledge, attitude, and practice are strongly associated with CD testing. That raises more responsibilities for not only the health care providers but also the community to make mothers aware of the importance of screening tests in endemic areas.

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PORTABLE SMARTPHONE-BASED MOLECULAR TEST TO SUPPORT THE ELIMINATION PROGRAM OF LEISHMANIA DONOVANI

Rea Maja Kobialka1, Arianna Ceruti1, Madhurima Roy2, Sutopa Roy2

New emerging diseases are a threat to public health systems worldwide. Low- and middle-income countries are facing additionally the burden of ancient pathogens, which were until still are circulating in human and animal populations. To control these diseases, the World Health Organization (WHO) created a list of so-called neglected tropical diseases (NTDs) together with a program to eliminate these pathogens. One of the NTDs is Leishmaniasis, caused by the parasite Leishmania donovani (LD). LD is a huge problem in different countries in Asia and Africa causing kala-azar (visceral leishmaniasis, VL) and Post kala-azar dermal Leishmaniasis (PKDL). Accessibility to accurate diagnostic methods is the essential first step to achieve the elimination goals. There is a need for sensitive, affordable and portable diagnostic systems for the field. The aim of this study was to test the accuracy of a handheld Minoo device connected to a smartphone for the detection of LD. It is based on isothermal DNA amplification and fluorescence detection in less than 20 minutes. Kassandra, a Phyton-based algorithm, is utilized for fluorescence signal analysis, including data processing, feature extraction and result classification. Limit of detection (LOD) was determined using a ten-fold dilution range of whole LD genome and LD molecular standard. Pathogens considered for differential diagnosis were tested to identify possible cross-reactivity. For clinical performance, 170 human samples from India and Bangladesh were screened. DNA extracted from peripheral blood (n = 98) and skin biopsies (n = 72) was tested. As control all samples were simultaneously examined with real-time PCR. The Minoo devices detected down to 11.2 genome equivalents and 134 copies of the molecular standard per reaction. Other Leishmania species were detectable, while no other pathogens were identified. Minoo can offer a convenient, sensitive, cheap alternative to real-time PCR. The devices are easy to handle and ideal for regular testing in low resource settings to monitor the progress of elimination.

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REACHING THE LAST MILE IN ONCHOCERCIASIS ELIMINATION IN MALI: RESPONDING TO PRE-STOP FAILURE

Yaya Ibrahim Coulibaly1, Abdoul Fatao Diabate1, Moussa Sangare1, Sekou Oumarou Thera1, Mamoudou Mahamadou Koureichi1, Dadje Tanapo1, Siaka Yamousa Coulibaly1, Fatoumata Koundou Maiga1, Salif Seriba Doumbia1, Housseini Dolo1, Yakouba Sangare2, Atzaza Rajabali1, Dukharmel Nazaire3, Thomas B. Nutman4, Alison Krentel5

Onchocerciasis is a parasitic disease caused by the filarial nematode Onchocerca volvulus and transmitted by the bites of black flies of the genus Simulium. In Mali, the impact of mass ivermectin administration has shown progress in onchocerciasis elimination in all operational transmission zones except the KA05. The aim of this study is to accelerate onchocerciasis elimination using an implementation research approach to understand the complexity and socio-economic barriers that hinder the elimination process in KA05 which failed the pre-stop survey. We conduct a mixed methods study with community cross-sectional surveys, community mapping, focus group discussions (FGDs) and in-depth interviews (IDIs). Community mapping took place with stakeholders and community leaders to highlight patterns of migrations in/out of communities, geographic features and notable landmarks. A questionnaire was administered to understand perceptions of mass drug administration (MDA) and other health interventions at the community level, reasons for missing out health campaigns, preferences and intent to participate in future MDA. 921 persons participated in the survey; 10 FGDs and 24 IDIs were conducted concurrently. Farmers (66.4%), gold miners (15%) and daily workers (6.1%) represented the main occupations of survey respondents. 41.6% of respondents reported participation in the most recent MDA campaign. 51.1% of respondents reported not remembering the most recent MDA campaign. Reasons for nonparticipation in the most recent MDA included lack of information about the campaign and distrust of drugs. In some areas, there were insufficient community drug distributors (CDDs) to cover all villages and hamlets. As a result, CDGs did not have enough time or resources to cover all sites. This study highlights the possibility that populations miss multiple MDA rounds hampering elimination efforts. Reasons include low CDD numbers, multiplicity of difficult-to-access and remote hamlets, frequent movement of populations in the area and poor social mobilization. Identifying and addressing these challenges will aid NTD elimination efforts in Mali.
supervised on implementation areas to track supervisory coverage. The success of this pilot has demonstrated that digitalizing MDA reporting forms is an innovation that offers significant potential for improving the availability of reliable and timely data for decision making, however, concerted efforts to overcome the challenges and ensure equitable access to these technologies is required.

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LESSONS LEARNED ON FINGER-INKING AS A MEANS OF COVERAGE VERIFICATION FOR MASS DRUG ADMINISTRATION FOR NEGLECTED TROPICAL DISEASES

Woubedle Alemayehu
Kantar Public, London, United Kingdom

When we think of finger-inking, most associate this with elections or when children are given polio drops. To independently verify the coverage of mass drug administration & MDAs; campaigns for neglected tropical diseases & NTDs; coverage surveys rely on self-reported responses from eligible individuals to report whether they took the drugs administered during the MDA. While this has been the standard approach followed widely by various governments and donors administering and funding MDAs globally, in a recent project, the Accelerate Resilient, Innovative, and Sustainable Elimination of NTDs across four countries Tanzania, Uganda, Kenya and Mozambique, finger-inking was introduced as part of the MDA activities. Eligible individuals who received drugs subsequently had their fingers inked. The independent coverage survey administered after the MDA captured information on self-reported coverage (where individuals self-report whether they took the drug) and verified coverage (where individuals who report taking the drug have their fingers checked for visible finger ink marking). The study provides valuable insights into the differences between self-reported and verified coverage, the operational challenges in applying finger-inking during MDAs, the cost implications for MDAs, the acceptability of finger-inking among different target beneficiaries (e.g., women, men, youth, and children) and what this means for future MDAs for NTDs.

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THE JOURNEY OF NTD DATA FROM LOCAL FRAGMENTED DATABASE SYSTEMS INTO STABLE AND SECURE HEALTH MANAGEMENT INFORMATION SYSTEMS

Diana M. Stukel1, Charles Brown-Davies2, Nissou Ines Dossa3, Ange Elvis Aba4, Babacar Banda Diallo5
1FHI 360, Washington, DC, United States, 2FHI 360, Accra, Ghana, 3FHI 360, Cotonou, Benin, 4FHI 360, Abidjan, Côte D’Ivoire, 5FHI 360, Dakar, Senegal

NTD Programs operate to eliminate or control diseases such as LF, Trachoma, OV, SCH and STH, in endemic countries around the world. Such programs collect routine monitoring and outcome data in relation to their Mass Drug Administrations and Disease Specific Assessments to assist with programmatic decision making, to inform program progress, to report to donors and funders and to provide an evidentiary basis when submitting elimination dossiers to WHO. It is essential that countries have stable and secure database systems with complete current and historical NTD data to protect investments in elimination and control programs. Despite this, many countries continue to use siloed Excel sheets for the day-to-day management of NTD program implementation, a situation which is fraught with numerous challenges including: lack of a shared single database across all diseases, lack of ability to access the database by multiple users simultaneously in a network-based setting to eliminate data divergence, lack of built-in data security mechanisms (password, virus protection, and regular back-ups), and limited NTDP personnel capacity to update and maintain the data systems over time. Typically, Ministries of Health employ HMIS - often but not always based on DHIS2 software - as a central repository for all health-related data, spanning programs relating to Malaria, TB, HIV-AIDs, etc. However, NTD data are often not included as part of such systems and the NTD programs are left to manage their own data systems independently. In recent years, however, there has been a push to integrate NTD data into centralized HMIS because of the obvious benefits, and countries are slowly moving in that direction - starting with integrating data relating to a handful of NTD indicators only. The movement towards full integration of all NTD data into existing HMIS is essential to ensuring a sustainable pathway forward as countries move towards full self-reliance in relation to the management of these debilitating diseases. This poster explores a diverse set of attempts towards integration of NTD data into HMIS across USAID’s Act to End NTDs West portfolio of countries in West Africa.

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CHAGAS DISEASE SCREENING OF MATERNAL DONORS IN PUBLICLY BANKED UMBILICAL CORD BLOOD IN NORTH CAROLINA, UNITED STATES 2007-2022

Naseem Alavian, Robert Rolfe, Susan Izatt, Jose Hernandez, Joanne Kurtzberg, Elizabeth Livingston
Duke University, Durham, NC, United States

Trypanosoma cruzi antibody screening in the perinatal setting has the dual benefit of identifying cases of Chagas Disease in women for whom treatment could reduce morbidity and providing opportunity for early intervention in the case of vertical transmission to infant. North Carolina (NC) has had substantial growth in population from Chagas endemic regions. Routine screening for T. cruzi is not performed in NC. However, T. cruzi antibody testing is performed on all donated umbilical cord blood by the largest public cord blood bank in NC, the Carolina Cord Blood Bank (CCBB). We aimed to identify the prevalence of positive T. cruzi screening serology within a cohort of donated umbilical cord blood. We performed a retrospective review of positive screening serology for T. cruzi in all cord blood samples donated to the CCBB in NC from 2019/02/07 – 2022. Screening was performed using chemiluminescent microparticle immunoassay for the qualitative detection of antibodies to T. cruzi. Descriptive statistics including T. cruzi serology, maternal race/ethnicity, and delivery site were tabulated. Among 25,706 cord blood donations screened over a 15-year period, 45 samples (0.18%) had a positive T. cruzi antibody screen. Among the 3,576 donations from patients identifying as Hispanic (13.9% of the entire cohort), 12 (0.34%) had a positive screen. Of the 45 mothers with a positive screen, 23 identified as Caucasian, 12 as Hispanic, 5 as Black, 3 as multiracial, and 2 were unknown. 35.5% of positive screens occurred at a single donation site and 75% of mothers with a positive screen delivered within three neighboring counties. The prevalence of a positive T. cruzi screening in donated cord blood was 1.7 per 1,000 cord blood donations. A higher proportion of positive screening was seen in Hispanic mothers, suggesting a role for screening for T. cruzi in the perinatal period, potentially in targeted populations or regions that are associated with higher proportions of positive screening found in this study. Further evaluation is needed to determine the rates of positive confirmatory testing and linkage to care for those with a positive screening test in this cohort.

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TSSETSE FLIES INFECTED WITH TRYPANOSOMES IN THREE ACTIVE HUMAN AFRICAN TRYPANOSOMIASIS FOCI OF THE REPUBLIC OF CONGO

Irina Anne Emmanuelle Bemba1, Arsene Lenga1, Herman Perfait Awono-Ambene2, Christophe Antonio-Nkondjio2
1Laboratory of Animal Biology and Ecology, Faculty of Science and technology, Marien Ngouabi University, Brazzaville, Congo, Republic of the, 2Institut de Recherche de Yaoundé (IRY), Organisation de Coordination pour la lutte Contre les Endémies en Afrique Centrale (OCCEA), Yaounde, Cameroon

Human African trypanosomiasis (HAT) is a neglected tropical disease still endemic in the Republic of Congo. Despite the continuous detection of HAT cases in the country, there is still not enough data on trypanosomiasis infections in tsetse flies, trypanosome species and tsetse flies’ species distribution in endemic foci. The present study was intended to fill this gap and improve understanding of trypanosome circulation in three active foci in the centre and south of Congo. Pyramid traps were set in various places...
in villages to collect tsetse flies both during the rainy and dry seasons. Once collected, tsetse flies were identified using morphological keys. DNA extracted from flies was processed by PCR for species identification and for detection of trypanosome presence. A second PCR was run for different trypanosome species identification. A total of 1291 tsetse flies were collected. The average apparent density of flies per day was 0.043 in Mpoouya, 0.73 in Ngbabé and 2.79 in Loudima. Glossina fusca fusca quanzensis was the predominant tsetse fly collected in Ngbabé and Mpoouya, while Glossina palpalis palpalis was the only tsetse fly found in Loudima. A total of 224 (17.7%) flies were detected infected by trypanosomes; 100 (7.91%) by Trypanosoma congolense savannah, 22 (1.74%) by Trypanosoma congolense forest, 15 (1.19%) by Trypanosoma vivax, 83 (6.56%) by Trypanosoma brucei (s.l.) and 2 (0.16%) undetermined species. No T. Trypanosoma brucei gambiense was found. A total of 57 co-infections between T. brucei (s.l.) and T. congolense savannah or T. brucei (s.l.) and T. congolense forest were found only in G. p. palpalis. Loudima recorded the highest number of infected tsetse flies. The study provided updated information on the distribution of tsetse fly populations as well as on Trypanosoma species circulating in tsetse flies in the different active HAT foci in Congo. These data suggested a high risk of potential transmission of animal trypanosomes in these foci, thus stressing the need for active surveillance in this endemic area.  

RESULTS FROM PATIENT INSIGHTS RESEARCH EXPLORING DISEASE AWARENESS, PATIENT JOURNEY, AND CURRENT MANAGEMENT OF VISCERAL LEISHMANIASIS IN BIHAR, INDIA  
Kirsten Carter1, Shyam Sundar2, Koert Ritmeijer3, Margriet den Boer4, Clare Zamble5, Colin Forsyth6, Fabiana Alves7, Gerhild Angyalosi1  
1Novartis Pharma AG, Basel, Switzerland, 2Institute of Medical Sciences, Banaras Hindu University, Varanasi, India, 3Medecins Sans Frontieres, Amsterdam, Netherlands, 4Lumanity, London, United Kingdom, 5Drugs for Neglected Diseases initiative - North America, New York, NY, United States, 6Drugs for Neglected Diseases initiative, Geneva, Switzerland  
Visceral leishmaniasis (VL) is a neglected tropical disease, lethal if untreated, with 50,000-90,000 cases per year worldwide. India is one of the most affected endemic countries. As VL affects almost exclusively an underserved population with limited healthcare access, there is a major gap in understanding patient perspectives. The ongoing research seeks insights from patients’ life experiences. Given the high illiteracy rate in VL patients, oral interviews with patients and caregivers of pediatric patients were held in Hindi, in Bihar, India. The objectives were to evaluate patient disease awareness, to determine drivers and barriers to seeking health care, and to understand the impact of the disease on patient’s lives. Participants were asked their understanding about VL, including symptoms, diagnosis, treatment and measures to prevent infection. Their insights regarding barriers to diagnosis and treatment and their experiences of the treatment process were collected. In order to inform the clinical development of a new, oral therapy, opinions on a potential new treatment were gathered. Recruitment of the planned 30 participants is ongoing. Preliminary results from the first six participants show a poor understanding of the disease, and of its transmission, even after being treated for VL. The time from symptom onset to diagnosis varies among participants (from several days up to one month), resulting in delays to effective treatment for VL. In the short-term, the impact of the disease is reported as greatly affecting participants’ daily social and practical lives, their finances and their emotional well-being. Patients received intravenous and oral therapies in hospital settings to treat their disease. An oral therapy, given in an outpatient setting, was viewed as a preferable treatment option. Full results from all interviewed patients and caregivers will be presented in the poster. This research aims to improve understanding of patient needs and expectations, in view of a patient-centric clinical development program, for a potential new oral treatment.

SEROPREVALENCE AND RISK FACTORS OF TOXOPLASMA GONDII IN WOMEN OF REPRODUCTIVE AGE (15-44 YEARS) — NIGERIA, 2018  
Dawn Blackburn1, Nwando Mba2, William Nwachukwu3, Hong Zhou1, Andrew Abbott1, Nishanth Parameswaran1, Samuel Awala2, Stacie Greby1, Matthias Alagi1, Nnaemeka Iriemiren1, McPaul Okoye5, Mahesh Swaminathan1, Jeffrey Priest1, Diana Martin1, Anne Straily1, Chikwe Ihekweazu1  
1US Centers for Disease Control and Prevention, Atlanta, GA, United States, 2Nigeria Centre for Disease Control and Prevention, Abuja, Nigeria, 3Institute of Human Virology, Abuja, Nigeria, 4US Centers for Disease Control and Prevention, Abuja, Nigeria  
Toxoplasmosis is caused by an obligate intracellular protozoan, Toxoplasma gondii which may cause illness in infants born to mothers who become newly infected during or just before pregnancy. Infection can also occur via contaminated food or water. The nationally representative 2018 Nigeria HIV/AIDS Indicator and Impact Survey included blood specimen and survey data collection. Blood specimens were tested by multiplex bead assay (MBA) for antibodies against antigens such as SAG2A from T. gondii. We evaluated seropositivity to SAG2A (defined as median fluorescent intensity >211) in a sample of 9,752 women of reproductive age (WRA) aged 15–44 years. Bivariate logistic regression was performed to identify potential risk factors. Anti-SAG2A seropositivity overall was 27.4% (95% CI 26.1–28.7%) and was lower in 15-24-year-olds (21.9%, 95% CI 20.3–23.6%) than 25-44-year-olds (31.8%, 95% CI 30.9–33.5%). A similar proportion of pregnant (24.6%, 95% CI 21.5–28.0%) and non-pregnant women (27.5%, 95% CI 26.2–28.9%) were seropositive. WRA from all states had evidence of T. gondii exposure; seroprevalence ranged from 7.4% (95% CI 3.6-14.7%) in Yobe to 70.3% (95% CI 58.1-80.1%) in Bayelsa. Lower seroprevalence was associated with owning livestock (OR 0.66, 95% CI 0.58-0.75) and being Muslim (OR 0.62, 95% CI 0.54-0.72) compared to being Christian. Those in higher wealth quintiles had greater odds of seropositivity (OR 2.51, 95% CI 2.07-3.05) compared to the lowest. Unimproved sanitation and drinking water sources were not associated with a difference in odds. This is Nigeria's first population-based estimate of T. gondii exposure in WRA, obtained by leveraging MBA to test specimens from a prior survey. However, data on specific risk factors were not collected, limiting interpretation. Women who have been exposed to T. gondii (i.e., seropositive) before pregnancy have minimal risk of transmitting the infection to their fetus in subsequent pregnancies. Among this sample, almost 75% of WRA are at risk of congenital transmission. Future studies should further investigate potential risk factors to inform development of effective prevention measures.

CLINICAL IMPLICATION OF REGIONAL LEISHMANIA SPECIES DISTRIBUTION IN ECUADOR: A CROSS-SECTIONAL STUDY  
Henk Schallig1, Jaap Beumer1, Byron Freire2, Manuel Calvopina2, Henry de Vries1  
1Academic Medical Centre, Amsterdam, Netherlands, 2Universidad de las Américas, Quito, Ecuador, 3Universidad de las Américas, Quito, Ecuador  
Among the cutaneous leishmaniasis (CL) causing Leishmania species in Ecuador, Leishmania guyanensis and L. braziliensis are dominant. Earlier studies on CL species in Ecuador focused on the Pacific areas, included only few patients from the Amazon region, and did not study patient characteristics. The resulting lack of knowledge impairs a region specific diagnosis and therapy for CL possibly leading to treatment delay and patient suffering. Patients were included by private and public primary health care centers and hospitals in the Pacific part of the Pichincha province and in the Amazonian Napo, Pastaza, and Morona Santiago provinces. All patients were subjected to a microscopic smear slide examination of a skin lesion suspected for CL in the participating centers. A skin scraping and filter paper imprint sample was taken from the border of the lesion for

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smear slide microscopy and qPCR. Leishmania species was determined by Cytochrome B sequencing. Additional patient and geographic variables were collected. Presence of Leishmania parasites was confirmed with PCR and/or microscopy in 245 patients who were included for this study. 154 patients (63%) were infected in the subtropical Pacific region and 91 (37%) in the Amazon. Infesting Leishmania species could be determined in 135 (73%) patients. L. guyanensis was the main CL causing species (93%) in the subtropical Pacific, but more than half of the patients with species determination from the Amazon was either infected by L. braziliensis (46%) or L. lainsoni (13%). Patients infected in the Pacific region had significantly higher concentrations of Leishmania DNA in the samples. Median health seeking delay for patients infected in the Amazon was 1 month longer. Lesion type and number of lesions was not significantly different across regions. L. guyanensis was the dominant species in CL patients in the Pacific region and health seeking delay was relatively short leading to a low risk of mucosal leishmaniasis (ML). The majority of CL lesions in the Amazon was caused by L. braziliensis (causative agent of ML) or L. lainsoni, health seeking delay was longer.

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THE ASYMPTOMATIC DOG WITH VISCERAL LEISHMANIASIS: IS THIS THE “REAL BAD DOG”? A SYSTEMATIC REVIEW

Ana Izabel Passarella Teixeira1, Debora Marcelino Silva2, Leila Ullmann3, Juliana Arena Galhardo1, Adriana Caroprezo Morini1, Gustavo Adolfo Sierra Romero4
1 UFMS, Paranaiba, Brazil, 2 OPAS, Brasilia, Brazil, 3 UFMS, Campo Grande, Brazil, 4 NMT/UnB, Brasilia, Brazil

The case of the asymptomatic canine visceral leishmaniasis (aCVL) has drawn attention because its epidemiological role in the persistence of visceral leishmaniasis in urban areas is not yet well understood. With that in mind, we conducted a systematic review of epidemiological reports on aCVL. We used the descriptors “asymptomatic” and “canine visceral leishmaniasis.” We retrieved 365 articles in Pubmed and 504 in ScienceDirect. After reading the abstracts and full articles, we selected 49 that met the criteria for the epidemiological aspects of aCVL. 55.2% (27) were cross-sectional studies, 22.4% (11) were experimental, 16.4% (8) were diagnostic evaluations, 4.1% (2) were mathematical models, and 2.1% (1) were cohort studies. The proportion of aCVL in cross-sectional studies ranged from 4 to 60% in countries from the Global South, such as Brazil and Iran. The experimental studies showed that aCVL dogs can infect the vector and have amastigotes identifiable in blood, skin, liver, lymph nodes, and bone marrow. The development of new diagnostic techniques focused on the ability to identify asymptomatic dogs. Also, 2 studies (1 cross-sectional and 1 cohort) indicated that the owner profile of an aCVL dog is middle to upper-class. Two mathematical models considered that intervention in aCVL could have a positive impact on the transmission ratio in a given area, and showed that the presence of aCVL must be taken into account when developing strategies for disease control. The burden of aCVL can be heavy, and infected dogs can continue to transmit for a long time before developing symptoms and receiving adequate interventions. The analyzed studies revealed that the owner profile of aCVL dogs is mostly wealthy, and they also tend to not adopt particular measures against visceral leishmaniasis with their dogs. Therefore, emphasizing this owner profile and encouraging them to take the necessary precautions could be an effective approach to help achieve disease control.

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ADDRESSING AFRICAN SLEEPING SICKNESS TRANSMISSION THROUGH STREET THEATRE

Hannah C. Bialic1, Walt Adamson1, Annette MacLeod1, Nicola Veitch2, Janelisa Musaya2, Lumbani Pete2, Alan Richardson3, Karen Veitch4, John Alufandika4, Bwanalori Bwamlima5, Garry Chillingar4
1 University of Glasgow, Glasgow, United Kingdom, 2 Kamuzu University of Health Sciences, Blantyre, Malawi, 3 SURGE, Glasgow, United Kingdom, 4 Voices Malawi, Chikwawa, Malawi, 5 Rumphi District Hospital, Rumphi, Malawi, 6 Nhokotaka District Hospital, Nhokotaka, Malawi

Human African Trypanosomiasis, or African sleeping sickness, is caused by the parasite Trypanosoma brucei which is transmitted by the Tsetse fly. Community engagement surrounding this disease and education in methods of control and elimination of the insect vector has proved lacking in endemic regions. Mistrust in research in sub-Saharan African regions also poses a barrier to intervention methods. This mistrust can often be traced to the ebola outbreaks of the past decade and can lead to a hostile environment. Beliefs, fears, and unsubstantiated rumours such that healthcare workers and researchers were responsible for disease spread, have resulted in the injury and death of scientific and medical field workers. Trust in scientists is crucial in working toward the World Health Organization’s target of eliminating sleeping sickness transmission by 2030. Our innovative project has addressed these barriers through a unique model of community engagement and epidemiological data collection: street theatre. The project provides a model for stirring the public’s interest in science, breaking down trust barriers and involving the community in developing strategies that can reduce transmission. This project is readily transportable and accessible across geographical and language boundaries. The performance was developed by a diverse team of Malawian and Scottish scientists, performers, and health officials, and was performed both in Glasgow and throughout the Rumphi and Nhokotaka regions in Malawi, which has brought an important educational component to underserved communities but also demonstrates the value of and enhances the trust in, scientific research. This unique community engagement project, and the methodology behind its development, have provided a platform for future artistic and theatrical endeavours that can be adapted to other parasite-driven diseases – such as malaria, schistosomiasis, and leishmaniasis. Our process and outcomes now serve as a toolkit to support the development of future collaborations and interactive performances in local communities, resulting in a strong legacy for the project for years to come.

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EPIDEMIOLOGY OF SPOTTED FEVER GROUP RICKETTSIA AND CHAGAS DISEASE INFECTION IN A RURAL COMMUNITY IN BOYACÁ, COLOMBIA

Lidia Gual-González1, Omar Cantillo-Barraza2, Manuel Medina3, Sara Patiño4, Stella CW Self5, Melissa S. Nolan1
1 University of South Carolina, Columbia, SC, United States, 2 Universidad de Antioquia, Medellín, Colombia, 3 Secretaria de Salud Departamento de Boyacá, Tunja, Colombia

Neglected tropical diseases (NTD) are described by the World Health Organization (WHO) as a group of communicable diseases that occur mostly in tropical and subtropical regions affecting people in under-resourced areas. The WHO has recognized twenty conditions as NTDs, such as Chagas disease (CD); however, there has been debate on adding other diseases to the list. Rickettsial infections are a group of vector-borne bacterial pathogens prevalent in tropical and subtropical regions that affect mostly poor communities, and despite not being included in the WHO’s NTD list, are understudied and remain a blind spot for public health efforts. Among rickettsioses, spotted fever group Rickettsia (SFG) are of public health importance due to the wide species distribution, with R. rickettsii being a highly pathogenic species. Colombia is affected by these infections that require simultaneous efforts: Chagas disease is a notifiable disease that requires public health interventions due to the emergence...
of vector species that circumvent previous vector-control strategies, and gain terrain in areas where the primary species was eliminated; on the other hand, SFGR infections are non-reportable, and despite growing emergence globally, local epidemiological factors that are driving its emergence still remain unknown. A collaboration with the Boyacá Health Department revealed concerns about the underdiagnosis and thus lack of treatment among patients exposed to those infections, especially in rural communities. A serosurveillance study was performed between 2021-2022 in the municipality of Miraflores, Boyacá evaluating human and canine serological samples, to better understand the epidemiology of these infections. Despite different ecology and epidemiology, both infections require adequate evaluation and understanding to reduce the risk they pose for the community. Our presentation will bring the first evidence of SFGR exposure in the department of Boyacá and will discuss the results of the cross-sectional serological survey evaluating Chagas disease and SFGR exposure.

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MODELLING SLEEPING SICKNESS AT DIFFERENT SPATIAL SCALES: A HEALTH AREA ANALYSIS

Christopher N. Davis1, Ronald E. Crump1, Samuel A. Sutherland1, Simon E. F. Spencer1, Alice Corbella1, Erick Mwamba Miaka2, Kat S. Rock1

1University of Warwick, Coventry, United Kingdom, 2PNLTHA-RDC, Kinshasa, Congo, Democratic Republic of the

The intensification of control and surveillance activities relating to gambiense human African trypanosomiasis in the last two decades has led to a large decline in the number of annually reported cases. However, while the disease moves closer to achieving the ambitious target of elimination of transmission, pockets of infection remain. The distribution of cases is highly heterogeneous and dependent on many factors, such as the density of the disease vector, the tsetse, and access to diagnostics and treatments and health facilities. We present a stochastic mathematical model for qHAT in the Democratic Republic of Congo (DRC) that captures the dynamics at the health area level, a finer scale than previous modelling studies, to better understand the geographical variation in case numbers. All fitted parameters of the model are matched to health area data and the stochastic properties allow us to better quantify the uncertainty in our results. The model allows greater flexibility in simulating projections to target specific regions for control interventions and we are working with PNLTHA-RDC to tailor our modelling strategies to realistically match planned activities, as well as the plausible alternatives. The analysis focuses on the 16 health areas of Mosango health zone as a case study, with the ability to scale up the method to approximately 1200 analysable health areas within the DRC. The modelling approach in Mosango highlights the benefit of using smaller scales, since approximately one third of cases in the health zone come from a single health area. And this is reflected in the modelling results, where in this health area, Kinzamba II, we predict a later expected year of elimination of transmission, under the current intervention strategies. Aggregating all our health area results to the larger spatial unit of the health zone, accurately recovers the results of earlier analyses, but fine spatial scale models could be pivotal in understanding exactly where remaining transmission is occurring.

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FROM THE PLAINS TO THE MOUNTAINS: A NEW FRONTIER FOR LEISHMANIASIS IN NORTH INDIA

Harnoor Singh, Prateindersinh Singh Manshahia, Pathik Dhangar, Prasan Kumar Panda

All India Institute of Medical Sciences, Rishikesh, India

Leishmaniasis, a disease traditionally endemic to Bihar, Jharkhand, West Bengal, and Eastern UP, has recently been observed in Northern Himalayas. To investigate this trend, we conducted a retrospective analysis of all cases of Leishmaniasis that presented to a tertiary care hospital in this region. Our study included 14 patients, all of whom had Visceral Leishmaniasis (VL) and presented with fever, vomiting, malaise, weight loss, and abdominal pain were other frequently reported symptoms. Hemophagocytic lymphohistiocytosis (HLH) was the most common complication, occurring in 71.4% of patients. All patients were pancytopenic, had deranged liver function tests, and had splenomegaly. The diagnosis was made based on rK39 antigen detection or LD bodies on bone marrow aspiration or both. We identified several risk factors for VL, including living in rural areas and organic matter around the household being the most common, followed by the presence of peri-domestic animals, precarious living conditions, sleeping outside or near vegetation, dense vegetation cover, contact with people from endemic areas, presence of nearby water bodies, and nearby construction work. Low educational status, proximity to households of VL patients, and travel to endemic regions were less commonly reported risk factors. Treatment with conventional or liposomal Amphotericin B or a combination of both was effective, with only one septic patient being unable to receive treatment. Three patients (21.4%) died. Our findings suggest that diagnosing VL in non-endemic areas can be challenging, particularly when it occurs with HLH. The recent increase in cases in the mid-Himalayan range of North India is likely due to the area's ecological similarities to endemic regions, allowing the sandfly to move up the Ganges river. Therefore, our study highlights the need for a reassessment of endemicity in the mid-Himalayas region of North India and the importance of early diagnosis and treatment of VL in non-endemic areas.

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EPIDEMIOLOGICAL, SEROLOGICAL, AND ENTOMOLOGICAL ASPECTS OF VISCERAL LEISHMANIASIS IN SUSPECTED NEW VISCERAL LEISHMANIASIS FOCI IN BANGLADESH

Debashis Ghosh1, Md Utba Bin Rashid1, Soumik Kha Sagor1, Md Rasel Uddin1, Shomik Maruf1, Prakash Ghosh1, Rajashree Chowdhury1, M M Akhtaruzzaman1, Abu Nayeem Mohammad Sohel2, Megha Raj Banjara2, Axel Kroeger2, Abraham Assefa3, Dinesh Mondal4

1International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B), Dhaka, Bangladesh, 2Communicable Disease Control (CDC), Directorate General of Health Services (DGHS), Dhaka, Bangladesh, 3UNICEF/UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR), World Health Organization, Geneva, Switzerland, 4University Medical Centre Freiburg, Centre for Medicine and Society, Freiburg, Germany

The study aimed to explore epidemiological, serological, and entomological aspects of visceral leishmaniasis (VL) in suspected new VL foci and assess the knowledge, attitude, and practices of the community living in the alleged new VL foci. The study investigated new VL cases reported between 2019 and 2020 in four sub-districts where we tested 560 members using the rK39 rapid test and conducted vector collections in six neighbouring houses of the index to assess sandfly density and distribution, examined sandflies’ infection, and determined the spatial relationship with VL infection. Furthermore, we highlighted the importance of early detection, community awareness, and targeted interventions in controlling the spread of the disease. The study screened 1073 people from 231 households in four upazilas for fever, history of VL, and PKDL-like skin lesions. Sava’s rK39 rapid test positivity rate was the highest (3.51%). The sandfly was present across all areas except Charmapasha, but all 21 collected female P. argenteipes were negative for the Leishmania parasite DNA. We found that one person from Islampur with a history of VL, and one from Islampur and Savar had PKDL-like lesions. After the awareness intervention, more people became familiar with VL infection (91.27%), and the knowledge of the participants concerning sandflies being the vector of the disease and the risk of having VL increased significantly (30.14%). The study found no active case in the new foci, but some asymptomatic patients. As sandfly vectors exist, the National Kala-azar Elimination Programme (NKEP) should consider these areas as kala-azar endemic and start the control activities as per national guidelines.
MODELING THE RELATIONSHIP BETWEEN PRECIPITATION AND TEMPERATURE ON THE INCIDENCE OF CUTANEOUS LEISHMANIASIS IN NORTHERN MOROCCO

John William Carew1, Meryem Lemrani1
1Yale University, New Haven, CT, United States, 2Laboratory of Parasitology and Vector-Borne Diseases, Institut Pasteur de Maroc, Casablanca, Morocco

Leishmaniasis is a Neglected Tropical Disease of global epidemiological importance due to its castration of large open wounds during infection, which lead to severe social stigma. Cutaneous leishmaniasis (CL), caused by Leishmania species, is endemic in Morocco and is associated with high levels of geographic and secular variation. Because CL is transmitted by dipteran vectors which are poikilotherms, we hypothesize that environmental factors play a key role in its transmission. Therefore, we examined historical associations between rainfall and temperature patterns and CL due to Leishmania tropica incidence in four northern provinces of Morocco and fitted time series models using meteorological covariates to predict CL cases in those provinces from 1995 to 2014. The regression models showed that the level of rainfall most strongly predicted CL incidence after a time lag of 4 months, 3 months, 1 month, and 4 months, respectively, in Moulay Yacoub, Sefrou, Taounate, and Taza Provinces; and that temperature most strongly predicted CL incidence after a time lag of 5 months, 4 months, 6 months, and 5 months, respectively, in Moulay Yacoub, Sefrou, Taounate, and Taza Provinces. Taza Province had the highest rate of CL incidence during the final three years of the study, but the highest CL incidence rate over the course of the study was in Moulay Yacoub in 2000 (55.71/100,000). Changes in temperature, precipitation patterns, and humidity due to climate change will likely have a significant influence on future CL transmission patterns in endemic regions. These models provide evidence that meteorological data can be used to predict future CL incidence.

HAEMOGLOBIN DYNAMICS FOLLOWING TREATMENT OF VISCERAL LEISHMANIASIS: AN INDIVIDUAL PATIENT DATA META-ANALYSIS USING THE INFECTIOUS DISEASES DATA OBSERVATORY DATA PLATFORM

Prabin Dahal1, Abdalla Munir2, Rishikesh Kumar2, Sauman Singh-Phulendra1, Niyamat Ali Siddiqui2, Gemma Buck1, Caitlin Naylor1, Matt Brack1, Manju Rahi3, Paritosh Malaviya4, Monique Sciences, Banaras Hindu University, Varanasi, India, 5Drugs for Neglected Diseases initiative, Geneva, Switzerland, 6Infectious Disease Initiative, Nairobi, Kenya, 7Division of Tropical and Humanitarian Medicine, Paris, France, 8Department of Community Medicine, University of Brasilia, Brasilia, Brazil, 9Faculty of Medicine, University of Khartoum, Khartoum, Sudan

Chagas disease (CD) is a neglected tropical disease endemic to Latin American countries. It is transmitted by triatomine insect vectors, mostly in rural and poor areas. Our objective is to describe the available CD seroprevalence data in Latin America. We conducted a systematic review of CD seroprevalence from 1980 through 2019. We included CD seroprevalence studies in humans in endemic locations, using PubMed and reference mining. We included surveys from representative populations or censuses, using two or more diagnostic methods, unless the single method was ELISA or ICT (per WHO guidelines). The systematic review protocol was published in Open Science Framework (DOI 10.17605/OSF.IO/USWC3). This review is currently being updated through 2021, as described in a separate protocol (PROSPERO ID CRD42022368900). We found 3,723 papers in this search. During the title and abstract screening, we included 439 papers. 307 papers were excluded because they were not retrievable (19) or did not meet inclusion criteria (289). 132 studies were included in the final analysis, comprising 189 location-years of data. We found studies from 17 of the 20 endemic countries. There were 38 studies with data from the 1980s, 47 from the 1990s, 62 from the 2000s, and 12 from the 2010s. No data were available for 10 of the 20 countries after 2010. Most of the studies were conducted in highly endemic, rural areas. Diverse levels of endemicity made it difficult to compare prevalence across countries and years without adjusting for the population at risk. CD data are sparse, and many endemic countries have little or no recent published seroprevalence data. Most seroprevalence studies were conducted in rural areas, but migration from rural to urban areas in Latin American countries and the chronic nature of CD make it challenging to estimate the true total prevalence from these data. The small number of recent studies demonstrates the need for new surveys. Additional data on seroprevalence in large urban areas would help to quantify the effect of rural-to-urban migration on CD epidemiology.
Cutaneous Leishmaniasis (CL) is considered an overlooked public health threat in Saudi Arabia. Many Saudi regions are highly endemic including Al Ahsa. No previous published species identification of Leishmania parasite in the vectors in Al Ahsa. This study aimed to molecularly identify Leishmania species from sand flies collected from Al Ahsa, Eastern Region, Saudi Arabia. This is a cross-sectional study that was conducted in Al Ahsa from July 2020 to May 2021. Eastern and southern towns were the targeted areas of collection that considered to have the highest rate of sand flies and CL cases according to the data from the Vector Borne Diseases Prevention Center in Al Ahsa. Sand flies were collected from indoor and outdoor habitats using CDC miniature light trap (John W. Hock Company, USA) and sticky traps. After recording the sampling date, locations and weather parameters, collected female sand flies were sorted and gathered for further identification and analysis. DNA was extracted from all sand flies using commercial genomic DNA extraction tissue kits (QiAamp Fast DNA Tissue Kit, Qiagen, Germany) following the manufacturer’s instruction. Pools of 3-10 female sand flies were used for DNA extraction according to the number of sandflies collected from each region. All extracted DNA was amplified using a protocol targeting Leishmania genus and species-specific primers. Post PCR-Restriction Fragment Length Polymorphism (PCR-RFLP) technique was used for species identification. 113 pools of sandflies were included in the study. 10 were positive for Leishmania genus following our experimental protocol. Characterization of Leishmania species by PCR-RFLP established Leishmania major as the species found in the collected sand flies. In conclusion, to the best of our knowledge, this is the first study that provides a preliminary molecular characterization of leishmania species in the sand fly vector at Al Ahsa region. Leishmania major was the only species found in our study. Further comprehensive research about the vector and reservoirs is vital to establish Leishmania transmission dynamics in Al Ahsa.
and developed thematic codes from the transcribed data for analysis based on the grounded theory approach. The farmers use antibiotics for any kind of sickness of their birds without diagnosis of diseases. They believe that antibiotics do not have any side effects or transfer to humans. Farmers purchase chicks, poultry feeds, and antibiotics from poultry dealers on credit, they repay the debt by selling their poultry to these dealers. However, farmers are obligated to sell their poultry exclusively to these dealers. Dealers offer a reduced price and sometimes refuse to purchase their poultry. Dealers wield significant influence over farmers regarding antibiotic usage, as they decide which antibiotics to use and how frequently they should be administered. Occasionally, veterinary professionals of pharmaceutical companies provide treatment to poultry. Farmers reported that if they do not use antibiotics recommended by these veterinarians, who often represent a specific company, then the veterinarians do not provide further advice. Poultry farmers are often forced to use antibiotics by poultry dealers and veterinarian of pharmaceutical companies. Recommended interventions that offer economic benefits and increase farmers’ awareness on antibiotics usage and impose stringent regulations on use of antibiotics in poultry farming to prevent emergence of antibiotic resistance.

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ASSESSING TRANSBOUNDARY ZOONOTIC DISEASE THREATS AT POINTS OF ENTRY BETWEEN IRAQ AND JORDAN: A ONE HEALTH APPROACH

Alanna S. Fogarty1, Alexander G. Linder1, Aso Kareem Zangana2, Madhi Sinan3, Hudhaifa Abdulmahdi Hadi Al Jumiei4, Rasul Hamadi5, Rachel Dodeer6, Alaa Hamdallah7, Erin M. Sorrell8


The One Health Systems Assessment for Priority Zoonoses (OH-SAPZ) tool was created to facilitate a systematic assessment of One Health infrastructure and coordination mechanisms to prevent, detect, and respond to national priority zoonotic diseases. This framework identifies gaps and develops recommendations for action towards One Health systems strengthening. Our team adapted this tool to prioritize and assess systems for detection, reporting, coordination and response of transboundary zoonotic diseases (TZD) at Points of Entry (PoE). The One Health Transboundary Assessment of Priority Zoonoses (OH-TAPZ) tool was successfully piloted at the Libya-Tunisia border and the methodology was formalized. The OH-TAPZ has now been applied to identify priority transboundary zoonoses, and assess One Health coordination mechanisms at PoEs between Iraq and Jordan, countries which have previously conducted OH-SAPZ assessments and continue to build national One Health capacities. Through a phased approach, our team sat out to assess, evaluate and strengthen preparedness, detection and response plans as they relate to transboundary zoonotic threats between Iraq and Jordan using the OH-TAPZ tool as the guiding framework. With the completion of Phase 1, we have updated analysis on Iraq and Jordan’s national priority zoonotic diseases and mapped progress to date to measure and identify where gaps still exist or have been created over time. In addition, we have identified a joint priority TZD list and using the OH-TAPZ tool assessed mechanisms in place at PoEs to identify areas for capacity building.

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USING A ONE HEALTH SYSTEMS ASSESSMENT TOOL TO STRENGTHEN TRANSBOUNDARY ZOONOTIC DISEASE DETECTION, SURVEILLANCE AND RESPONSE BETWEEN LIBYA AND TUNISIA


1Georgetown University, Washington, DC, United States, 2Kenya Snakebite Research and Intervention Center, Nairobi, Kenya, 3Kenya National Centre for Disease Control, Tripoli, Libyan Arab Jamahiriya, 4National Centre for Animal Health, Tripoli, Libyan Arab Jamahiriya, 5Ministry of Agriculture, Tunisia, Tunisia, 6Ministry of Public Health, Tunis, Tunisia, 7Johns Hopkins University, Baltimore, MD, United States

Points of entry (PoE), such as land border crossings, are prime locations for detection and surveillance of zoonotic disease threats with the potential for transboundary spread. The border between Tunisia and Libya is challenged by illicit movement of materials, livestock and people increasing the risk of transboundary zoonotic diseases (TZD). This project aims to determine how current national frameworks for zoonoses in Libya and Tunisia can be strengthened to build sustainable One Health strategies for biannual prevention, detection, surveillance and response of TZDs at PoEs. The established One Health Systems Assessment for Priority Zoonoses (OH-SAPZ) methodology was first adapted for the cross-border setting. Following prioritization of five TZDs, we developed systematic maps outlining the current operations and intersections of communication and coordination between public and veterinary health sectors within.
and across the two countries. We assessed the current 1TZ detection, surveillance and response operations at the PoEs, identified strengths in capacity and limitations to timely information-sharing, and developed targeted recommendations for both national and joint action planning. After completing our transboundary assessment plot and receiving feedback from in-country partners, we determined evident gaps in existing international guidance and assessment tools for PoEs, which provide limited instruction on and integration of One Health practices for cross-border coordination. Following an in-depth literature review, we developed the One Health Transboundary Assessment for Priority Zoonoses (OHTAPZ) tool which supports binational strengthening of transboundary zoonotic disease detection, surveillance and response systems.

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TAILORING A ONE HEALTH COURSE FOR AN ESTABLISHED NON-ONE HEALTH GRADUATE PROGRAM IN BRAZIL

Tereza Magalhaes1, Ana M. B. P. Barreto2, Jamerson Mesquita-Silva3, Kamille M. L. Serravalle2, Marcela Valente de Andrade1, Rita C. L. Gomes1, Romero J. Nazaré5, Rosa M. G. A. Calado2, Guilherme S. Ribeiro1, Uriel Kifron6

1Texas A&M University, College Station, TX, United States, 2Universidade Federal da Bahia, Salvador, Brazil, 3Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil, 4Department of Health of Lauro de Freitas, Lauro de Freitas, Brazil, 5Instituto Gonçalo Moniz, Fundação Oswaldo Cruz, Salvador, Brazil, 6Emory University, Atlanta, GA, United States

One Health (OH) approaches recognize the interconnectedness and interdependence of human, animal, and environmental health and aim at improving the health and well-being of living beings and the ecosystems in which they live. While OH initiatives initially focused on infectious and zoonotic diseases, the broader concepts extend beyond this, and cover a wide range of complex health issues at the interface of human, animal, and environmental health. Given the potential for OH to be tailored and applied to a broad range of fields and disciplines, it is critical to introduce the OH concept in diverse settings, so that various audiences can adapt and use OH to address the specific challenges they face. Teaching students about OH is crucial, as they are the future generation that will be developing, implementing, and likely modifying OH approaches. Integrating OH into the curriculum of academic programs has been identified as a fundamental strategy for promoting OH principles and enhancing human capacity building. However, integrating OH into established non-OH graduate programs can be challenging as the OH content may not initially align with the program’s main research lines and goals. To address this challenge, we present a case study of tailoring an OH syllabus to the Graduate Program in Health, Environment, and Work at the Federal University of Bahia in Salvador, Brazil. The syllabus was developed from scratch by identifying relevant OH content from the literature and tailoring it to meet the specific needs and goals of the program while teaching critical OH elements. By doing so, the syllabus content became more naturally integrated into the program, rather than appearing as an isolated or unconnected topic. As a result, the course offered faculty members and students of the program an opportunity to learn and discuss OH concepts, which intersects with several ongoing projects and lines of research in the program. We believe our work contains significant information that may assist others in tailoring syllabuses to implement OH concepts in diverse educational settings.

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RIFT VALLEY FEVER VIRUS AND GENOME STABILITY IN RAW MILK

Brian E. Dawes1, Alina M. De La Mota-Peynado1, Izabela Rezende1, Esra Buyukcangaz1, Keli Gerken2, Christabel Winter3, Bethel Bayrau1, Dana N. Mitze1, William C. Wilson3, A. Desiree LaBeaud1

1Stanford University, Stanford, CA, United States, 2United States Department of Agriculture, Manhattan, KS, United States, 3University of Liverpool, Liverpool, United Kingdom, 4Käsmu, Kenya

Rift Valley fever virus (RVFV) is a zoonotic arbovirus which represents a threat to both humans and ruminants across Africa and the Middle East. In humans, RVFV causes a spectrum of mild to lethal disease including self-limiting febrile illness, retinitis, hepatitis, meningoencephalitis, and hemorrhagic fever. One significant gap in the understanding of RVFV epidemiology is the risk of handling and consumption of animal sourced foods, including milk, which have strong epidemiologic links to exposure, including in urban settings in the absence of direct animal contact. In various populations in Kenya, 5-36% of participants report consuming raw milk, and there are many actors in the informal milk value chains that handle milk daily, representing a public health risk. Recent studies detected RVFV by rt-PCR in cow milk in East Africa; however, the stability of infectious virus in milk has not been assessed. In this study, the stability of infectious RVFV and viral RNA was measured in fresh cow’s milk across a range of temperatures to simulate field relative storage and ambient temperatures in Kenya. In milk, RVFV titers decreased 10-100-fold over 24 hours, but infectious virus was still detectable after 96 hours at 4C. Furthermore, at 25C virus was infectious up to 72 hours and up 24 hours when incubated at 30C. Viral RNA was detectable at 96 hours with minimal changes in CT values throughout all tested temperatures. Despite this stability, RVFV was inactivated by standard pasteurization techniques of heating milk to 60C for 30 minutes or 72C for 15 seconds as well as boiling. These findings support mounting epidemiologic data suggesting unpasteurized milk products containing RVFV may carry risk even after several days. More research on the infectious potential of milk is required, and public education highlighting pasteurization of milk, particularly during outbreaks, could reduce the risk of RVFV direct infection. Regardless of public health risk, testing milk from animals could potentially be leveraged as a non-invasive surveillance method to monitor RVFV transmission levels and should be further assessed.

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GENOMIC EPIDEMIOLOGY OF CAMPYLOBACTER JEJUNI AND C. COLI ISOLATED FROM INDUSTRIAL AND HOUSEHOLD POULTRY IN IQUITOS, PERU

Francesca Schiaffino1, Katia Manzanares Villanueva1, Lucero Romaina Cachique1, Tacksheny Pinedo Vasquez1, Maribel Paredes Olortegui1, Paul F. Garcia Bardales2, Steven Huynh3, Pablo Perhataro Yori4, Evangelos Mourkas5, Ben Pascoe6, Kerry K. Cooper6, Craig T. Parker6, Margaret N. Kosek6

1Faculty of Veterinary Medicine, Universidad Peruana Cayetano Heredia, Lima, Peru, 2Associacon Benellica Prisma, Iquitos, Peru, 3Agricultural Research Service, U.S. Department of Agriculture, Produce Safety and Microbiology Research Unit, Albany, CA, United States, 4Division of Infectious Diseases, University of Virginia, Charlottesville, VA, United States, 5Imperial Oxford Institute for Antimicrobial Research, Department of Biology, University of Oxford, Oxford, United Kingdom, 6Centre for Genomic Pathogen Surveillance, Big Data Institute, University of Oxford, Oxford, United Kingdom, 7School of Animal and Comparative Biomedical Sciences, University of Arizona, Tucson, AZ, United States

Campylobacter is the leading cause of bacterial gastroenteritis in children living in LMICs. C. jejuni and C. coli are adapted to the avian gut, and poultry meat and by-products are the main source of Campylobacter related outbreaks in high-income countries. In LMICs, industrial poultry
production is rapidly increasing, as well as the unrestricted use of antibiotic within the system. In rural settings, the markets are also serviced by informal poultry production and backyard production. These non-industry poultry production streams are highly relevant activities given that small-scale backyard production provides a source of income and protein for low-resource households. The multiplicity of production scenarios and lack of regulatory practices are expected to contribute to important differences in the ecology, diversity and MDR risk of poultry derived human campylobacteriosis. Fecal samples from industrially produced chickens were sampled monthly 18 months. Fecal samples from backyard raised poultry were sampled as part of an ongoing cohort during the same time frame. All Campylobacter isolates were sequenced using Illumina short-read technology. Colonization heterogeneity, phylogeny and genomic determinants of AMR were assessed and compared between both production systems. A total of 200 isolates from backyard poultry 800 isolates from industrial poultry were sequenced. Campylobacter sequence types from industrial poultry were distinct from those of backyard poultry, with only few shared STs (such as ST 609). Newly assigned ST such as ST-11957 - ST-11967 have not been isolated in other parts of the world, suggesting a unique ecology of C. jejuni and C. coli in poultry of the Peruvian Amazon. AMR was higher in industrially raised poultry.

Phylogenetic comparisons and prevalence of resistance genes and point mutations are presented. These distinct characteristics of Campylobacter epidemiology in backyard and industrially produced poultry will impact source attribution models that estimate the risk of human disease, and as a result, intervention strategies to reduce the overall burden of Campylobacter.

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**FACTORS ASSOCIATED AND PREVALENCE OF ABNORMALITIES OF VENTILATORY FUNCTION IN ATTECÔUBÉ, ABIDJAN, CÔTE D’IVOIRE, JANUARY-FEBRUARY 2022**

Affou Seraphin Wognin1, Loukou Leandre Konan2, Wilnine Pierre3, Joseph Blaise Otahudianleka1, Issaaka Tiembre1, Vroh Joseph Beni1*

1CIAPOL, Abidjan, Côte D’Ivoire, 2MOH, Abidjan, Côte D’Ivoire, 3AFENET, Abidjan, Côte D’Ivoire, 4INHP/ UFHB, Abidjan, Côte D’Ivoire, 5INHP/UFHB, Abidjan, Côte D’Ivoire

Ventilatory function abnormalities are one of the causes of the public health problems worldwide. In Côte D’Ivoire, the prevalence of respiratory diseases differs in the professional sector, in particular bakers, sales assistants, cashiers, cleaners and administrative staff, respectively from 9.7%, 2.3%, 0.6% and 1.2%. This study aim to determine the prevalence of respiratory symptoms & ventilatory function abnormalities in the population of the lagoon district of Attécoubé in Abidjan, Côte d’Ivoire. A cross-sectional study with an analytical aim was conducted among 170 people in the town of Attécoubé lagune. A questionnaire set up on the Kobo collect tool relating to socio-demographic, environmental characteristics & respiratory symptoms was inspired by that of the American Thoracic Society (ATS). Pulmonary function tests were performed in the subjects investigated by baseline spirometry and a beta 2 mimetic test. Data analysis was done with Epi-Info software version 7.2.1. The study population was composed of 103 women and 67 men with a sex ratio (Male/Female) of 0.65. The average age was 35.92 years ± ET15.28. The most common respiratory symptoms were chest tightness (29.41%), dyspnea (28.82%), sneezing (22.94%) & cough (22.35%). The prevalence of ventilatory function abnormalities was 43.24% among residents of Attécoubé lagune. The most common ventilatory function abnormality was ventilatory restriction (35.2%). Obstruction (4.85%) was the second ventilatory anomaly. The factor associated with abnormalities in ventilatory function was heavy pollution [OR=2.581; CI: 1.051 - 6.342; P=0.039]. Residents of the lagoon district of Attécoubé presented many respiratory symptoms & had a high prevalence of ventilatory function abnormalities. The associated factor was heavy pollution. Improving air quality is urgently needed in this municipality.

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**TUBERCULOSIS MORTALITY: A SCOPING REVIEW**

Luz Quevedo Cruz1, Sumona Datta2, Carlton A. Evans3

1Imperial College London, Perú, United Kingdom, 2Liverpool School of Tropical Medicine, Perú, United Kingdom, 3Imperial College London, UK, United Kingdom

Tuberculosis (TB) has killed more people than any other infection and reducing deaths caused by tuberculosis is a United Nations priority. The aim of this scoping review is to describe and critically appraise the published literature concerning TB mortality. The PRISMA-ScR checklist was used. We systematically searched the PubMed database with the following search terms: “tuberculosis” AND (“death” OR “fatality” OR “mortality”). Eligibility criteria and categorisation were applied by three investigators using the Rayyan tool. Of 1730 articles found, 841 fulfilled the eligibility criteria, including 18 trials. There were far fewer publications concerning TB mortality than other similarly frequent causes of death, and other aspects of TB such as diagnosis or therapy. TB mortality publications principally originated in high-income countries, relatively neglecting countries where tuberculosis causes most deaths. Also, children were under-represented as the focus of only 4.3% of TB-mortality publications, much less than the 14% of tuberculosis deaths that occur in children. TB mortality publications...
DISPROPORTIONATE COVID-19 DISEASE SEVERITY AND MORTALITY IN A DIVERSE POPULATION OF HOSPITALIZED PATIENTS

Alexandra Do¹, Jordan West¹, Molly Biggs¹, Marshall Reviere¹, Gaealan Montoya¹, Dominic Lundquist¹, Samuel B. Anyona², Douglas J. Perkins³, Ivy Hurwitz⁴, Jens O. Langsjoen¹

¹University of New Mexico, School of Medicine, Albuquerque, NM, United States; ²Maseno University, Maseno, Kenya; ³University of New Mexico HSC, Center for Global Health, Albuquerque, NM, United States; ⁴University of New Mexico HSC, Dept of Internal Medicine, Albuquerque, NM, United States

Limited data exist on the relationship between presenting symptoms, comorbidities, and the development of severe COVID-19 across hospitalization in diverse patient cohorts, despite COVID-19 tracking information highlighting disparities according to race/ethnicity. This observational study included all adult symptomatic COVID-19 patients (n=1319) admitted to the University of New Mexico Hospital from 3/2020 to 7/2021. Self-reported race/ethnicity, presenting symptoms, comorbidities, and clinical events were obtained by manual chart review and aggregate data queries. Severe COVID-19 included ICU admission and/or death during hospitalization, while non-severe cases survived without requiring ICU support. The distribution for the three primary racial/ethnic groups was: 39.8% American Indian/Alaska Native (AI/AN), 44.0% Hispanic, and 16.2% non-Hispanic White (NHW). The average age was 57.7±16.1 years, with 45.9% being female. Presenting symptoms differed among the groups with Hispanic patients having the highest proportion of nausea (P<0.015), vomiting (P=0.013), and headache (P=0.001). Altered mental status was most prevalent in the NH-W group (P<0.001), while shortness of breath was highest in AI/AN (P=0.017). At admission, NH-W patients had significantly more comorbidities, including hypertension (P<0.001), stroke (P<0.001), chronic obstructive pulmonary disease (P<0.001), hyperlipidemia (P<0.001), and hypothyroidism (P<0.001). During hospitalization, 839 patients had non-severe COVID-19 and 480 patients developed severe disease. The AI/AN group experienced higher rates of secondary bacterial pneumonia (P<0.001), acute respiratory distress syndrome (P=0.022), cardiac failure (P<0.001), and myocardial infarction (P<0.001), consistent with longer hospitalization. Logistic regression modeling revealed that AI/AN ancestry is a strong predictor of severe COVID-19 (OR=1.93, CI=1.23-3.02, P<0.004) and is associated with higher mortality (OR=1.74, CI=1.06-2.86, P=0.028). Interventions aimed at reducing COVID-19 disparities are critical for improved health outcomes in diverse communities.
delivery. There was no significant difference observed in all other antenatal or delivery complications including SARS-CoV-2 infection (<2% in each group). Neonatal parameter low birth weight (LBW) had significant values in neonates whose mother have administered SARS COVID-2 vaccine during their pregnancy (OR 0.52, 95% CI 0.35 to 0.78 p=0.001). The rate of perinatal outcome like Still Birth (0.8%), IUD (1.2%) and foetal distress (6.3%) were not different than the national averages in India also, there was no significant differences for those outcomes between vaccinated and unvaccinated compared groups. There was no significant difference observed for all other neonatal outcomes between both the groups. From the present study it can be concluded that SARS-COVID-2 vaccination during pregnancy was not associated with adverse perinatal outcomes. These observations may assist health care provider and pregnant women to make informed decision regarding administration of vaccine.

WEIGHTED FIDELITY OF DELIVERY OF AN INTERVENTION IN THE HEALTH FACILITY: A CASE OF TUBERCULOSIS SCREENING AMONG HIV CLIENTS IN SELECTED HOSPITALS IN GHANA

Solomon A. Narh-Bana1, Mary Kawonga2, Esnat D. Chinwa2, Selase A. Ofori1, Frank Bonsu4, Latifat Ibisomi2, Tobias F. Chirwa2
1Dodowa Health Research Centre, Dodowa, Ghana, 2University of the Witwatersrand, School of Public Health, Faculty of Health Sciences, Johannesburg, South Africa, 3Gender & Health Research Unit, Medical Research Council, Johannesburg, South Africa, 4National TB Control Programme, Accra, Ghana

Fidelity assessment is essential in health intervention implementation. Studies have assessed fidelity separately; either healthcare provider or facility levels with managers. However, there might be variations in facility resources and their utilization, skewing such comparisons. We assessed weighted fidelity of implementing the TB screening guidelines among PLHIV and examine its effect on TB screening coverage in HIV clinics in Ghana. We used cross-sectional study with 226 HIV care providers, 27 managers of district hospitals implementing the TB screening intervention, and extracted information from TB registers monthly for 2018. Weighted fidelity was measured based on the extent to which the intervention was implemented, considering both the facility and the provider level assessments. Response scores and extracted data on fidelity were analyzed and summarized using the median and inter-quartile range for non-normal data such as fidelity scores and coverage and frequencies and percentages for categorical data such as resources availability. Linear regressions models were fitted for TB screening coverage using the fidelities separately. The study revealed that the weighted fidelity median score was 67% (IQR: 59.9 - 74.9%), and the TB screening coverage was 71.3% (IQR: 56.9 - 96.7). Weighted fidelity of delivery was statistically associated with TB screening coverage (p<0.01). All the moderating factors investigated have no statistical association with weighted fidelity of delivery (p>0.05) except for IE&C. Facilities with TB IE&C materials available had a significantly (p=0.025) higher median fidelity score 75.4% (74.9 - 88.5) than their counterparts 65.7% (59.4 - 72.6). The combined provider-facility level assessment of fidelity demonstrated that weighted fidelity of delivery is positively associated with TB screening coverage and provided a better platform for assessing implementation fidelity. It also showed that the availability of IE&C materials significantly moderates the weighted fidelity of delivery. Weighted fidelity is an efficient way of holistically assessing fidelity within health facilities.

SURVEILLANCE OF INFLUENZA-LIKE ILLNESS AT THE U.S. MILITARY CAMP LEMONNIER, DJIBOUTI

Mayar Maged Said
US. Naval Medical Research Unit #3 (NAMRU-3), Cairo, Egypt

Respiratory tract infections are the most common cause of infection in adults, and influenza-like illness (ILI) accounts for most of these infections. From May 2021 till end of 2022, ILI surveillance was conducted at the U.S. military base Camp Lemonnier, located in Djibouti (CLDJ). 1332 NP/OP samples from both deployed U.S. military and civilians were collected and tested. Eligible cases were those matching the WHO case definition of 2014. Samples were tested using the Biofire® FilmArray® with the Respiratory Panel 2.1 (RP2.1) assay. From the 1332 tested samples an etiology was identified in 869 (65.2%) samples. Co-infection with 2 pathogens was identified in 13/869 positive cases. Data showed that 492/1332 (36.9%) were positive for SARS-CoV-2 with 8/492 (1.6%) were co-infection with other respiratory virus. For Influenza A, 25/1332 (1.9%) were positive for A(H1N1), 3/1332 (0.2%) were positive for A(H1N2), 33/1332 (2.5%) were positive for A(H3N2), and 1/33 (3%) was reported with co-infection with Human Rhinovirus. Influenza B was detected in 6/1332 (0.5%) of the tested samples. Other respiratory pathogens were detected including Human Rhinovirus 193/1332 (14.5%) of samples tested. Additionally, 10/193 (5.3%) of the Rhinovirus cases reported co-infection with another respiratory virus. Human Respiratory Syncytial Virus was detected in 17/1332 (1.3%) of the samples. Seasonal corona viruses, human para influenza viruses, Adeno virus and HMPV were also detected. While quite challenging to undertake accurate near real-time respiratory surveillance in deployed austere military settings, the importance of establishing an early warning system that would help in identifying pathogens of pandemic potentials is critical. Furthermore, this type of surveillance provides the necessary data for enhanced risk mitigation and improved force health protection. It also has the potential to both decrease disease non-battle injury, and aid in maintaining medical readiness of the warfighter that help break the chain of disease transmission, by further reducing the risk to the deployed forces stationed in Djibouti and the Horn of Africa.

SENSITIVITY PROFILE OF FUNGAL PATHOGENS RESPONSIBLE FOR LOWER RESPIRATORY TRACT INFECTIONS IN YAOUNDE

Claris Killa
The University of Yaounde 1, Yaounde, Cameroon

Respiratory tract infections are usually further classified as an upper respiratory tract infection (URI or URII) or a lower respiratory tract infection (LRI or LRTI). LRIs are the leading cause of death among all infectious diseases. The objective of our study was to bring out the sensitivity profile of fungal pathogens responsible for lower respiratory tract infections in Yaounde. We carried out a transverse and descriptive study during a 6 month period. (February to June 2021), at the Jamot hospital in Yaounde. Included in this study were patients suffering from a LRI from whom the medical practitioner had requested a sputum or broncho alveolar liquid analysis. A macroscopic, microscopic, fungal culture of the sample was carried out and a germ tube test, fungal sensitivity test as well as specie identification using the ID 32 C gallery was carried out on the positive cultures. Statistical analysis was carried out using the R version 3.6.1 software. The mean was calculated with the aid of the Kruskal Wallis rank sum test. 300 patients participated in this study. They had mean age ± standard deviation of 41.59 ± 17.5 years and extremities of 1 and 91years. The male /female ratio was 2:1 Fungal infection was positive in 127 patients (42.33 %), 75(59%) Candida albicans, 25 (19.68%) Cryptococcus humicola, 10 (7.87%) Candida tropicalis, 6 (4.72%) Candida krusei, 4(3.14%) Candida famata, 4 (3.14%) Candida sake and 3 (2.36%) Cryptococcus curvatus. As far as antifungigam is concerned, the total drug susceptibility was ; Nystatin (98.47%), Amphotericine B (86.91%), Miconazole (55.42%), Econazole (52.61%), Katoconazole (52.57%) and Fluconazole (14.42%).The prevalence of fungal pathogens was 42.33%. Of the 300 patients, 71 had tuberculosis, 24 were HIV positive and 6 were diabetic. We had 5 patients with pulmonary tuberculosis and positive data for enhanced risk mitigation, 16 with HIV and fungal co-infection and 6 with HIV and tuberculosis co-infection. This study shows a relative high prevalence (42.33%) of the colonisation of the respiratory tract by the above listed fungal pathogens. The drug of choice is Nystatine and Fluconazole presents a very limited activity. 
TUBERCULOSIS IS A GOOD POINT OF ENTRY FOR THE SCREENING OF CARDIOVASCULAR EVENT RISK FACTORS IN A LOW-MIDDLE-INCOME COUNTRY: LESSON LEARNED FROM INTEGRATED HEALTHCARE IN GABON

Bayode Romeo Adegbite
Centre de Recherches Médicales de Lambarené, lambarene, Gabon

Many Low-middle income countries currently experience an epidemiological transition including a double burden of infectious and non-communicable diseases. Gabon is a high-burden tuberculosis country and 46% of tuberculosis patients carried cardiovascular disease comorbidities. This study aimed to assess the prevalence of high and intermediate risk of developing cardiovascular events (coronary heart disease, stroke, peripheral arterial disease, aortic disease) in ten years in patients consulting for tuberculosis symptoms. We performed a cross-sectional analysis among adult patients with tuberculosis symptoms, consulting in the two referral hospitals in Mvoung Ogooué region in Gabon between 2020 and 2022. The American, ASCVD (atherosclerotic cardiovascular disease) score was used to identify patients at high risk of developing cardiovascular events at the next ten years. A total of 405 patients have been included, with 151 (37%) having pulmonary tuberculosis. The majority of patients were men (216;53%); More than half (277,68%) of patients leave in an urban area. HIV infection accounted for 29% (119). A total of 31 (7.7%) and 51 (13%) have a medical history of diabetes, and high blood pressure respectively. Overall, 6% and 14% of study patients were at High-risk and Intermediate risk of cardiovascular events in the next 10 years. Men (aOR 4.5, 95% CI 2.6-6.5), a medical history of diabetes (aOR 7.5, 95% CI 5.5-10.5) and a medical history of Hypertension (aOR 6.5, 95% CI 3.5-10.5) were significantly associated with a considerable risk of having a CVE. Four out of five patients with considerable risk of developing a CVE were newly diagnosed with diabetes or hypertension. In conclusion, there is an epidemiological transmission in Gabon. Four out of five patients who are at risk to have a CVE in the next ten years would be saved if there is integrated care for communicable and non-communicable diseases. High-burden and endemic infectious diseases such as tuberculosis could be a good point of entry for earlier diagnosis of CVE in many African countries.

EFFECT OF POST PCV13 ON VACCINE TYPE INVASIVE PNEUMOCOCCAL DISEASE AMONG CHILDREN IN RURAL GAMBIA

rasheed A. salaudeen1, Usman Barjo2, Momodou Drammeh2, wutor Baleng Mahama3, Yekini A. Olatunji4, isaac osei5, Mohammad Ililas Hossain1, Grant A. Mackenzie1

Medical Research Council Unit, The Gambia, Basse, URR, Gambia
Medical Research Council Unit, The Gambia, Basse Santa su, Gambia

Introduction of pneumococcal conjugate vaccines (PCVs) has reduced the incidence of vaccine-type (VT) invasive pneumococcal disease (IPD) but non-vaccine type (NVT) serotype replacement disease remains a concern. PCV7 was introduced for routine use in Gambia in 2009 but was non-vaccine type (NVT) serotype replacement disease was not a significant issue in IPD. However, more recent surveillance suggests that replacement disease may be developing in the under-5 year age group. Higher valency PCVs should be prioritised.

THE ADDED VALUE OF USING PULSE OXIMETER ROUTINELY INTO THE INTEGRATED MANAGEMENT OF CHILDHOOD ILLNESS GUIDELINES TO BETTER IDENTIFY AND MANAGE SEVERE CASES AMONG CHILDREN UNDER-5 YEARS OLD IN WEST AFRICA, JUNE 2021 TO JUNE 2022

Gildas Boris HEDIBLE1, Desire Neboua2, Lucie Peters Bokol1, Gildas Anogo1, Zineb ZAIR1, Severin Lenaud3, Honorat Agbeci1, Abdoul Guanjiy Sawadogo4, Désiré Kargougou5, Bertrand Meda5, Jacques Séraphin Kolié6, Sandrine Busiere5, Franck Lamontagne9, Sarah Louart10, Valéry Riddel11, Valériane Leroy1

The Integrated Management of Childhood Illness (IMCI) guidelines for children under 5 is a symptom-based algorithm guiding health care workers at the primary health center (PHC) level in resource-limited countries. Hypoxemia is a life-threatening event often underdiagnosed clinically. The AIRE project, UNITAID-funded, has implemented the routine Pulse Oximeter (PO) use into IMCI consultations at PHCs in Burkina Faso, Guinea, Mali & Niger. We measured the added value of PO use as part of IMCI compared to IMCI alone to improve the diagnosis & care-management of hypoxemia at PHC level. In 16 AIRE PHC research sites (4/country), all children aged 0-59 months attending IMCI consultations, except those aged 2-59 months classified as green case without cough or breathing difficulties were eligible for PO use, and enrolled in a cross-sectional study with parental consent. Severe cases were followed-up during 14 days. From June 2021 to June 2022, 39,360 children attended IMCI consultations at the research PHCs of whom 31,600 (80.2%) was eligible for PO use. Overall, 9.8% were identified as severe cases using IMCI alone (3,103/31,600; 95% CI: 9.5-10.2) & 60 cases newly identified using IMCI+PO. Prevalence of severe case using IMCI+PO was at 10% (3,103;60/31,600; 95% CI; 9.7-10.3). Overall +1.9% (60/3,103; 95% CI: 1.5-2.5) were detected using PO alone. This added value was heterogeneous between countries: +0.9% (95% CI: 0.1-3.4), +0.3% (95% CI: 0.09-0.9), +3.2% (95% CI: 2.3-4.2), +2.9% (95% CI: 1.4-5.2), in BF, Guinea, Mali, Niger, respectively. Among the 60 additional severe cases identified only with PO, 27 were included & followed, 20 (74%) were transferred to district hospital but only 14 (52%) received an oxygen therapy. In conclusion, the added value of IMCI+PO was estimated globally to +2% to better identify severe cases, with a similar effect except in Guinea and Burkina Faso. Their appropriate management remains challenging for West African governments.

FINE-SCALE SPATIOTEMPORAL DYNAMICS OF SARS-COV-2 INTRODUCTION AND SPREAD IN NAIROBI COUNTY, KENYA

Edith Chepkorir1, John Mwita2, Solomon Langat1, Silvanos Mukunzi3, Limbaso Konongai1, Samwel Lifumo1, Samwel Khamadi1, George Githinji2

1Kenya Medical Research Institute (KEMRI), Nairobi, Kenya, 2Kenya Medical Research Institute (KEMRI)-Welcome Trust Research Programme, Kilifi, Kenya

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative virus of coronavirus disease 2019 (COVID-19), is a highly transmissible virus that was first reported in Wuhan in December 2019 threatening human populations, health systems and economies worldwide. As of March 2023, Africa has reported over 12 million confirmed cases
of COVID-19 and over 200,000 deaths although this is likely to be major underreporting of actual cases and deaths. Kenya reported the first case of SARS-CoV-2 on the 13th of March 2020 comprising one the first SARS-CoV-2 introductions in Kenya. Thereafter additional introductions and intense within country transmission contributed to increase in the number of infections (n= 341,235) over multiple successful waves and reported mortality (n= 5,684), Nairobi, East-Africa’s largest City (population =4,397,073) covering an estimated 692 square Kilometres (2019 Census) was a major epicentre of COVID-19 cases in the country. In this study, we sequenced and analyzed 168 Real Time- Polymerase Chain Reaction (RT-PCR) positive samples selected based on PCR cycle threshold score ($\Delta$Ct)$^0$. The samples were collected from seventeen sub-counties in Nairobi County between April 2020 and January 2022, yielding 141 sequences suitable for phylogenetic analysis to provide the region’s fine-scale genomic epidemiology of SARS-CoV-2. Full genome sequencing was carried out using Oxford Nanopore Technologies (ONT) GridIon machine. The sequence reads were assembled using the ARTIC bioinformatics pipeline. Sequence quality checks and mutation calling were performed using NextClade and then lineage analysis with Pangolin v4.0.6. The sequences were aligned using Nextalign version 1.4.1, and the phylogenetic and phylogeographic analyses performed. We describe the clustering patterns of the different SARS-CoV-2 variants found in the different Nairobi sub-counties, the transmission patterns of the strains within the sub-counties and the impact of the containment measures on the spread on SARS-CoV-2 in Nairobi. The study is part of the strategy to develop a COVID-19 genomic surveillance system for the country.

SCALING RISK FOR A GLOBAL TUBERCULOSIS PROGRAM IN AN OIL AND GAS COMPANY

Susan Ngunjiri, Malick Diara, Candace McAlester, Tammy Pipes
ExxonMobil Corporation, Spring, TX, United States

Workers in high-risk tuberculosis (TB) locations with incidence of more than 20 active cases per 100,000 per year may be at an increased risk for TB infection. This risk is greater in congregated settings (i.e., camps, offshore rigs, and vessels) with a mix of people from high and low endemic areas. To safeguard workers assigned to sites in high-risk settings, ExxonMobil requires enrollment of such workers in the company TB Control Program which consists of awareness, screening, and case/contact management. Screening includes annual questionnaire and testing with a skin test or preferably a blood test using Interferon Gamma Release Assays (T-SPOT or QuantIFERON). Chest X-rays are accepted as an indicator of no active pulmonary TB in specific situations. World Health Organization (WHO) estimated that a third of the world’s population had latent tuberculosis infection (LTBI), with low rates in countries like the US at 4.7% but much higher in countries with higher TB incidence. These rates are reflective of what is seen in workplace screening and guidance is provided based on national program guidelines for LTBI. The critical objective of the workplace program is to have zero transmission at the workplace. During the past 12 years of implementation, over 100 active TB cases were diagnosed among our workers. They were all promptly identified and treated with no reported workplace TB transmission, following contact tracing. In 2021, WHO updated its guidance on systematic screening for tuberculosis. We used this to review our TB program including consultations with external experts and company executives. As a result, we adopted a more fit for risk TB screening approach by updating its periodicity from annual to two years for offshore, mobile assets and onshore congregated settings in high-risk locations. Offshore fixed sites in areas with low risk discontinued the screening. The program guidance was updated, and communications with roll out completion are expected by end of 2023. In conclusion, using a risk-based approach for our TB program, we have an improved fit for risk screening guidance that amplifies efficiencies and maintains effectiveness.

INVESTIGATION OF SUSPECTED PULMONARY TUBERCULOSIS CASES IN KATIALI IN THE DIANRA HEALTH DISTRICT, CÔTE D’IVOIRE, JUNE 2021

Pegmontaye Moussa Soro1, Kouadio Felix Koffi2, Wilnique Pierre3, Joseph Blaise Otschudjangaka1, Issaka Tiembre4, Vroh Joseph Beni Bi4
1FETP, Abidjan, Côte d’Ivoire, 2INHR, Abidjan, Côte d’Ivoire, 3AFENET, Abidjan, Côte d’Ivoire, 4UFETP, Abidjan, Côte d’Ivoire

On June 10, 2021, the health district of Dianra notified the epidemiological surveillance service of the National Institute of Public Hygiene four suspected cases of pulmonary tuberculosis residing in the village of Katiali located at 7 km from the district. We conducted an investigation to confirm the disease & to implement control and prevention measures. We conducted a descriptive cross-sectional study from June 23 to 26, 2021, in the village of Katiali with an estimated population of 719 in 2020. A suspected case was defined as any person in Katiali with a cough lasting more than 14 days, whether or not associated with any of the following signs: fever in the evening or afternoon; sweating at night; intense fatigue; lack of appetite; weight loss or weight loss; cessation of menstruation without pregnancy in women of childbearing age, between 18 March and 10 June 2021. A confirmed case was defined as any suspected case with a positive laboratory result for BAAR by microscopy or Xpert MTB/RIF test. Proportions and ratio were calculated by using EpInfo 7. 12 suspected cases were recorded and one confirmed with pulmonary tuberculosis for a positivity rate of 8.3%. The median age was 7 years (1-46). The sex ratio was 0.5 male for 1 female. The confirmed case was a 7-year-old girl, in a household of 3 members. 16 contacts of the confirmed case were identified & followed up, of which six (37.5%) tested negative by microscopy,eight contacts(50%) were not tested due to difficult of sampling collection and 2 were positive (12.5%). The confirmed case was isolated and treated with antituberculosis drugs & the close contacts under 5 years of age were put on isoniazid prophylaxis. A young girl was confirmed for pulmonary tuberculosis and two other additional contacts. The isolation of the confirmed case, its management, the prophylaxis of the contacts and their follow-up allowed limiting the spread of tuberculosis in Katiali. We recommend strengthening the technical platform of the general hospital of Dianra for early detection of tuberculosis; enhance testing capacities & sensitizing the community to massively adhere to the extended vaccination program.

PRE-CLINICAL VALIDATION STUDIES OF NOVEL POINT-OF-CARE RNA TEST FOR COVID-19 DIAGNOSIS AND UTILITY FOR SARS-COV-2 GENOMIC SURVEILLANCE IN GHANA

Charles Narh1, Maame E. Acquah1, Gloria Amegatcher1, Deborah Tetteh1, Ethel Debrah1, Bridget Quist1, Gideon Twienu2, Sarmuel Aumco2, Bill Hopper1, Lydia Mosi1, Jack Richards2
1West African Centre for Cell Biology of Infectious Pathogens (WACBIP), University of Ghana., Accra, Ghana, 2Water Research Institute, Council for Scientific and Industrial Research, Accra, Ghana, 3ZIP Diagnostics, Melbourne, Australia, 4ZIP Diagnostics, Accra, Australia

Rapid public health response to identify local and imported COVID-19 cases will need cost-effective RNA amplification tests with comparable sensitivity as PCR but without the requirements for expensive equipment. To fill this need, we developed ZIP-CoVx-P2 (zip-test), a CE-certified SARS-CoV-2 RNA test, integrated into a portable isothermal device, for COVID-19 diagnosis at the Point-of-Care, with a 30 mins sample-to-results capability. A pre-clinical trial (ACTRN12623000666684) was conducted to validate the zip-test performance in field settings, and to assess its integrability into SARS-CoV-2 genomic surveillance programs in Ghana. This report discusses the trial implementation, study demographics and preliminary test performance. From Oct-Dec 2022, we conducted a multi-site prospective cross-sectional survey to enrol, and collect two oropharyngeal/nasal swabs and clinical data from 1,000 consented participants residing in
12 communities in Greater Accra and Central regions of Ghana. Where permitted and within biosafety limits, zip-testing was performed on site, else the swabs were transported to COVID-19 testing laboratory for zip-testing followed by RT-PCR confirmation. Positive test samples were further processed for sequencing. Demographically, 92.8% of the participants were above 18 yrs and 66.5% were females. Additionally, 55.0% reportedly received ≥ 1 dose of a COVID-19 vaccine, and 73.5% reported having no COVID-19 symptoms. Among suspected cases (26.5%), fever, cough and head were the most reported symptoms (>54%), with 67.8% of these cases reportedly confirmed COVID-19-positive by RAT/RT-PCR test. Using the zip-test, the overall positivity rate among 980 study samples was 15.5%, with 13.6% giving positive tests for the two SARS-CoV-2 targets - orf1ab and M genes. RT-PCR testing (ongoing) on the paired study samples will be used to determine sensitivity and specificity of the zip-test but the positivity rates in our study population are comparable to the mean 12% positivity rate reported by the Ghana Health Services in August 2022. The zip-test presents prospects for COVID-19 diagnosis at the Point-of-Care.

A CASE OF TUBERCULOSIS PERICARDITIS

Bethlehem Atoma1, Kinfu Debele2, Dawi Girma2, Obse Deressa3, Anteneh Zezewde1
1 University of Minnesota, Minneapolis, MN, United States, 2Adama Hospital Medical Center, Adama, Ethiopia, 3St Paul Hospital, Addis Ababa, Ethiopia

Tuberculosis pericarditis is one of the most critical complications in 1-2% of patients with pulmonary tuberculosis infection. The pathophysiology of Tuberculosis pericarditis occurs by extension of infection from the lung or tracheobronchial tree adjacent to lymph nodes, spine, or miliary spread. Pericardiocentesis and abscess drainage are effective surgical procedures for managing disseminated tuberculosis with complications such as pericarditis and abscess formation. A 7-year-old male child presents to Adama Hospital with a persistent dry cough for a week and fever. Later patient's condition worsened with dyspnea at rest and orthopnea. The patient was evaluated, and the initial vital signs were BP: 118/74 PR: 138 BPM, T: 38c Sap02: 96%, weight: 24kg. Physical examination showed accessory muscles use, coarse crackles over the right lung, and hepatomegaly. Lab work showed a white blood count of 2.4x103/UL with 13% lymphocytes, hemoglobin count of 10.0g/dl, and no parasite on the blood film. Abdominal and chest ultrasound revealed complex pericardial collection, bilateral pleural effusion, and ascites. An echocardiogram showed an extensive exudative pericardial collection with internal pericardial thickening. Due to worsening respiratory distress, the patient was transferred to the ICU. Ceftriaxone, Vancomycin, Azithromycin, internal pericardial thickening. Due to worsening respiratory distress, the patient was transferred to the ICU. Ceftriaxone, Vancomycin, Azithromycin, prednisolone, and anti-TB medications were initiated along with intranasal oxygen. The patient was suspected of having severe community-acquired pneumonia and cardiac tamponade secondary to disseminated TB. Pericardiocentesis and thoracentesis were noted with pus-containing fluid, but the culture was inconclusive. Despite pericardiocentesis, ultrasound still revealed pericardial fluid accumulation, necessitating pericardiostomy, and abscess drainage. The patient was hospitalized for 42 days and was discharged with outpatient anti-TB treatment; currently in stable condition. This care demonstrates the importance of prompt and accurate diagnosis and treatment of disseminated TB-related pericardial effusion in improving patient outcomes in developing countries.

SCHISTOTRACK: A COMMUNITY-BASED, PROSPECTIVE COHORT IN RURAL UGANDA TO EXAMINE CAUSES OF PERIPORTAL FIBROSIS ASSOCIATED WITH INTESTINAL SCHISTOSOMIASIS

Goylette F. Chami
Big Data Institute, Nuffield Department of Population Health, University of Oxford, Oxford, United Kingdom

Periportal fibrosis is a severe morbidity associated with intestinal schistosomiasis, including Schistosoma mansoni and S. japonicum. These parasitic blood flukes live in the mesenteric venules. The eggs produced by mature female flukes must traverse the intestinal mucosa to be excreted. Only ~50% of the eggs are successfully excreted with the remaining eggs being swept back into the liver and spleen. The inflammation, granulomas, and subsequent fibrosis in and around the portal veins contributes to vascular restructuring within the liver and distinct patterns of fibrosis associated with intestinal schistosomiasis. Understanding the risk factors and predictors of progression within and across individuals is challenging due to imperfect diagnostics, confounders of ongoing treatment, and interacting co-infections within the liver. SchistoTrack is a community-based, prospective cohort in three rural districts of Eastern and Western Uganda that aims to assess the within and across individual causes of periportal fibrosis. Nearly ~4000 individuals randomly sampled from 2080 households in 52 rural villages are being followed over five years with detailed clinical examinations and a further ~6000 individuals are assessed through medical histories. This talk will introduce the SchistoTrack Cohort and share findings on the risk factors for periportal fibrosis, focusing on the challenges of developing clinical phenotypes within the context of a cohort and on the contributions of co-infections with hepatitis B, malaria, and HIV for predicting the likelihood of periportal fibrosis.

THE MEASURING OF TREATMENT COVERAGE AS COMPARED TO ADMINISTRATIVE COVERAGE FOR SCHISTOSOMIASIS WITH PREVENTIVE CHEMOTHERAPY IN BONG, LOFA, AND NIMBA COUNTIES IN 2018 IN LIBERIA

Anthony Kerkula Bettee
Ministry of Health, Monrovia, Liberia

Schistosomiasis or Bilharzia is a parasitic disease caused by infection with the trematode blood-flukes schistosomes. There are two major forms of human schistosomiasis that occur in Sub-Saharan Africa, which include intestinal schistosomiasis caused by Schistosoma mansoni infection and urinary schistosomiasis due to Schistosoma haematobium infection. Schistosomiasis is among the neglected tropical diseases (NTDs), which remain one of the serious public health problems, posing unacceptable threats to human health where there is a high prevalence of the disease. The World Health Assembly Resolution 54.19 urges all member states to regularly treat at least 75% of all school-aged children who are at risk of morbidity. To determine if these global goals are being reached, each
national program routinely reports administrative drug coverage from the annual mass drug administration. In order to monitor and support the NTDs program performance, independent drug coverage surveys are recommended by the World Health Organization to compare the administrative coverage and an independent survey coverage to validate the reported coverage by the national program for control and elimination. The result of the survey assists in the identification of recommended actions to improve program delivery. The aim of the coverage survey was to evaluate the effectiveness of preventive chemotherapy in reaching the targeted population in endemic counties. The survey was based on the SCH data collected during the 2012 baseline survey in the 15 counties in Liberia. During the surveys, the total number of children interviewed was 651 while the number of adults was 1,023. The survey contains 14 villages per county (42 in total) and 15 households per village. The PZQ coverage for SAC and adults was 75% while the administrative coverage was 76% which shows the validity of the data.

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MAPPING THE TRANSMISSION DYNAMICS OF SCHISTOSOMIASIS: INSIGHTS FOR CONTROL AND ELIMINATION STRATEGIES

Obiageli Josephine Nebe1, Juliana A. Amanyi Enegelaj, Jacob Solomon1, Rita Ochombo Urude1, Rinpan Ishaya2, Girja Sankar2, Bosede Eunice Ogundipe3, Abdurrahman Sadiq Tsimiri4, Abubakar Abba5, Joseph Kumbur6, Bright Ekweremadu7, Chistopher Ogochisi8, Moses Aderogba9, Louise Makau-Barasa10

1Federal Ministry of Health, Abuja, Nigeria, 2CBM Christoffel Blindenmission CBM e.V, Cambridge, United Kingdom, 3Health and Development Support Programme (HANDS), Jos, Nigeria, Jos, Nigeria, 4Department of Public Health, Federal Capital Territory Administration, Abuja, Nigeria, 5CBM Christoffel Blindenmission CBM e.V, Abuja, Nigeria, 6The END Fund, Abuja, Nigeria, 7The END Fund, New York, NY, United States

Schistosomiasis (SCH), is a parasitic infection caused by several species of flatworms or blood flukes of the genus Schistosoma. While several species of freshwater snails are the intermediate host, the infection are transmitted to humans. SCH is a neglected tropical disease prevalent in tropical countries and estimated to affect more than 200 million people worldwide. Nigeria accounts for over 25% of the global burden of schistosomiasis, with an estimated 29 million people infected. SCH is endemic in the 36 States and Federal Capital Territory (FCT) in Nigeria. The results of the epidemiological baseline survey conducted in FCT showed prevalence ranging from 7%-52%. Mass administration of medicine (MAM) using Praziquantel commenced in FCT in 2014. In 2020, an outbreak of SCH was reported in some communities in AMAC, Gwagwalada and Bwari area councils (ACs) thus, prompting a new round of survey to: validate the claim of the outbreak, determine the current prevalence and endemicity of SCH in the reported communities and provide data for informed decision making on the appropriate intervention strategy. A purposive sampling of 26 communities across AMAC, Gwagwalada and Bwari ACs were conducted. About fifty-five children and adults were systematically and conveniently sampled respectively in each community. Urine samples collected from selected school children were examined in the laboratory for Schistosoma eggs using urine filtration technique; while stool samples were examined for parasite eggs using the Kato-Katz technique. A total of 2,364 participants were sampled. The mean prevalence were 27.7%, 8.9% and 26.4% in AMAC, Bwari and Gwagwalada ACs respectively. Community prevalence ranged from as high as 64% to as low as 2.1. While the result revealed marginal reduction of prevalence in Gwagwalada, more effort is required to reduce SCH prevalence in AMAC and Bwari ACs. This is a pointer that MAM alone cannot halt interruption of transmission. There is cogent need for prioritization and integration of Water Sanitation and Hygiene Services and investment in snail intermediate hosts control if we are to achieve the 2030 NTDs elimination goals.

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COMPARATIVE GENOMIC EVOLUTIONARY ANALYSIS OF BIOMPHALARIA SUDANICA, A NEGLECTED INTERMEDIATE HOST VECTOR OF SCHISTOSOMIASIS

Tom Pennance1, Javier Calvelo2, Jacob A. Tennessen3, Stephanie R. Bollmann4, Michael S. Blouin5, Johannie M. Spaan1, George Ogara6, Fredrick Rawogo7, Kennedy Andiego8, Boaz Mulonga9, Meredith Odhiambo10, Eric S. Loker11, Andréis Iriarte12, Maurice Odiero13, Michelle L. Steinauer14

1Western University of Health Sciences, Lebanon, OR, United States, 2Universidad de la Republica, Montevideo, Uruguay, 3Harvard T.H. Chan School of Public Health, Boston, MA, United States, 4Oregon State University, Corvallis, OR, United States, 5Kenya Medical Research Institute (KEMRI), Kisumu, Kenya, 6University of New Mexico, Albuquerque, NM, United States

Biomphalaria sudanica is a major vector host of Schistosoma mansoni in the highly endemic African Great Lakes region, notably the largest transmission sites where current snail control methods are impractical to apply. New targets for vector control and reducing transmission of schistosomes to humans, perhaps through utilizing natural resistance mechanisms in snails, may provide approaches that are scalable to these vast transmission sites. Here, we report the first genomic and transcriptomic characterization of B. sudanica, providing a foundation for understanding the biology of this species and evolutionary history of this important group of vectors. Our PacBio genome assembly size of 946.6 Mb for B. sudanica, is larger than other Biomphalaria species and is comprised of 23,998 genes representing a close to complete genome annotation for Mollusca. Gene family expansion/contraction analysis (genetically orthologous relationships estimated in Phylogenetically Hierarchical Orthogroups) show a clear trend toward the simplification of the genome of several species of Biomphalaria, and phylogenetic analysis of specific genes of interest support the phylogenetic position of B. sudanica as diverging more recently relative to B. pfeifferi after colonization of Africa several million years ago. Intraspecies nucleotide diversity was calculated through the sequencing and alignment of four additional genomes of B. sudanica isolates. The resulting alignment was used to search for molecular signatures of balancing selection, that may be the result of host-pathogen coevolution and therefore present in immune genes. Our bioinformatic pipeline identified 1,047 of the top most diverse genes, ~25% of which were broadly immune related (AIG molecules, lectins, redox proteins, TLRs) and orthologs to gene families conferring resistance to S. mansoni in closely related B. glabrata (PTC1, PTC2, PEPs, SOD, Bmpyhalysin). These results illuminate the complexity and diversity of snail molecular biology which underlies their interaction with schistosomes and the transmission of these parasites.

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RE-ASSESSING THERMAL SENSITIVITY OF SCHISTOSOMIASIS TRANSMISSION RATES IN THE ERA OF CLIMATE CHANGE: EVIDENCE OF A HIGHER THERMAL OPTIMUM THAN PREVIOUSLY PREDICTED

Giulio A. De Leo1, Ibrahim H. Aslan1, Andrew J. Chamberlin1, Kaitlynn Mitchell1, Alyson L. Singleton2, Caroline K. Glidden3, Erin A. Mordecai4, Roberta Lima Caldeira5, Miguel AMV Monteiro6, Vivian Silva7, Adriano Pinter8, Roseli Tuan9, Raquel GS Palacio10, Eliizer K. N’Coran11, Nana Rose Diakite11, Mamadou Ouattara11, Fiona Alian11, Andrew S. Brierley12, Dave Little12, Rachel Norman12, Reed Ozretich11, Ping Liu13, Kamazima Lwiza13, Thiago A. Pereira14, Susanne H. Sokolow1, Chelsea C. Wood15

1Stanford University, Program for Disease Ecology, Health and the Environment, Pacific Grove, CA, United States, 2Stanford University, Emmett Interdisciplinary Program in Environment and Resources, Stanford, CA, United States, 3Stanford University, Department of Biology, Stanford, CA, United States, 4Oregon State University, Dept of Biology, Stanford, CA, United States, 5Fiocruz Minas, Belo Horizonte-MG, Brazil, 6Instituto Nacional de Pesquisas Espaciais, São José dos Campos, São Paulo, Brazil, 7Stanford University, Program for Disease Ecology, Health and the Environment, Pasteur Institute, Brazil, 8Pasteur Institute, São Paulo, Brazil, 9USP School of Public Health, Brazil, 10Stanford University, Program for Disease Ecology, Health and the Environment, Pasteur Institute, Brazil, 11Stanford University, Program for Disease Ecology, Health and the Environment, Pasteur Institute, Brazil, 12Stanford University, Program for Disease Ecology, Health and the Environment, Pasteur Institute, Brazil, 13Stanford University, Program for Disease Ecology, Health and the Environment, Pasteur Institute, Brazil, 14Stanford University, Program for Disease Ecology, Health and the Environment, Pasteur Institute, Brazil, 15Stanford University, Program for Disease Ecology, Health and the Environment, Pasteur Institute, Brazil
The geographical range and seasonal transmission risk for schistosomiasis is affected by the ecology of schistosome parasites and their obligate host snails, and their response to temperature. Previous thermal sensitive models predicted optimal transmission at 21.7 °C, which is at odds with field observations of disease prevalence in sub-Saharan Africa where schistosomiasis is endemic. We performed an extensive literature search and identified the most comprehensive set, up to date, of experimental studies reporting the effect of temperature on physiological and epidemiological parameters regulating the dynamics of the free-living stages of S. mansoni and S. haematobium and of their corresponding parasite burden and prevalence of infection in the human population as a function of temperature. By resampling experimental data to account for uncertainty in model parameters' thermal response, we found that thermal optimum ranges were 23.1-27.3°C for S. mansoni and 23.6-27.9°C for S. haematobium, in both cases at significantly higher temperature than previously determined. A large data set on schistosomiasis prevalence in Africa validates our model projections. Using these more accurate nonlinear thermal-response models will improve our understanding of the effects of current and future temperature regimes on schistosomiasis transmission risk under climate change scenarios.
A SURFING PARASITE: HOW SCHISTOSOME CERCARIAE COUPLE TO INTERFACIAL BOUNDARIES TO EXTEND DISPERSION AND DISEASE SPREAD

Melanie Hannebelle, 1 Ian Ho, 2 Alassane Ndiaye, 2 Manu Prakash 1
1 Stanford University, Stanford, CA, United States, 2 Station d’Innovation Aquacole, Saint Louis, Senegal

Schistosomiasis remains a persistent public health concern despite mass drug administration efforts, highlighting the importance of understanding the biophysical mechanisms contributing to infection risk upon contact with contaminated water. Schistosome cercariae exhibit a unique swimming behavior, characterized by active upward swimming and passive vertical sinking. In this study, we report a previously unrecognized swimming mode, termed “surface swimming,” wherein cercariae swim laterally directly beneath the air-water interface. To accurately characterize cercarial swimming motion, we developed portable microscopes using modified low-cost action cameras, enabling us to examine their behavior in both laboratory settings and natural environments in Senegal, encompassing 3D spaces with water flows, vegetation and wind. Furthermore, we modeled the swimming hydrodynamics of cercariae using a linkage model and derived the swimming efficiency as a function of ecological parameters such as viscosity and surface tension. We demonstrate that surface swimming enables efficient active lateral displacement of the parasite and facilitates long-distance passive travel through wind-driven water surface flows, both of which being important factors determining the local concentration of parasites near potential human hosts within a water body. Our findings emphasize the significance the cercarial swimming behavior in the context of schistosomiasis infection and provide crucial insights for assessing risks and devising effective environmental interventions.

INVESTIGATING THE PRESENCE OF SALMONELLA SPP. IN LOCALLY MADE CHEESE IN MONTERÍA, CORDOBA, COLOMBIA IN 2021

Linda M. Chams 1, Jorge D. Arieta 2, Carlos J. Castro 1
1 Universidad de Córdoba, Montería, Colombia, 2 Universidad de Córdoba, Barranquilla, Colombia

Fresh coastal cheese is a fundamental part of the diet of the population of Cordoba, Colombia. However, there is a concern about the presence of Salmonella spp. in artisanal cheese, which can pose a public health risk. This study aims to detect, isolate, and characterize Salmonella spp. in fresh artisanal cheese sold in Monteria, the capital of Cordoba, using the method suggested by INVIMA (National Institute of Food and Drug Surveillance, 2021) and evaluate its resistance or susceptibility to specific antibiotics. This study is a descriptive exploratory cross-sectional study that took place in Monteria, Cordoba, during the first quarter of 2021. A total of 25 retail outlets were selected from the list provided by the Health Department of Cordoba. The INVIMA method was used for the isolation and identification of Salmonella spp. The black colonies suspected of Salmonella spp. were identified using conventional biochemical tests. Serological identification was performed using the Kaufman-White scheme. The sensitivity or resistance to specific antibiotics was determined using the agar diffusion method, following the international standards set by the CLSI in 2008. Out of the 75 samples analyzed, 18 (24%) showed growth of black colonies, which were classified as suspected for Salmonella spp. The results of this study indicate the presence of Salmonella spp. in fresh artisanal cheese sold in Montería, Cordoba, Colombia. This study revealed the resistance of Salmonella spp. to specific antibiotics, namely amikacin (62.5%) and gentamicin (15%). In conclusion, the study highlights the need for measures to control the production and sale of fresh artisanal cheese to prevent the transmission of Salmonella spp. to humans. The findings of the study can provide valuable information to public health authorities and policymakers to design appropriate interventions to control the transmission of Salmonella spp. through fresh artisanal cheese.

IMPACT OF THE WASH IN SCHOOLS FOR EVERYONE (WISE) PROGRAMME ON THE HEALTH AND SCHOOL ATTENDANCE OF KINDERGARTENERS IN ADDIS ABABA, ETHIOPIA: A CLUSTER-RANDOMISED CONTROLLED TRIAL

Sarah Bick 1, Charles Opondo 1, Baptiste Leurent 1, Oliver Cumming 1, Alam Ezezew 2, Elizabeth Allen 1, Robert Dreibelbis 1
1 London School of Hygiene and Tropical Medicine, London, United Kingdom, 2 University College London, London, United Kingdom, 3 Holster International Research and Development Consultancy, Addis Ababa, Ethiopia

Water, sanitation and hygiene (WASH) interventions in schools (WinS) have been proposed to reduce morbidity in schoolchildren, including gastrointestinal and respiratory infection, and improve school attendance. However, evidence of the impact of WinS interventions on pupil health and educational outcomes has been mixed. We evaluated the WASH in Schools for Everyone (WISE) programme implemented by US-based NGO Splash in partnership with the Government of Ethiopia, which aims to achieve universal WASH coverage in schools in Addis Ababa, Ethiopia over a five-year period. Within the context of a cluster-randomised trial assessing intervention impacts on primary school-aged children (WISE evaluation), we conducted a sub-study to specifically assess impacts on kindergarten (KG) pupils. We included schools enrolled in the main trial with KG classes and randomly selected and allocated additional schools with KG classes until 20 in total were each enrolled in the intervention and control arms. Schools were randomly assigned 1:1 to receive the intervention during the 2021/22 academic year or the following year (waitlist control). The intervention comprised WASH infrastructure improvements, including water storage and filtration, drinking water / handwashing stations and upgraded sanitation facilities, and behaviour change promotion. Within each participating school, we randomly selected 20 KG pupils (ages 3 - 6), and recorded caregiver-reported diarrhoea, respiratory illness and school absence over four consecutive weekly telephone interviews with caregivers of selected pupils between April and June 2022. We found consistent reductions in caregiver-reported diarrhoea, respiratory illness and school absence, although only reduction in absence was significant at the 5% level (OR: 0.67, 95% CI: 0.46, 0.99). Dropout of three schools and the considerable challenges of telephone-based data collection resulted in large losses to follow-up, impacting statistical power. Further research should examine how the use of mobile health technology can be improved to obtain sufficient follow-up data for evaluating school-based interventions.
Assessment of Knowledge, Attitudes and Practice: KAP of Hand Washing Hygiene and the Role of Prevention Project of Infection Control, Gezira State, Sudan 2022

Dania Akasha1, Mazin Osman2
1University of Gezira, Faculty of Medicine, Wad Medani, Sudan, 2King Fahad Hospital Albaha, Al Baha, Saudi Arabia

Infection due to hand-transmitted microbes is considered as a dangerous problem worldwide. Most infections today thought to be transmitted by hands particularly the school aged children in Gezira state. The application of hand hygiene in Gezira villages is too poor with prevalence of infectious diseases in Gezira. The aim of study is to assess knowledge, attitude and practice regarding hand hygiene practices among schools’ children before and after extensive intervention program , and to identify areas of gaps in their knowledge and attitude. This is KAP study ,a three months study period from August until December 2022 in alhafsay village . The study composed of three phases-initial assessment - The Intervention-Post interventions assessment. The intervention program depend on the health education and practical skills teaching (hand washing techniques ) to school children and to their family’s at school and home. Their level of knowledge was assessed based on the hand hygiene by self-designed knowledge questionnaire and then assessed by direct case presented to village health center. A total 239 respondents were studied about their knowledge and attitude towards hand hygiene practices. The KAP score change is generally optimistic. First, knowledge improved by about 80% to reach about 92% in study pupils, followed by an improvement in attitude of about 85% to reach 100% in study pupils, and finally, practice improved by about 74 percent to reach 98 percent in study pupils. Most of them practice and know the international hand washing technique with soap and water from 3% to 67%. The majority of the respondents accept the training sessions regarding hand hygiene practices (81%). Decrease hospitalization, which is due to infectious disease (80% to 35%). The majority of the students learned the importance of infection control by hand washing (27% to 95%). Present study highlights the need of extensive interventional programs regarding hand hygiene practices among the primary school students to provide the current knowledge in the area with behavioral change in attitudes and practices to reduction of infection and effective control.

Use of Enrichment mNGS Method to Identify Respiratory Pathogens in Wastewater from Treatment Plant Location in Salvador, Bahia, Brazil

Luciane Amorim Santos1, Laisa Eduarda Paixão de Moraes1, Pablo Alessandro Barbosa Viana2, Pedro Millet Meirelles1, Tiago Gräf3, Pablo Ivan Pereira Ramos1, Manoel Barra-Netto4, Ricardo Khouri5
1Instituto Gonçalo Moniz, Fiocruz, Salvador, Brazil, 2Instituto de Biologia da Universidade Federal da Bahia, Salvador, Brazil, 3Instituto de Biologia da Universidade Federal da Bahia, Salvador, Brazil, 4Instituto Carlos Chagas, Fiocruz, Curitiba, Brazil

Wastewater-based epidemiology has emerged as a promising tool for pathogen monitoring, including the detection of endemic microbes and the identification of outbreaks earlier than clinical surveillance. This allows for timely interventions to prevent disease spread and reduce healthcare burdens. Additionally, frequent sampling can provide a comprehensive picture of disease spread, which can guide public health decision-making for intervention, control, and prevention of respiratory pathogen outbreaks. In this study, we aimed to evaluate the use of mNGS for wastewater-based surveillance to detect and monitor the circulation of pathogens in a population. Starting in March 2022, sewage samples were collected for six months from ten treatment plant locations in the city of Salvador, Bahia, Brazil, for respiratory pathogen surveillance. The genetic material of each sample was extracted followed by sequencing using the Respiratory Pathogen ID/AMR (RPIP) method (llumina, Inc.). The generated data were analyzed using CZID platform to identify the circulating pathogens. The most frequent pathogenic bacterial identified at a genus level in all sewage locations and at most evaluated time points were: Acinetobacter, Citrobacter, Enterococcus, Escherichia, Enterobacterium, Klebsiella, Moraxella, Pseudomonas, Salmonella and Streptococcus. Vibrio cholerae was identified in two locations and at two time points calling attention to monitoring the possible emergence of this infection. The only pathogenic virus identified was HHV-4 in one location and time point. RPIP sequencing also revealed the prevalence of various types of antimicrobial resistance genes (AMR) demonstrating the antibiotic profile of the population. Our findings provide valuable insights into the potential of wastewater-based surveillance as a complementary tool for respiratory pathogen surveillance, which could help inform public health decision-making and support outbreak control efforts.

Cholera Outbreak in the Mifi Health District of Cameroon, A Case-Control Study, July 2022

Ncham Evaristus Ngong1, Florence Eloula Estelle Basa1, Patricia Mendjirme2, Etoudi Evouna Antoine De Padoue3, Evouna Armel Mbang3
1Cameroon Field Epidemiology Training Program, Yaoundé, Cameroon, 2National Tuberculosis Control Program, Yaounde, Cameroon

Since 2018, Cameroon has experienced a resurgence of cholera epidemics. On May 15th, 2022, four cholera cases were confirmed in the Mfi Health District (MHD). By July 5th, there were 82 cases in 14 out of 20 health areas (HAs) of the district and the case fatality rate (CFR) was 3.7%. We conducted an investigation to identify cholera risk factors and evaluate the cholera surveillance system (SS) in the MHD. We conducted a 1:2 matched case-control study in the MHD in July 2022. Cases were patients with acute watery diarrhea having an epidemiological link with a confirmed case from May 5th to July 12th, 2022, or who were positive on culture. We identified cases by reviewing registers in health facilities and from the regional line list. We selected controls in cases’ neighborhoods. We collected data using a semi-structured questionnaire and analyzed it using Epi Info. We evaluated the SS using the CDC updated guidelines. We identified 172 cases in 15 HAs. Kouougouo (41/172, 24%), Famla (35/172, 20%), and Djeleng (26/172, 15%) were the most affected HAs. The median age of cases was 32 years (4 months – 85 years) and for controls was 31 years (4 months – 87 years). The attack rate was 0.39%, and CFR was 2.33% (4/172). Drinking untreated water (OR=13.1; 95% CI:2.3-75.1) and not washing hands with soap (OR=4.7; 95% CI:1.6-13.7) were identified as risk factors. Among staff, 83% (24/29) mastered cholera case definitions and found notification forms easy to fill. Notification forms were available in 28% (8/28) health facilities, and 80% (26/29) of staff considered cholera surveillance part of their routine activities. The cholera epidemic in the MHD affected 75% of HAs. Drinking untreated water and poor hand hygiene were associated with cholera. The SS was acceptable, simple, unstable, and not sensitive. Sensitizing the population on water purification and hand hygiene could limit the spread of cholera. 

Controlled Before-and-After Study of a Multi-Modal Hand Hygiene Intervention in Healthcare Facilities in Cambodia

Elisabeth Tadiri1, Leang Supheap2, Naisim Sum3, Senghort Ret3, Channara Thea4, Bernice Sarpong5, Robert Dreibleib6
1LSHTM, London, United Kingdom, 2NHPI, Phnom Penh, Cambodia, 3WaterAid Cambodia, Phnom Penh, Cambodia, 4WaterAid Australia, Melbourne, Australia

Hand hygiene (HH) is a critical component of infection prevention and control in healthcare facilities. However, intervention models to improve HH behavior in low resource settings are limited. Building on previous learnings from Cambodia, we adapted and tested a multimodal intervention
to improve HH among healthcare workers (HCW) during childbirth in referral hospitals in Kampong Cham Province, Cambodia. The intervention included a low-dose high-frequency participatory training, installation of HH facilities, visual cues and nudes, and a system of peer support among midwives. The intervention was tested in a controlled before-and-after study (3 intervention facilities, 3 control) and lasted approximately 4 months. Across the study period we observed 92 births for a total of 801 events requiring aseptic technique (hands washed with soap, clean gloves worn, and potential recontamination avoided). HH of HCWs was classified at each event into three categories: adequate HH (aseptic technique maintained), inadequate HH (clean gloves worn, but hands not washed with soap), or HH invalidated. In control facilities, at baseline, only 12% of all observed aseptic procedures were conducted with adequate HH, dropping to 8% at endline; 21% were performed with inadequate HH at baseline, increasing to 23% by endline. In intervention facilities, 5% of all observed aseptic procedures were conducted with adequate HH, increasing to 13% at endline; 32% were performed with inadequate HH at baseline, which dropped to 22% at endline. In a differences-in-differences logistic regression model, after controlling for repeated observations on the same mother and shift time of HCWs, the odds that a HCW would perform an aseptic procedure under adequate HH was 5.3 times higher in intervention facilities compared to control facilities [CI 1.20-23.62; p=0.029]. Although absolute improvement in adequate HH was low, the observed effect suggests the potential for a multimodal behavior change intervention to improve HH among HCWs. Forthcoming analyses will explore how sequencing of aseptic events and additional factors are associated with adequate HH in this context.

Factors Influencing Household Wash Practices

Andrea L. Smith, Alessandra Bazzano, Emily W. Johansson, Udochisom Anaeba, Paul L. Hutchinson
Tulane University, New Orleans, LA, United States

Healthy WASH behaviors are key to reducing the burden of childhood diarrheal disease, which in Nigeria remains a leading cause of child mortality. Lack of WASH infrastructure is a serious hurdle; however, low utilization of improved water and sanitation sources poses a significant threat to improving child health outcomes through WASH interventions. In Nigeria, particularly in the northwest region, use of improved WASH is low among households, and there is conflicting evidence regarding the relative importance of different factors driving healthy WASH practices. Literature reviews have indicated that WASH behavior change models overemphasize psychosocial factors, neglecting environmental context and technological features of WASH infrastructure; other studies indicate that psychosocial factors indeed play a larger role in behavior change and contextual factors contribute very little. Undoubtedly, WASH behaviors are multidimensional and there is sparse information on potential behavioral determinants within the northwestern Nigerian context. Therefore, this study examines a spectrum of contextual, psychosocial, and technological factors quantifying their association with using improved water sources and sanitation facilities. Data were collected in September 2019 using an interviewer-administered, cross-sectional survey. A stratified, multi-stage sampling strategy targeted pregnant women and non-pregnant women with a child under two years. Respondents were asked about access to WASH resources and WASH practices, prevalent cases of under-two diarrhea, and diarrhea-associated ideations. Crude bivariate estimates are calculated for the water and sanitation outcomes over each contextual, psychosocial, and technological factor. Mixed-effects logistic regression models are used to identify potential determinants of health WASH practices. The results of this study could have important implications for the programmatic development of WASH interventions by informing behavior change strategies for the northwestern region of Nigeria, particularly among households with children under two years.

Behavioral Determinants of Arsenic-Safe Water Use Among Great Plains Indian Nation Private Well Users: Results from the Community-Led Strong Heart Water Study Program

Kelly Endres, Tracy Zacher, Francine Richards, Lisa Bear Robe, David Harvey, Lyle G. Best, Reno Red Cloud, Annabelle Black Bear, Steve Ristau, Dean Aurand, Leslie Skinner, Christa Curvy, Marie Gross, Elizabeth D. Thomas, Ana Rule, Kellogg Schwab, Lawrence H. Moulton, Marcia O'Leary, Ana Navas-Acien, Christine Marie George
1Johns Hopkins Bloomberg School of Public Hlth, Maryland, MD, United States, 2Missouri Breaks Industries Research Inc., Eagle Butte, SD, United States, 3Indian Health Service, Rockville, MD, United States, 4Environmental Resource Department, Ogala Sioux Tribe, Rapid City, SD, United States, 5Missouri Breaks Industries Research, Inc Eagle Butte, SD, United States, 6Mid Continent Testing Labs, Inc, Rapid City, SD, United States, 7Missouri Breaks Industries Research, Inc, Eagle Butte, SD, United States, 8Columbia University Mailman School of Public Health, New York, NY, United States

The objective of this study was to evaluate the behavioral determinants associated with exclusive use of arsenic-safe water in the Strong Heart Water Study (SHWS) program. The SHWS is a randomized controlled trial designed to reduce arsenic exposure among private well users in American Indian Great Plains communities. All households received point-of-use arsenic filters at baseline and were followed for 2 years. Behavioral determinants selected were those targeted during the development of the SHWS, and were assessed at baseline and follow-up. Exclusive use of arsenic-safe water for drinking and cooking at follow-up was associated with higher self-efficacy for accessing local resources to learn about arsenic (OR: 5.19, 95% CI: 1.48-18.2) and higher self-efficacy to resolve challenges related to water arsenic using local resources (OR: 3.11, 95% CI: 1.11-8.17). Higher commitment to use the arsenic filter faucet at baseline was a significant predictor of exclusive arsenic-safe water use for drinking (OR: 32.6, 95% CI: 1.42-746.7) and cooking (OR: 15.90, 95% CI: 1.33-189.5) at follow-up. Over the study period, the SHWS significantly increased perceived vulnerability to arsenic exposure, self-efficacy, descriptive norms, and injunctive norms. Changing one’s arsenic filter cartridge after installation was associated with higher self-efficacy to obtain arsenic-safe water for drinking (OR: 6.22, 95% CI: 1.33-29.1) and cooking (OR: 10.65, 95% CI: 2.48-45.68) and higher perceived vulnerability of personal health effects (OR: 7.79, 95% CI: 1.17-52.0) from drinking arsenic-unsafe water. The SHWS conducted a theory-driven approach for intervention development and evaluation that allowed for behavioral determinants to be identified that were associated with the use of arsenic-safe water and changing one’s arsenic filter cartridge. These results demonstrate that theory-driven, context-specific formative research can influence behavior change interventions to reduce water arsenic exposure. The SHWS can serve as a model for the design of theory-driven intervention approaches that engage communities to reduce arsenic exposure.

Microplastics Exposure and Their Association with Diarrhoea, Growth, and Development of Children in Mali

1University of sciences, Techniques and Technologies of Bamako, Bamako, Mali, 2School of Geography, Earth and Environmental Sciences, University of Birmingham, Birmingham, United Kingdom, 3Institute of Applied Health Research, University of Birmingham, Birmingham, United Kingdom

Micro- and nanoparticles (MNPs) are omnipresent in the natural and urban environment. Despite progress in identifying MNPs pollution sources, as well as their presence in water, drinking water, food and soils, the accumulation and exposure by people, consequences for health effects have not been
explored epidemiologically or for childhood diseases. Prior literature indicates the ingestion of MnP by the adult body, and into the gut has a correlation to cause gut inflammation in relation to MnP concentration found in stool. Our study aims to explore this further, by assessing MnP in drinking water and from the stool of children aged 6-36 months in Mali. We are also examining the presence of any geographical gradient (regional, urban vs. rural). Cross-sectional random samples from children’s drinking water (n=110) and stools (n=100) were taken from children (6-36 months) who were sampled in 60 urban and 60 rural communities during the baseline data collection of the MaaCiwara 120 cluster RCT. Fluorescence microscopy and Raman spectroscopy were conducted to identify and quantify MnP abundance, size and polymer type, respectively. Results show that MnP are detected in both water (>90%) and stool in Mali (>60%). Water samples indicate an increased level of MnP in urban areas with a higher frequency of fibre-type MnP found. In stool both fibre and fragments are found, with abundant polymer types identified as: Polyvinyl Chloride (PVC), Polyethylene terephthalate (PET) and Polyamide (PA). Our results highlight that MnP pollution is prevalent in drinking water and stool. Further findings and implications to health are to be presented at the conference. Presence of MnP in young children’s drinking water and stool is of concern, warranting further investigation at larger scale.

**BEHAVIORAL AND ENVIRONMENTAL FACTORS ASSOCIATED WITH SELF-REPORTED DIARRHEA AND ENTERIC PATHOGEN DETECTION IN INFANTS DISCOVERED BY COMPARING HOUSEHOLD CLUSTERS WITH DISTINCT LIVING CONDITIONS**

**Kelly K. Baker**, Daniel Sewell, Jane Mumma, Oliver Cumming, Robert Dreibelbis, Sheillah Simiyu

1University of Iowa, Iowa City, IA, United States, 2Great Lakes University of Kisumu, Kisumu, Kenya, 3London School of Hygiene and Tropical Medicine, London, United Kingdom, 4African Population and Health Research Center, Nairobi, Kenya

Designing effective strategies to prevent enteric infections in disease endemic populations is challenging due to the diverse viral, bacterial, and parasitic pathogen species transmitted by multiple host species through many interconnected transmission pathways. This study used data from the Safe Start cluster randomized controlled trial in Kisumu, Kenya to examine how household environmental and behavioral conditions influenced self-reported 7-day diarrhea and the detection of enteric pathogens in feces for enrolled infants. Correlation between exposure conditions and between pathogens in infant feces was common, so latent class analysis (LCA) was used to identify 5 distinct Clusters of participants who shared similar living conditions to each other. Correlation in pathogen detection, measured using a 19-pathogen molecular diagnostic tool, was examined as species diversity in feces at 6 and 9 month age timepoints. After adjusting for intervention effects, logistic regression analysis of 7-day diarrhea in infants between Cluster groups showed that diarrhea was significantly less likely at 6 months and 9 months of age for Clusters 1, 2, and 5 compared to Cluster 3, which had the lowest frequency of food-related handwashing. Regression comparisons of pathogen diversity in infants by Cluster identified Cluster 4 as having significantly higher rates of pathogen diversity compared to other Clusters at 6 months age, although these differences disappeared by 9 months age. Norovirus GII and typical Enteropathogenic E. coli contributed most to pathogen diversity at 6 months of age. Cluster 4 was unique from other Clusters in having the highest numbers of households using unimproved latrines, owning domestic animals, living on dirt floors, using water and pasteurized cow milk for infant feeding, and low rates of handwashing after defecation. Results suggest that in high transmission environments improving food-related handwashing behaviors may be effective for reducing diarrhea burden in infants but reducing infection rates may require comprehensive upgrades of multiple living conditions and hygiene behaviors in targeted households.

**TARGETING INFLAMMATION IN CHILDHOOD MALNUTRITION: A RANDOMIZED CONTROLLED CLINICAL TRIAL OF ADDITION OF FISH OIL TO THE STANDARD OF CARE NUTRITIONAL INTERVENTION**

**Mark Muchina**, Mary Inziani, Beatrice Olack, Mary Muriu, Finnley Osuna, Chris L. Melby, Margaret Mbuchi, Thaddeus Egondi, Linet Ouma, Ruka Kibaya, Adam Chicco, Asma Omar, Peter C. Melby, Phelgonia Otieno

1Kenya Medical Research Institute, Nairobi, Kenya, 2Colorado State University, Ft. Collins, CO, United States, 3Colorado State University, Ft. Collins, CO, United States, 4University of Texas Medical Branch, Galveston, TX, United States

Systemic inflammation and impaired intestinal barrier function contribute to the pathogenesis of malnutrition. Lipid (energy)-dense supplements are commonly used to treat malnutrition, but they are rich in pro-inflammatory omega-6 (n-6) fatty acids (FA). We hypothesized that adding anti-inflammatory n-3 FA (fish oil) to the standard nutritional intervention could reduce malnutrition-associated inflammation. We conducted a double-blind, randomized, controlled trial among children 9-24 months of age with moderate acute malnutrition (MAM), living in Kibera slums,
Nairobi. Children with MAM were randomized to receive a 12-week daily supplement of either Corn-Soy flour Blend (CSB) with soybean oil plus an additional 2 mL of fish oil (1136 mg total n-3) (n=40) or CSB with soybean oil (standard-of-care) plus an additional 2 mL of soybean oil (n=40; CSB-VO). A convenience sample of 20 healthy controls was also evaluated. The primary endpoint was a change in markers of inflammation and intestinal barrier function. Secondary endpoints included growth recovery and plasma n-3 FA concentrations. Growth recovery by 12 weeks was >92% in both study arms. Comparison of serum inflammatory markers at the end of the treatment to baseline (pre-intervention) showed that IL-1b, LPS binding protein, lipocalin, CXCL1, and CXCL2 were significantly reduced after the intervention. There was also post-intervention improvement in markers of intestinal barrier function, including soluble CD14 (p<0.0001), intestinal FA binding protein (p<0.05), and bacterial LPS (p<0.0001). Plasma FA analysis revealed that children with MAM had lower levels of plasma total n-3 FAs and a lower n-3/n-6 ratio compared to healthy controls (p<0.001). Children from both study arms fully and equally recovered from the n-3 deficiency after the nutritional intervention. Recovery from MAM led to less inflammation, improved intestinal barrier, and normalization of plasma n-3 levels. However, improvement was not enhanced by supplemental fish oil, suggesting that the soybean oil supplies adequate precursor FA substrates for endogenous synthesis of long-chain n-3 FAs.

CHILD STUNTING FROM BIRTH TO AGE TWO: A LONGITUDINAL COHORT STUDY IN AMHARA, ETHIOPIA

Frederick G. B. Goddard1, Bezawit M. Hunegnaw2, Jonathan Luu3, Sebastien Haneuse4, Mesfin Zeleke5, Yahya Mohammed6, Chalachew Bekele7, Delaye Wubayehu Bekele8, Grace J. Chan9

1Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, MA, United States, 2Department of Pediatrics and Child Health, St Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia, 3Department of Biostatistics, Harvard T.H. Chan School of Public Health, Boston, MA, United States, 4Birhan HDSS, St Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia, 5Department of Obstetrics and Gynecology, St Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia, 6Department of Medicine Critical Care, Boston Children's Hospital, Harvard Medical School; Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, MA, United States

The Sustainable Development Goals set out an ambitious goal to end all forms of malnutrition by 2030. Although there has been a reduction in stunting (length-for-age Z score <-2SD), the prevalence of malnutrition in Ethiopia is still high. To improve nutritional outcomes, accurate data are needed to determine key time points for child growth, vulnerabilities and potential for recovery. The Birhan maternal and child health cohort in Amhara, Ethiopia, enrolled children between December 2018 and November 2020, and followed them through age 2. On scheduled visits at home and in health facilities, children's lengths were measured and recorded to the nearest 0.1 cm. We investigated the burden, incidence and reversal of stunting at birth, four weeks, six, 12 and 24 months. We enrolled 4,354 children under the age of two, among which 3,961 (91.0%) had their length measured at least once and were included in this study. Our findings indicate that median population-level length in this population is consistently below global standards from birth to age two. Growth velocity was slowest compared to global standards during the neonatal period and after children reached six months of age. The observed prevalence of stunting was 16.5% at birth and highest at age two (57.9%). Among all newborns who were not stunted at birth, 24% developed stunting within the first month of life. The incidence of stunting increased over time and was highest between the ages of 12 to 24 months (52.4%) followed by the 6 to 12 months period (32.6%). Rates of stunting reversal were highest between the ages of birth to six months. Among children who were already stunted at birth and thus had a chance to reverse stunting later in life, 65.4% were able to do so by 6 months of life and 58.3% reversed by the end of the first year of life. Overall, the evidence from this study highlights a dynamic process of incidence and reversal resulting in a chronically malnourished population with much of the burden driven by growth faltering during the neonatal periods as well as after 6 months of age. To end all forms of malnutrition, growth faltering in populations such as that in young children in Ethiopia needs to be addressed.

MICRONUTRIENT STATUS DURING PREGNANCY IS ASSOCIATED WITH YOUNG CHILD TELOMERE LENGTH


1Department of Epidemiology, Mailman School of Public Health, Columbia University, New York, NY, United States, 2Infectious Diseases Division, International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh, 3Division of HIV, Infectious Diseases, and Global Medicine, University of California, San Francisco, San Francisco, CA, United States, 4Department of Epidemiology and Biostatistics, School of Public Health, University of California Berkeley, Berkeley, CA, United States, 5Department of Biochemistry and Biophysics, University of California San Francisco, San Francisco, CA, United States, 6Institute for Interdisciplinary Salivary Bioscience Research, University of California Irvine, Irvine, CA, United States, 7Department of Psychiatry & Behavioral Sciences, Sylvester Comprehensive Cancer Center, University of Miami, Miami, FL, United States, 8VIMLab, Wiltstaett, Germany, 9Department of Biobehavioral Health, Pennsylvania State University, University Park, PA, United States, 10Division of Infectious Diseases and Geographic Medicine, Stanford University, Stanford, CA, United States, 11Division of Community Health Sciences, School of Public Health, University of California Berkeley, Berkeley, CA, United States, 12Institute for Global Nutrition, University of California Davis, Davis, CA, United States

Antenatal nutrition, cortisol, inflammation, and estriol may impact child telomere length (TL) and health. In rural Bangladesh, we explored associations between these antenatal factors and child TL. In the WASH Benefits trial, maternal nutrition status (vitamin D, iron (ferritin and soluble transferrin receptor), and vitamin A (retinol-binding protein (RBP)); inflammation (C-reactive protein, alpha-1-acid glycoprotein, and 13 additional cytokines), and morning cortisol and estriol were analyzed during the first two trimesters. Children’s whole blood relative TL was measured at Year 1 (age 14 months) and Year 2 (age 28 months) using qPCR. We estimated associations using generalized additive models, adjusting for potential confounders, reporting estimated mean differences between the 75th and 25th percentiles of each exposure distribution. Telomeres were analyzed from 466 children at Year 1, 515 children at Year 2, and 403 children between Years 1 and 2. Maternal vitamin A deficiency (RBP < 0.70 μmol/L) during pregnancy was not significantly associated with child TL at Year 1 (adjusted difference: -0.42 z-score of T/S ratio, 95% CI: -1.17, 0.33), significantly associated with child TL at Year 2 in the same direction (adjusted difference: -0.75, 95% CI: -1.42, -0.09), and not significantly associated with change in TL between Years 1 and 2 (adjusted difference: -0.54, 95% CI: -1.35, 0.27). Higher vitamin D levels (nmol/L) were associated with less TL lengthening from Years 1 to 2 (adjusted difference: -0.49, 95% CI: -0.96, -0.02). We found no associations between iron status, cortisol, estriol, CRP, AGP, or cytokines and TL nor change in TL. These results suggest a potential association between vitamin A and vitamin D status during pregnancy and subsequent child TL. Because of increased cell division during the postnatal period, maternal vitamin A and D levels could affect children’s TL dynamics and long-term health.
 Poor immune function increases children’s risk of infection and mortality. Several maternal factors during pregnancy may affect infant immune function during the postnatal period. We conducted observational analyses within the WASH Benefits Bangladesh randomized controlled trial. We measured biomarkers in 575 pregnant women and postnatally in their children. Maternal biomarkers measured during the first and second trimester of pregnancy included: nutrition status via vitamin D (25-hydroxy-D$_{3}$), ferritin, soluble transferrin receptor (sTfR), retinol binding protein (RBP); cortisol; estriol. Immune markers were assessed in pregnant women at enrollment and their children at ages 14 and 28 months, including: C-reactive protein (CRP), alpha-1-acid glycoprotein (AGP), and thirteen cytokines (including IFN-γ). We generated a standardized sum score of log-transformed cytokines. We analyzed IFN-γ individually because it is a critical immunoregulatory cytokine. All outcomes were pre-specified. We used generalized additive models and reported the mean difference and 95% confidence intervals at the 25th and 75th percentiles of exposure distribution. At child age 14 months, concentrations of maternal RBP were inversely associated with the cytokine sum score in children (-0.34 adjusted difference between the 25th and 75th percentile [95% confidence interval -0.61, -0.07]), and maternal vitamin A deficiency was positively associated with the cytokine sum score in children [0.12 [0.13, 1.92]]. At child age 28 months, maternal RBP was positively associated with IFN-γ in children (0.07 [0.01, 0.14]) while maternal vitamin A deficiency was negatively associated with child AGP (-0.07 [-0.13, -0.02]). Maternal iron deficiency was associated with higher AGP levels in children at age 14 months (0.13 [0.04, 0.23]), and maternal sTfR concentrations were positively associated with child CRP levels at age 28 months (0.18 [0, 0.36]). Maternal deficiencies in vitamin A or iron during the first two trimesters of pregnancy may shape the trajectory of child immune status.

**SEROPREVALENCE FOR NOVEL ORAL POLIOVIRUS VACCINE TYPE 2 (nOPV2) FOLLOWING OUTBREAK RESPONSE IN LIBERIA: FINDINGS, IMPLICATIONS AND FUTURE DIRECTIONS**

**MICRONUTRIENT STATUS DURING PREGNANCY IS ASSOCIATED WITH CHILD IMMUNE STATUS IN RURAL BANGLADESH**

**FUTURE DIRECTIONS**

**RESPONSE IN LIBERIA: FINDINGS, IMPLICATIONS AND VACCINE TYPE 2 (nOPV2) FOLLOWING OUTBREAK SEROPREVALENCE FOR NOVEL ORAL POLIOVIRUS**
achieved with >97% accuracy using ~100 SNPs. Our data suggest that TCBZ-R is a polygenic trait, and provide valuable resources for developing a genetics-based resistance surveillance for sustainable control.

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NATURAL VARIATION IN A PARASITIC FLATWORM ION CHANNEL UNDERPINS DIFFERENTIAL SENSITIVITY OF PARASITES TO PRAZIQUANTEL

Claudia M. Rohr, Daniel J. Sprague, Sang-Kyu Park, Nicholas J. Malcolm, Jonathan S. Marchant
Medical College of Wisconsin, Milwaukee, WI, United States

The drug praziquantel (PZQ) is the primary treatment for infections caused by parasitic flatworms. A target for PZQ was recently identified in schistosomes, a transient receptor potential ion channel in the melastatin subfamily (TRPMPZQ); however, little is known about the properties of TRPMPZQ in other flatworms. TRPMPZQ orthologs were scrutinized in all currently available parasitic flatworm genomes and functionally profiled. TRPMPZQ is present in all parasitic flatworms, and the consensus PZQ binding site is well conserved. Three loci of variation were identified across the parasitic flatworm TRPMPZQ pocketome, including an acidic residue in the TRP domain that acts as a gatekeeper residue impacting PZQ residency within the TRPMPZQ ligand binding pocket. Functional profiling of trematode, cestode, and free-living flatworm TRPMPZQ orthologs revealed differing sensitivities to PZQ, matching the varied sensitivities of flatworms to PZQ documented clinically. In trematodes and cyclophyllidean cestodes, which display high sensitivity to PZQ, the gatekeeper TRP domain residue is an aspartic acid, allowing for nemoral activation by PZQ. However, the presence of a glutamic acid residue, found in pseudophyllidean cestode TRPMPZQ, was associated with lower PZQ potency. Functional profiling of a pseudophyllidean channel revealed micromolar potency of PZQ at Spirometra erinaceieuropaei TRPMPZQ. The definition of these different binding pocket architectures explains why PZQ shows high therapeutic efficacy against specific fluke and tapeworm infections. Effort to identify new therapeutics that tolerate this TRP domain residue would be immensely valuable if analogous variation were to ever occur in natural trematode populations, and for the development of targeted therapies towards specific infections.

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CHARACTERIZATION OF AN ENDOGENOUS ION CHANNEL ACTIVATED BY PRAZIQUANTEL WITHIN A LIVE, ADULT SCHISTOSOME

Evgeny G. Chulkov, Jonathan S. Marchant
Medical College of Wisconsin, Milwaukee, WI, United States

Praziquantel (PZQ) serves as the main clinical treatment for schistosomiasis, and other infections caused by parasitic flatworms. PZQ causes a rapid, spasitic contraction of schistosomes ex vivo that results from PZQ action on a parasite transient receptor potential ion channel known as TRPMPZQ. While the properties of TRPMPZQ have been characterized following on a parasite transient receptor potential ion channel known as TRPMPZQ, we recently resolved for the first time the properties of an endogenous ion channel activated by PZQ within a live adult schistosome, and the properties of this ion channel are consistent the properties of TRPMPZQ in vitro.

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CHROMOSOME LEVEL ASSEMBLY OF BAYLISASCARIS PROCYONIS GENOME

William Sears1, Sriveny Dangoudoubiyam2, Sasikethan Bernuru3, Pedro Gazzinelli-Guimarães4, Myndi Holbrook5, Craig Martens6, Kent Barbian7, Kimmo Virteneva7, Shellsie Brooks8, Gerard Bouffard9, Thomas Nutman1
1NIAD, Bethesda, MD, United States, 2Purdue, West Lafayette, IN, United States, *NIAD, Hamilton, MT, United States, *NISG, Rockville, MD, United States

Baylisascaris procyonis, the raccoon roundworm, is a zoonotic nematode causing a visceral larval migrans syndrome complicated by severe neurological manifestations that oftentimes are fatal. Despite the severity associated with human infection, little understanding of the molecular nature of this parasite is available nor has there been a full-length genome assembled. To this end, nanopore long-read sequencing was employed to generate a chromosome level genome assembly of B. procyonis. Roadside racoon carcass was dissected to obtain B. procyonis females. The uteri of these females were removed, and eggs allowed to embryonate in culture after which in vitro hatching led to the production of viable L3 larvae. High molecular weight (HMW) DNA was extracted from these L3 larvae, and 2 sequencing rounds were performed. The first used the MinION sequencer with an ultra-high molecular weight (UHMW) protocol; the second used the PromethION platform with a nanopore ligation sequencing kit. Basecalling was conducted with Guppy, and assemblies were created using Fyfe, SMARTdenovo, Shasta, and Canu. Assembly contiguity was improved with Purge Haplotigs and Quickmerge, and 3 rounds of Pilon polishing was performed. The final assembly comprised 51 contigs. The B. procyonis assembled genome has a total length of ~250MB organized. The assembly has an N50 of 7,029,608, and a GC content of 37%. Repetitive sequences, as identified by RepeatModeler and RepeatMasker, composed 6% of the total nucleotide content and 5’ and 3’ telomeric sequences could be identified. We identified ~19,000 genes with the BrakeR annotation pipeline that incorporated transcriptomic data obtained from RNAseq on the L3s and the adults. This resulting genome will support further investigation into host-pathogen interactions and expand the capacity of metagenomic diagnostics to detect B. procyonis in clinical cases.

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A UNIVERSAL METABARCODING PIPELINE AND HOST SIGNAL REDUCTION METHOD FOR CHARACTERIZATION OF VERTEBRATE SYMBIONT/PARASITE ASSEMBLAGES

Leah Ann Owens1, Sagan Friant2, Bruno Martorelli Di Genova3, Laura J. Knoll4, Monica Contreras5, Oscar Noya5, Maria G. Dominguez-Bello6, Tony L. Goldberg7
1University of Wisconsin-Madison, Madison, WI, United States, 2The Pennsylvania State University, University Park, PA, United States, 3The University of Vermont, Burlington, VT, United States, 4Venezuelan Institute of Scientific Research, Caracas, Venezuela, Bolivarian Republic of, 5Universidad Central de Venezuela, Caracas, Venezuela, Bolivarian Republic of, 6Rutgers University, New Brunswick, NJ, United States

Our understanding of host-associated bacterial, archaeal, and fungal communities far exceeds our understanding of eukaryotic symbiont/parasite communities. Whereas metabarcoding of prokaryotes and fungi are commonplace, no single method for eukaryotic symbiont/astmh.org
parasite metabarcoding has been adopted due to issues with primer complementarity and high off-target read abundance. We assessed published eukaryotic symbiont/parasite metabarcoding protocols and created new primers to recognize all symbionts/parasites of vertebrates. In silico PCR and single organism PCR of published primer sets (n = 22) alongside our new primers (n = 4) showed that only our new primers successfully amplified all clades of symbionts/parasites and had the highest overall taxonomic coverage. We then created a novel mock community standard of 16 cloned parasite DNAs and used it to show that one of our new primer sets more closely recovered the underlying community than any other. When applied to human (n = 12) and nonhuman primate (n = 40) clinical samples, our new protocol (Vertebrate Eukaryotic endoSymbiont and Parasite Analysis, or VESPA) outperformed the “gold standard” method of microscopy, with 51.3 % of identifications made by VESPA alone. VESPA identified taxa not found with microscopy, resolved a cryptic species complex not resolved by microscopy, and revealed greater prevalence and richness of parasitic organisms than microscopy. To reduce off-target PCR amplification, we designed a novel CRISPR-Cas9 based method that selectively digests host DNA. When applied to blood and tissue samples, our method resulted in a mean 92 % decrease in host read abundance compared to no treatment, a 61 % decrease compared to the most commonly published blocking method, and allowed for detection of hemoparasite infections that would otherwise have been missed.

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THE PROGNOSTIC POTENTIAL OF BIOMARKERS ASSOCIATED WITH FILARIAL LYMPEDEMA DEVELOPMENT

Derrick Adu Mensah1, Linda Batsa Debrah1, Vera Serwa Opoku1, Jubin Osei Mensah2, Charles Gyasi3, Patricia Jebett Korir4, Manuel Ritter5, Tomabu Adjobine6, Ute Klarmann-Schulz7, Achim Hoerau8, Alexander Yaw Debrah9

1Department of Clinical Microbiology, Kwame Nkrumah University of Science and Technology, KUMASI, Ghana, 2Department of Veterinary Pathobiology, Kwame Nkrumah University of Science and Technology, KUMASI, Ghana, 3Institute for Medical Microbiology, Immunology and Parasitology, University Hospital Bonn, Germany/German Center for Infection Research (DZIF), partner site Bonn-Cologne, Germany, Bonn, Germany, 4Institute for Medical Microbiology, Immunology and Parasitology, University Hospital Bonn, Germany, Bonn, Germany, 5Department of Medical Diagnostics, Kwame Nkrumah University of Science and Technology, KUMASI, Ghana

Lymphatic Filariasis causes chronic morbidity, which usually manifests as lymphedema (LE) or hydrocele. MDA to control LF infection primarily does not treat patients with chronic morbidities. Such people will continue to suffer from the disease even after a community is declared “LF-free”. Thus, a hygiene-based LE Morbidity Management and Disability Prevention (MMDP) plan has been established by the WHO to reduce LE morbidity. Nonetheless, there are no established biomarkers for LE progression and thus predicting the outcome of LE after MMDP measures have been initiated has become difficult. Lymphangioplasminogen molecules such as VEGF-C, sVEGFR3, TIMP-1, etc. are reported to be involved in LE development. The study aimed at assessing the prognostic potential of biomarkers associated with LE development. A total of 175 LE-affected individuals from the Upper East Region of Ghana were enrolled, strictly trained, and resourced with hygiene-based LE morbidity management materials, and followed up for 24 months. The Friedman test with Dunn’s posthoc comparison, receiver operating characteristic curve, and logistic analyses were performed to estimate the association between baseline and follow-up biomarker levels and LE outcomes. Overall, 26.8% saw LE improvement, while 96.6% lacked progression. The mean plasma levels of VEGF-C, sVEGFR3, CEACAM-1, MMP-2, and IGF-1 in study participants who experienced LE progression rose, with a rise in one marker up-regulating the others. TIMP-1 up-regulation resulted in lower levels of VEGF-C, sVEGFR3, CEACAM-1, MMP-2, and IGF-1 in participants who had LE improvement. Reduced TIMP-1 levels, on the other hand, resulted in up-regulation and over-expression of these biomarkers leading to lymphatic vessel dilation, extracellular matrix remodeling, and LE progression. To conclude, the study demonstrated that lymphangiogenic biomarkers have a prognostic value in LE pathology and could be used for prognosis after MMDP measures have been initiated. In mitigating the best care for LE-affected individuals, efforts aimed at drug development can be focused on increasing TIMP-1 levels in blood plasma.

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MULTIANTIGEN PRINT IMMUNOASSAY: TOWARDS A GLOBALY ACCESSIBLE SEROLOGICAL DIAGNOSIS OF NEUROCYSTICERCOSIS

Luz M. Toribio1, Carolina Guzman1, Sassan Noazin2, Herbert Saavedra3, Javier Bustos1, Sukwan Handali1, Hector H. Garcia1

1Center for Global Health, Universidad Cayetano Heredia, Lima, Peru, 3Department of International Health, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD, United States, 4Instituto Nacional de Ciencias Neurologicas, Lima, Peru, 5Division of Parasitic Diseases, Centers for Disease Control and Prevention, Atlanta, GA, United States

Neurocysticercosis (NCC) is the most common helminthic infection of the human central nervous system. The antibody detection assay of choice is the enzyme-linked immunoelectrotransfer blot assay (EITB, Western blot) using lentil-lectin purified parasite antigens, an immunocassay with exceptional performance in clinical settings. However, its use is mainly restricted to a few research laboratories because the assay is labor-intensive and requires sophisticated equipment, expertise, and needs large amounts of parasitic material. We report a new multiantigen print immunocassay (MAPIA), which consists in directly spraying of antigen into a nitrocellulose membrane, that overcomes most of these barriers by using recombinant antigens that correspond to the three diagnostic protein families GP50, T24/42, and 8kDa. We initially compared the performance of five antigen combinations in a subset of defined samples in the MAPIA format. After selecting the best performing format, 148 archive serum samples were tested, including 40 from individuals with parenchymal NCC, 40 with subarachnoid NCC and 68 healthy controls with no evidence of neurologic disease. MAPIA using three antigens (rGP50 + rT24H + sTs14), was highly efficient for antibody detection in NCC, detecting 39/40 parenchymal NCC cases and 40/40 subarachnoid cases in relation to the EITB, with a specificity of 98.53% (67/68). Thus, MAPIA using three recombinant and synthetic antigens is a simple, reproducible, and economical tool with a performance equivalent to the LLGP-EITB assay; even more, the EITB protein family responses were concordant with its recombinant form responses. We propose the MAPIA test (rGP50, rT24H, and sTs14) as a new affordable, globally available diagnostic tool for detecting antibodies in serum, providing a suitable option to overcome the availability limitations of the current standard assay, and that could support the clinical diagnosis of NCC or contribute to epidemiological studies in endemic populations.

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EPIDEMIOLOGY OF THE HOOKWORM, NECTATOR AMERICANUS, IN BEPOSO, GHANA: APPLICATION OF MOLECULAR AND SEROLOGIC METHODS TO DEFINE THE PREVALENCE OF INFECTION

Savanna Randi1, Irene O. Owusu Donkor2, Jeffrey Sumboh3, Dickson Osabutay4, Rahmat bin Yusuf5, Francis Appiah-Twum2, Amanda Lampetey2, Christina Paraggio3, Lisa M. Harrison1, Debbie Humphries1, Michael D. Wilson2, Michael Cappello1

1Yale School of Public Health, New Haven, CT, United States, 2University of Ghana, Accra, Ghana, 3Yale School of Medicine, New Haven, CT, United States

In 2022, a study was initiated to characterize the epidemiology of human hookworm infection in Beposo, Ghana (Bono East Region). After obtaining informed consent, 1,047 subjects (age 3-100 years; 48% female) were enrolled and completed a questionnaire to assess demographic, behavioral and socioeconomic characteristics. Baseline hookworm prevalence was 28.6% (285/996) as measured by Kato Katz (KK) fecal microscopy, with 92% (262/285) of infections defined as light intensity by WHO standards.
The highest prevalence of infection was noted in subjects aged 11-20 years (33.5%) and in those over 60 years old (36.4%). Individuals who reported having taken anti-parasite medication fewer than 30 days before screening were more likely to have hookworm positive than those who did not (31.7% vs 25.3%; p=0.04). Subjects who reported regular use of water from boreholes were more likely to have hookworm than those using piped or well water (p<0.05), based on a y2 unadjusted analysis. Analysis of 110 fecal samples using a probe-based TaqMan RT-qPCR assay detected Necator americanus genomic DNA in 39% (22/56) of KK-negative samples, while 98% (63/64) of KK-positive samples were confirmed by qPCR. An ELISA assay was developed to quantify serum antibody (IgG) responses to larval protein extracts (LEX) or adult worm excretory/secretory proteins (ES) isolated from a Beposso strain of N. americanus recently adapted to an animal model. Preliminary data (n=227) showed that antibody levels to LEX (p<0.03) and ES (p<0.0001) both correlated with KK infection status, with an AUC of 0.77 derived from Receiver Operator Curve analysis of anti-ES IgG levels (p<0.0001). These data confirm the poor sensitivity of KK microscopy for measuring the prevalence of hookworm in endemic communities, especially those characterized by low-intensity infections. The study also demonstrates the potential for serologic testing as an accurate, high throughput and potentially cost-effective tool for monitoring the impact of mass drug administration on hookworm prevalence in endemic communities.

**BIOMETRIC DATA CAPTURE DURING SOIL-TRANSMITTED HELMINTHS AND SCHISTOSOMIASIS MASS DRUG ADMINISTRATION IN A LARGE-SCALE GESHIYARO PROJECT**

Ewnetu Firdawek Liyew, Melkie Chernet Leikun, Rosie Maddren, Roy Anderson

Ethiopian Public Health Institute, Addis Ababa, Ethiopia; Imperial College, London, London, United Kingdom

Neglected tropical diseases are a diverse group of infectious diseases that affect one billion people globally. The Geshiyaro Project is a seven-year intervention-based research program that aims to identify the optimal design of soil-transmitted helminths (STH) and schistosomiasis (SCH) elimination programs. The project assesses different combinations of Water, Sanitation, and Hygiene interventions and community-wide mass drug administration (MDA) in the Wolaita Zone of Ethiopia. This project uses biometric fingerprint technology to evaluate the impact of different treatment strategies and WaSH interventions. Biometric fingerprint technology enables accurate identification and tracking of participants who will receive deworming tablets during the Geshiyaro Project. To interrupt the transmission of STH and SCH, community-wide MDA is targeted with reaching 90% of the population at each round. Since 2019, Albendazole and praziquantel were administered across the Wolayita zone. During each treatment round, Geshiyaro census records were used to identify participants through a tiered hierarchy of methods; biometric identification, subject ID card identification, or name search of the database. The realities of fieldwork have highlighted issues with implementing electronic data capture. Over time, participants often lose their ID cards and swap them with family members leading to unreliable identification. Name searching is hindered primarily by the translation from the local dialect to English spellings, similarly named individuals in a village. Biometric registration and identification of participants begin to alleviate these named issues, however, come with their own caveats. For example, manual laborers and young children are unable to provide a valid fingerprint, due to “illegible” or inadequately-sized prints. Ensuring community acceptance of the technology required additional sensitization methods, beyond the scope of traditional MDA protocols. The Geshiyaro project has had the unique opportunity to integrate this novel biometric capture as part of a large-scale control program.

**RAPID SPREAD OF TRICHURIS TRICHIURA INFECTION FOLLOWING TREATMENT AMONG POPULATION LIVING IN RURAL AREAS OF GABON, CENTRAL AFRICA**

Jean Claude Dejon Agobé, Christian Chassem Lapue, Jean Ronald Edo, Jeanne Fréjus Zinsou, Yabo Josiane Honkpéhèdji, Romeo Bayodé Adegbité, Straversky Térence Boussougou-Sambe, Tamirat Gebru Woldearega, Benjamin Mordmüller, Ayôla Akôm Adegïrika

1Centre de Recherches Médicales de Lambaréné (CERMEL), Lambaréné, Gabon; 2Institut für Tropenmedizin, Universitätsklinikum Tübingen, Tübingen, Germany; 3Department of Medical Microbiology, Radboud University Medical Center, Nijmegen, Netherlands

Soil-transmitted helminths (STH) remain a public health issue in sub-Saharan countries. As their elimination is not yet possible in the region, the current WHO’s recommendations are the control of the disease burden, through large-scale treatments of at-risk populations and implementation of measures to reduce transmission. However, the effectiveness of those programs seems to depend on STH species. The objective of the present analysis was to assess the occurrence of different STH species before and after anthelmintic treatment, using the incidence rate (IR). The study was longitudinal. Participants were followed-up (FU) in 2 consecutive phases of 6 and 9 months. Stool samples were collected at baseline, at 6 and 15 months of FU. STH diagnosis was by Kato-Katz and Coproculture techniques. At the 6-month FU, all participants infected with STH were treated with albendazole 400mg once a day for three consecutive days. The IR was calculated before treatment among participants negative at baseline, and after treatment among participants negative or treated at month 6. We included 262 participants with a mean (SD) age of 12.1 (4.8). Of them, 53% tested positive for STH infection over the study course. Before treatment, the IR was 41 (95%CI: 28 - 55) cases per 100 person-years for any STH and 14 (95%CI: 6 - 26), 15 (95%CI: 6 - 29), and 13 (95%CI: 5 - 25) cases per 100 person-years for A. lumbricoides, T. trichiura, and hookworm, respectively. After treatment, a statistically significant increase in the IR was observed for T. trichiura (36 cases per 100 person-years, 95%CI: 27 - 47, p-value<0.001). For A. lumbricoides and hookworm infection, the IR following treatment was 15 (95%CI: 9 - 24) and 12 (95%CI: 7 - 20) cases per 100 person-years, respectively. In conclusion, our results indicate a rapid spread of T. trichiura infection in our community after treatment, with a potential effect on the effectiveness of programs implemented for the control of STH. This calls for further investigation of the epidemiology and morbidity of T. trichiura infection and for the assessment of the efficiency of those programs on T. trichiura specifically.

**NATURAL AND VACCINE-MEDIATED IMMUNITY TO NIPPOSTRONGYLUS BRASIILIENSIS**

Neima Briggs, Aurobind Vidyarthi, Joseph Craft

Yale University, New Haven, CT, United States

Hookworm infects over 220 million people worldwide. As a leading cause of iron-deficiency anemia in children and pregnant women, hookworm infection reinforces the cycle of poverty through growth stunting, physical and cognitive delays, and a decline in work performance. Vaccines remain a promising preventative strategy, with ongoing clinical trials of vaccine candidates against hookworm. However, one candidate, recombinant protein (r)Na-ASP-2, failed in trials due to an unanticipated preformed IgE-mediated systemic urticarial response to immunization in a cohort endemic for Hookworm. Two subsequent vaccine candidates, rNa-APR-1 and rNa-GST-1, needed to be recently re-formulated with additional co-stimulatory TLR-4 and TLR-9 agonists, in hopes of inducing a more robust and durable immune response. Given the substantial cost to bring a vaccine candidate to clinical trials targeting low-resource populations, a poor understanding of vaccine immunity against helminths may be detrimental to finding a successful candidate. It remains unclear how protective immunity develops after natural infection to Hookworm in certain animal models or how prior
infection influences the immune response to vaccination. We have adapted a murine model for Nippostrongylus brasiliensis, the rat hookworm, and have found that recurrent challenge in wild-type C57BL/6J mice induces near sterilizing immunity. RAG-1 deficient mice, which lack mature B or T cells, remain completely susceptible to repeated challenges. This will be a framework to compare the acquired immune pathway to natural infection as opposed to the human-relevant vaccine candidates rNa-GST-1 and rNa-ASP-2, which we have found induce at least partial protection, a 54% and 52% intestinal worm burden reduction, respectively when co-formulated with an Alum-based adjuvant. We have characterized the correlative roles of antigen-specific T cells (type 1, type 2, T follicular helper, germinal center, and T regulatory) and antigen-specific B cells (plasma cells, IgG1+, memory, and germinal center) in natural and vaccine-protected mice.

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WASTEWATER-BASED EPIDEMIOLOGY FOR SOIL-TRANSMITTED HELMINTH SURVEILLANCE IN BENIN AND INDIA

Heather K. Amato1, Edoux JE Siko2, Malathi Manuela2, Zayina Zonderenenvii, Michael Harris1, Christopher LeBoa4, Partait Houngbegnon2, Rohan M. Ramesh2, Gideon J. Israel3, Venkateshprabhu Janagaraj1, Nils Pilotte3, Steve Williams5, Adrian JF Luty7, Moudachirou Ibikounlé2, Sitara SR Ajampur3, Amy J. Venkateshprabhu Janagaraj3, Michael Cappello6, Michael D. Wilson1

1Department of Civil and Environmental Engineering, University of California, Berkeley, CA, United States, 2Institut de Recherche Clinique du Bénin, Abomey-Calavi, Benin, 3The Welcome Trust Research Laboratory, Division of Gastrointestitual Sciences, Christian Medical College, Vellore, India, 4Division of Environmental Health Sciences, Berkeley School of Public Health, University of California, Berkeley, CA, United States, 5Department of Biological Sciences, Quinnipiac University, Hamden, CT, United States, 6Department of Biological Sciences, Smith College, Northampton, MA, United States, 7Institut de Recherche pour le Développement, Paris, France

Surveillance of soil-transmitted helminth (STH) infections is critical for targeting interventions like mass drug administration (MDA). While microscopy-based stool surveillance of STH in humans is resource-intensive and may miss most asymptomatic or mild-intensity STH infections or clusters that contribute to continued transmission, wastewater surveillance of STH environmental DNA (eDNA) may be a useful, low-cost approach for detecting ongoing STH transmission in communities, even in low prevalence, high aggregation scenarios. We leveraged the cluster-randomized DeWorm3 trial to pilot a wastewater-based molecular surveillance approach in Benin and India. We optimized methods to detect STH eDNA in wastewater samples (grab, sediment, and Moore swab) collected from non-networked open drains in Benin (N=68) and India (N=60) in June-July 2022. Using qPCR to detect STH targets from purified eDNA, we detected hookworm (Necator americanus) in 28% (17/60) of samples and roundworm (Ascaris lumbricoides) in 15% (9/60) of samples in India. In Benin, hookworm (N. americanus or Ancylostoma duodenale) was detected in 3% (2/68) and roundworm (A. lumbricoides) in 9% (6/68) of samples. Whipworm (Trichuris trichiura) was not detected in any samples. Across both study sites, STH eDNA was most frequently detected in wastewater sediment samples at 9.4% (12/128), followed by grab samples at 5.5% (7/128), then Moore swabs at 4.7% (6/128). Wastewater surveillance of STH eDNA could offer national control programs a simpler and more cost-effective way of targeting communities for MDA and monitoring for recrudescence over time compared with the standard stool-based Kato-Katz method.

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TWENTY-FOUR MONTH LONGITUDINAL STUDY OF HOOKWORM INFECTION IN GHANAIAN SCHOOL CHILDREN

Irene A. Larbi1, Debbie Humphries2, Jana Lohrova2, Molly McLaughlin0, Luis Maldonado2, Joseph Otchere1, Josephine Quagrainie1, Sena Seddoh1, Hibbah Saeed1, George Mensah1, Dickson Osabutey1, Lisa M. Harrison3, Hector Samani1, Michael Cappello6, Michael D. Wilson1

1Noguchi Memorial Institute for Medical Research, Accra, Ghana, 2Yale School of Public Health, New Haven, CT, United States, 3Yale University, New Haven, CT, United States

Worldwide, 576 million people and 156 million children are estimated to be infected with hookworm in tropical regions, causing an estimated 3.2 million disability-adjusted life-years annually. We randomly selected school-aged children (4-16 yrs) to participate in a two-year longitudinal study of hookworm infection in rural Ghana (n=274) to identify nutritional status and environmental exposures that impact hookworm infection and response to treatment. After the longitudinal study we conducted a cross-sectional survey in the study communities to assess spillover effects of the longitudinal study. Every six months (baseline Jan 2013) we collected anthropometric measurements, fecal samples and venous blood samples. Household surveys and multiple pass twenty-four-hour food intake recalls were collected at baseline and 18 months. The overall prevalence of hookworm infection among study subjects was reduced from 21% (58/274) at baseline to 10% (21/208) two years later. Sixty percent (60.8%; n=166) of participants were hookworm negative every time they were tested and 16.4% (n=45) were infected two or more times. Fecal egg reduction rate in the 27.7% (23/83) of children who were hookworm positive at least once after treatment ranged from 0-99%. Hookworm status and albendazole treatment outcome at baseline was the dominant influence on infections at six months. Albendazole treatment efficacy was greater in older children and those from food-insecure households, and these relationships were nonlinear. The subsequent cross-sectional community study recruited additional subjects from households with a child who participated in the longitudinal study (n=131) and households without a participating child (n=136). Prevalence of infection in non-participating children with a sibling who participated in the longitudinal study did not differ significantly from children without participating siblings. While rates of hookworm infection in Africa have declined, additional strategies are likely needed for sustainable control. Results from this innovative study add to our current knowledge of hookworm infection in school-aged children.

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STRONGYLOIDES STERCORALIS COINFECTION IS ASSOCIATED WITH ALTERED SYSTEMIC CYTOKINE PROFILES, GREATER DISEASE SEVERITY, HIGHER BACTERIAL BURDENS AND INCREASED FREQUENCIES OF UNFAVORABLE TREATMENT OUTCOMES IN PULMONARY TUBERCULOSIS

Saravanan Munisankar

ICER-NIRT, Chennai, India

Helminth infections and tuberculosis are both major public health problems which share considerable geographical overlap affecting mostly lower and some middle-income countries worldwide. Helminth infections are known to induce modulation of T cell mediated immune responses to tuberculosis disease. Hence, we explored the systemic levels of cytokines, chemokines and growth factors in individuals with pulmonary tuberculosis (PTB) (n=483) with (n=74) or without (n=409) coexistent Strongyloides stercoralis (Ss) infection. Type 1, Type 2, Type 17, pro-inflammatory cytokines and growth factors levels were determined by multiplex ELISA. Chest x-rays were used to determine cavitory disease and smear grade was used to determine bacterial burdens in PTB individuals. Treatment outcomes were classified as favorable (recurrence free cure) or unfavorable (treatment failure, death during treatment or TB recurrence) during 18 months of follow up. PTB
individuals with Ss infection showed higher bacterial burden and cavi
tary disease compared to PTB individuals without Ss infection. PTB individuals with Ss infection also exhibited a significantly increased frequency of unfavourable treatment outcomes compared to PTB individuals without Ss infection. We observed a significant increase in type 2 (IL-4, IL-5 and IL-13), pro-inflammatory cytokines (IFNα, IFNβ and IL-12) and growth factors (CCL2, CCL11, CCL19, CCL20, CXCL1, CXCL8, CXCL10 and CX3CL1) in PTB individuals with coexistent Ss infection and a significant decrease in type 1 cytokines (IFNγ and IL-2) in PTB individuals with coexistent Ss infection. Therefore, our data demonstrate that coexistent Ss infection is associated with modulation of systemic cytokine profiles, greater disease severity, higher bacterial burden and increased frequencies of unfavorable treatment outcomes in PTB.

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ACUTE ENCEPHALITIS SYNDROME IN ADULTS IN EASTERN INDIA: AN AETIOLOGICAL STUDY
Jaya chakravarty Tapadar, Tulika Rai, shubham Kashyap, sumit chatterjee, Gopal Nath
Institute of Medical Sciences, Banaras Hindu University, Varanasi, India
Seasonal outbreaks of Acute Encephalitis Syndrome (AES) with high case fatality have been reported most frequently in the state of Uttar Pradesh, India. The surveillance conducted by the National Vector Borne Disease Control Programme of India for AES focuses mainly on detecting Japanese encephalitis (JE) virus. Most published studies of AES have been done on children while it is a common health emergency even among adults. Thus, this study was done to identify the common causes of AES among adults in a tertiary care centre in eastern Uttar Pradesh. CSF and blood samples were collected from adults presenting with fever and altered sensorium of less than 15 days. ELISA was performed in serum samples for IgM antibody detection of Dengue Virus, Chikungunya virus, West Nile Virus, Scrub Typhus, Leptospira, CSF samples were tested for IgM JE virus and tuberculosis by Gene Xpert. Malaria was detected by rapid kit. Among the 126 patients of AES, 54% were males, mean age was 32 years and duration of fever was 9.4 days. The common aetiology were Scrub Typhus (36.5%), Leptospira (36.5%), Chikungunya (25.4%), Dengue (25%), tuberculosis (14.38%) West Nile & JE virus was 6.3% each and only one had malaria. No aetiology could be identified in 20 patients and 42% had more than one infection. Mortality was 21/126 (16.6%). The most common cause of AES among adults in eastern Uttar Pradesh has shifted from JE to treatable causes like scrub typhus and leptospirosis. Vector borne viral diseases like dengue, chikungunya, west nile and JE has emerged as an important cause of AES. Tuberculosis should be ruled out in all cases of AES in adults in India.

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THE DIAGNOSIS OF SUBARACHNOID NEUROCYSTICERCOSIS IS OFTEN DELAYED AND OTHER FINDINGS OF A MULTICENTER RETROSPECTIVE IN THE USA
Janitizio Guzman1, Jessica Herrick2, Timothy J. Hatlen3, Jill Weatherhead4, Eva H. Clark4, Jose Serpa4, Laila Castellino4, Elise M. O’Connell4, Eva H. Clark4, Jose Serpa4, Felicia C. Chow5, Bowman9, Anna Cervantes-Arslanian10, Christina Coyle11, A. Clinton F. Henao-Martinez15, Carlos Franco-Paredes16, Glenn Mathisen17, 5University of California San Francisco Medical Center, San Francisco, USA, 6David Geffen School of Medicine at UCLA, Los Angeles, CA, United States, 71, Jesica Herrick2, Timothy J. Hatlen3, Jill
University of North Carolina School of Medicine, Chapel Hill, NC, United States, 2Boston University Medical Center, Boston, MA, United States, 3Albert Einstein College of Medicine, New York, NY, United States, 4University of Texas Medical Branch, Galveston, TX, United States, 12The Lundquist Institute at Harbor-UCLA Medical Center, Los Angeles, CA, United States, 13University of California San Diego Medical Center, San Diego, CA, United States, 14University of Colorado School of Medicine, Aurora, CO, United States, 15University of North Carolina School of Medicine, Chapel Hill, NC, United States, 19University of Texas Southwestern Medical Center, Dallas, TX, United States
Subarachnoid (racemose) neurocysticercosis (SANCC) is an uncommon but severe form of Taenia solium infection. There is limited evidence to guide clinical management of these patients. We performed a multicenter retrospective chart review of 15 U.S. sites. A total of 69 subjects with racemose disease was entered. The most common region of exposure was Mexico (67%) followed by Central America (24%). Median age was 43 years (range 15-76) and 71% were male. Common symptoms at the time of index admission were headache (80%), nausea/vomiting (46%), dizziness (44%), and blurry vision (33%). Cysts were intracranial in 64 (93%) subjects and exclusively intraspinal in 4. One patient had meningitis without visible cystic lesions. Incidental admission magnetic resonance imaging (MRI) demonstrated ventriculomegaly in 41 (59%) and focal findings in 9 (13%) including ischemic infarct, subarachnoid hemorrhage, and/or arterial aneurysm. For 55 (80%), SANCC was first diagnosed during the index admission. Of these, 23 (42%) had prior neuroimaging and subacute delay in diagnosis (i.e. previously seen with hydrocephalus [27%], stroke [5.5%], and/or meningitis [11%], missed diagnostic radiologic features [4%], or inadequate imaging [5.5%]). Of the 69 subjects, 54% underwent a neurological procedure during index admission (cyst removal n=16, EVD/ shunt/ventriculostomy n=24). At the time of discharge, 6 (8.6%) patients were not given albendazole and/or praziquantel due to cost or availability. Six months following discharge, <10% of follow up MRIs demonstrated cyst resolution. Planned treatment course of <4 weeks at discharge compared to >4 weeks was associated with increased risk for new cyst development on follow up imaging at a median of 3.8 years following discharge (range 2.6 months-8 years). Those with a delayed diagnosis received a significantly longer total duration of corticosteroids than those without a delay. The diagnosis of SANCC is often missed, and most patients require neurological intervention. Antiparasitic therapy is suboptimal, especially with regimens developed for parenchymal NCC.

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CO-INFECTION WITH STRONGYLOIDES STERCORALIS IN ADULTS WITH CRYPTOCOCCAL MENINGITIS IN AFRICA
Mary Foley1, Joseph Donovan2, Kwana Lechilile3, Ronan Doyle4, Monika Struebig5, David B. Meya4, Henry C. Mwandumba6, Cecilia Kanyama6, Graeme Meintjes7, Tshepo Leeme3, Chiratidzo E. Ndihlovu4, Conrad Muzuroa4, David S. Lawrence2, Thomas S. Harrison2, Joseph N. Jarvis2
1London School of Hygiene and Tropical Medicine, London, United Kingdom, 2Clinical Research Department, Faculty of Infectious and Tropical Disease, London School of Hygiene and Tropical Medicine, London, United Kingdom, 3Botswana Harvard AIDS Institute Partnership, Gaborone, Botswana, 4Infectious Diseases Institute, College of Health Sciences, Makerere University, Kampala, Uganda, 5Department of Medicine, Kamuzu University of Health Sciences, Blantyre, Malawi, 6Ulongwe Medical Relief Trust (University of North Carolina Project), Lilongwe, Malawi, 7Wellcome Centre for Infectious Diseases Research in Africa, Institute of Infectious Disease and Molecular Medicine, University of Cape Town, Cape Town, South Africa, 8Internal Medicine Unit, Faculty of Medicine and Health Sciences, University of Zimbabwe, Harare, Zimbabwe, 9Institute of Infectious and Immunity, St George’s University London, London, United Kingdom
Cryptococcal meningitis is responsible for 15% of AIDS-deaths globally, with 75% of these occurring in Sub-Saharan Africa. The soil transmitted helminth Strongyloides stercoralis infects 30-100 million individuals globally and its distribution overlaps with that of cryptococcal meningitis. Host
immunity adapts to pathogens through differentiation of host CD4+ T cells into subpopulations. Co-infection with pathogens that each elicit different CD4+ T cell responses (Th1 vs. Th2) provides an immune conundrum; how should the host respond? In meningitis, hemorrh rhinocerebral infection may modulate host immune responses. In a study of Vietnamese adults with tuberculous meningitis, S. stercoralis co-infection was associated with less severe inflammation and better outcomes, as immunomodulation towards Th2 appeared beneficial. However, in cryptococcal meningitis Th2 responses associate with impaired control of infection and poor outcomes. We hypothesize that S. stercoralis co-infection is associated with more severe disease and worse outcomes in cryptococcal meningitis. We will perform S. stercoralis serological testing on stored blood from 844 adults from AMBITION: a phase 3 clinical trial of cryptococcal meningitis therapy in five African countries. Stored serum will be defrosted and tested for S. stercoralis by enzyme immunoassay following manufacturer instructions. Testing will be performed at the London School of Hygiene and Tropical Medicine, Pre-treatment cryptococcal meningitis severity, routine cerebrospinal fluid (CSF) parameters, CSF cytokines, and mortality by 10 weeks, will be compared between S. stercoralis positive and negative groups. Additionally, clinical data will be analyzed stratified by optical density in S. stercoralis positive cases, to distinguish strongly positive cases from potential past infection. S. stercoralis serological testing and CSF testing will be performed in June 2023. If S. stercoralis seropositivity associates with worse outcomes, a future trial could evaluate ivemectin therapy for S. stercoralis eradication in cryptococcal meningitis - a low-cost high-impact intervention.

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SEROLOGIC RESPONSE USING ELISA ANTI-VI IGG ANTIBODIES AT SEVERAL TIME POINTS FOLLOWING IMMUNIZATION WITH THE TYPHOID CONJUGATE VACCINE, TYPBAR-TCV, AMONG HIV INFECTED CHILDREN IN KARACHI PAKISTAN

Zoya Haq1, Farah Qamar2, Sonia Qureshi2, Fatima Mir2, Mohammad Tahir Younas3, Rabab Batoo5
1, 2, 3 Liqaut National Medical College, Karachi, Pakistan, 4 Aga Khan University Hospital, Karachi, Pakistan

Associated with outbreaks, complications, and the evolution to novel strains that are extensively drug resistant, enteric fever remains a major public health concern in Pakistan. WHO recommends programmatic use of typhoid vaccines for the control of typhoid fever in typhoid endemic countries, however, individuals infected with HIV generally have a lower response to immunization as compared to the general population and there is a need to corroborate the post-vaccination serologic response of TCV in HIV-infected children. Therefore, this study was designed to assess the serological response of typhoid conjugate vaccine (TCV) in HIV-infected children in Ratodero, Larkana in Sindh. A prospective cohort study was conducted in HIV-positive children who received a single dose of the Typhbar-TCV at Talaqoa Hospital in Ratodero Larkana, Pakistan. A total of 336 HIV-positive children aged 6 months to 15 years were vaccinated and followed-up for 1 year, from December 2019 till January 2021. We measured the serological response using ELISA anti-Vi IgG antibodies at several time points following immunization with a single 0.5ml intramuscular injection of Typhbar-TCV. Blood samples were collected at baseline, 6 weeks, 6 months and 12 months post-immunization with TCV and tested for anti-Vi IgG titers and the seroconversion rates were calculated. The mean age of the participants was 52.04 months, and the mean CD4 count was 941. The GMT titers were significantly lower in children aged 6 months to 5 years at 6 months and 1-year post vaccination. Besides age, high immunosuppression was significantly associated with lower seroconversion rates in children with high and low immunosuppression. HIV-infected children with high immunosuppression and younger age were associated with low seroconversion rates and failure to remain seroconverted at 6 months and 1-year post-immunization with TCV. Thus, it can be concluded that the overall seroconversion rates of TCV among HIV-positive children are lower as compared to healthy children. Therefore, the need for a booster dose of TCV should be assessed and administered accordingly amongst immunocompromised patients.

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TRIM-HIV: TARGETING THE RESTORATION OF INNATE IMMUNITY IN MEN WHO HAVE SEX WITH MENS WITH EARLY INITIATION OF ANTIRETROVIRAL THERAPY IN HIV-1 INFECTION FOR MANAGEMENT IN TROPICAL REGIONS

Matrana Akiso Mbendo, Robert Langat1, Daniel Muema, Geoffrey Ombati, Omu Anzala, Marianne W. Mureithi
University of Nairobi, Nairobi, Kenya

Background: Natural killer (NK) cells play a crucial role in the immune response against viral infections, including HIV-1, and their impairment may contribute to disease progression. Early initiation of antiretroviral therapy (ART) is crucial for reducing morbidity and mortality associated with HIV-1 infection, particularly in tropical regions where the burden of HIV-1 is high. However, the effects of early ART on NK cell function and phenotype in early HIV-1 infection in men who have sex with men (MSM) in tropical regions are not fully understood. Methods: We longitudinally evaluated NK cell function and phenotype in five MSM in Nairobi, Kenya, who were initiated on ART immediately upon seroconversion. Blood samples were obtained fortnightly for three visits post-seroconversion. PBMCs were stimulated with K562 cell line and interleukins (IL-2 and IL-15), stained with antibodies for NK cell phenotype, activation, and functionality and assessed by flow cytometry. Results: We observed a significant reduction in NK cell production of IFN-γ, expression of CD69, and NK cell inhibitory receptor SigleC7 in early HIV-1 infection. On the other hand, there were significant decreases in NK cell degranulation and presentation of the cell exhaustion marker PD-1. Most of these changes seem to have been partially restored a few weeks after ART initiation. Conclusions: Our findings suggest that early ART initiation can partially restore some, but not all, perturbations of NK cell function and phenotype in early HIV-1 infected MSM in tropical regions. These findings have important implications for managing HIV-1 infection in resource-limited settings where early initiation of ART is not always possible and where the burden of HIV-1 is high. Further research is needed to fully understand how HIV-1 impairs NK cell effector functions and how ART can restore these functions in early HIV-1 infection in tropical regions. This knowledge can inform the development of effective HIV-1 vaccines and strategies to reduce the burden of HIV-1 in tropical regions.

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ASSOCIATION OF UROGENITAL SCHISTOSOMIASIS WITH HIV INFECTION IN A LARGE COMMUNITY-BASED COHORT IN ZAMBIA (THE ZIPIME WEKA SCHISTA STUDY)

Kwame Shanaube1, Rhoda Ndubani1, Olimpia Lamberti1, Nkayta Kasese1, Emily Webb1, Beatrice Nyondo1, Maina Cheeba1, Jennifer Fitzpatrick1, Helen Kelly1, Helen Ayles1, Amaya Bustinyc1
1 Zambart, Lusaka, Zambia, 2 Department of Clinical Research, London School of Hygiene and Tropical Medicine, London, United Kingdom, 3 Department of Infectious Disease Epidemiology, London School of Hygiene and Tropical Medicine, London, United Kingdom

Co-infection of HIV-1 and Schistosoma haematobium (Sh) is highly prevalent in sub-Saharan Africa with evidence of an association between prevalent HIV-1 and schistosome infection. There is also suggestion of increased HIV-1 transmission in women with Female Genital Schistosomiasis (FGS), caused by Sh egg deposition in the genital tract. The Zipime Weka Schista study is an ongoing longitudinal cohort aiming to integrate home-based genital self-sampling for Sh and High Risk Human Papillomavirus (HR-HPV) and self-testing for HIV and Trichomonas vaginalis (Tv) in two endemic areas in Zambia. Here we used baseline data to examine the association between Sh with HIV-1 prevalence. Sexually active women aged 15-50 years were randomly selected. Community health workers recruited women during a home visit and obtained two cervicovaginal self-swabs, a urine sample, self-test for HIV-1 and Tv and...
completed a questionnaire. Women were referred to the clinic where a midwife collected genital samples and obtained images using hand-held colposcopy. A urine test was analysed via microscopy for Sh ova detection and self-swabs analysed for Sh and HR-HPV DNA. Overall, 251 women were recruited (median age 28 [IQR: 22-36]); 17.5% (439/2510) were HIV-1 seropositive, of which 8.4% (37/439) previously undiagnosed. After adjusting for confounders (age, trichomonas result, marital status) there was no association between active egg-patent Sh status and HIV status (OR=0.97, 95% CI 0.54-1.76, p=0.93). There was a significant association between women with TV and prevalent HIV-1 (OR=2.0, 95% CI 1.40-2.80, p<0.001). Our preliminary results differ from previous findings on the association between urinary Sh status and HIV status. Ongoing analysis of genital samples and images for FGS will help refine these associations.

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FALLING THROUGH THE CRACKS: APPLYING THE 95-95-95 METRIC TO PROVIDE INSIGHT INTO HIV PROGRAMME GAPS IN HIV-INFECTED INFANT DEATHS INVESTIGATED IN KENYA, SOUTH AFRICA AND MOZAMBIQUE

Beth A. Tippett Barr1, Zachary Madewell2, Victor Akelo3, Dickens Onyango4, Inacio Mandomando5, Quique Bassat6, Shabir Madhi7, Sana Mahtab7, Portia Mutevedzi8, Cynthia Whitney8, Dianna Blau2

1Nyanja Health Research Institute, Salima, Malawi, 2US Centers for Disease Control and Prevention, Atlanta, GA, United States, 3US Centers for Disease Control and Prevention, Kisumu, Kenya, 4Centro de Investigacao em Saude do Manhica, Maputo, Mozambique, 5IsGlobal Universitat de Barcelona, Barcelona, Spain, 6ISGlobal Universitat de Barcelona, Johannesberg, South Africa, 7Emory University, Atlanta, GA, United States

National HIV programs aspire to achieve the 2025 UNAIDS 95-95-95 targets: 95% of people living with HIV know their status (known status’); 95% of those are on antiretroviral therapy (ART), and 95% of those are virally suppressed (VLS). This cascade provides a snapshot of national HIV-programme progress towards epidemic control but has not been applied to research findings to understand how HIV service delivery gaps may contribute to HIV mortality. The multi-country Child Health and Mortality Prevention Surveillance (CHAMPS) investigates under-five (<5) mortality using clinical records, minimally invasive tissue sampling, extensive laboratory testing including a molecular HIV test, verbal autopsy, and a multidisciplinary panel to determine causes of death. We applied the 95-95-95 cascade to CHAMPS infant (<1 year) deaths due to HIV in 3 high-burden countries between 2017-2023, and compared them to available national data on live infant programme coverage in the public domain from within the same time frame, and restricted to ages <1 or closest age group available. HIV prevalence in CHAMPS decedents ranged from 4.0% in South Africa to 8.5% in Kenya. Among the HIV deaths, nearly 3/4 had wasting, 88.3% (30/34) of which was extreme wasting. In South Africa, 70% of CHAMPS <1 HIV deaths had known status compared to 75% nationally; 35% were on ART compared to 68%, and just over one-quarter in both CHAMPS and national cascades had VLS (i.e., 70-35-26 vs 75-68-27). CHAMPS-Kenya’s cascade was 8%-0%-0% vs 79%-74%-49% in the national program, and CHAMPS-Mozambique was 54%-31%-0% compared to 72%-57%-36% nationally. Globally, pediatric HIV treatment lags behind adult coverage (30/34) of which was extreme wasting. In South Africa, 70% of CHAMPS <1 HIV deaths had known status compared to 75% nationally; 35% were on ART compared to 68%, and just over one-quarter in both CHAMPS and national cascades had VLS (i.e., 70-35-26 vs 75-68-27). CHAMPS-Kenya’s cascade was 8%-0%-0% vs 79%-74%-49% in the national program, and CHAMPS-Mozambique was 54%-31%-0% compared to 72%-57%-36% nationally. Globally, pediatric HIV treatment lags behind adult coverage in all countries. We found that HIV program gaps measured in CHAMPS decedents with HIV were even larger than those reported in national infants from national HIV programs, and the pattern of gaps observed in CHAMPS cases differed between countries. Findings indicate that programme gaps contributing to HIV-related infant mortality are not uniform across countries, and that countries could learn from each other’s best practices to further reduce pediatric HIV mortality.

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CULEX PIPIENS AND CX. MODESTUS ARE VECTORS FOR WEST NILE VIRUS AND USUTU VIRUS, RESPECTIVELY, IN BELGIUM

Alina Soto, Lander De Coninck, Celine Van De Wele, Anne-Sophie Devlees, Ana Lucia Rosales Rosas, Lanjiao Wang, Jelle Matthijnssens, Leen Delang

Rega Institute for Medical Research, KU Leuven, Leuven, Belgium

West Nile virus (WNV) and Usutu virus (USUV) are emerging arboviruses in Europe. While Culex (Cx.) pipiens is widely acknowledged as their primary vector, studies have suggested a role for the less commonly known Cx. modestus. Recently, our lab showed for the first time that Cx. modestus is established in Belgium, but the vector competence for Belgian Cx. mosquitoes to WNV and USUV Mosquitoes were captured in Leuven, Belgium using BG-Sentinel traps. Females were fed chicken blood containing WNV lineage 2 (a Netherlands chaffeekill isolate) or USUV European (USUV/EU) or African (USUV/AF) lineage 3 (Belgian blackbird isolates). After 14 days at 25°C, mosquito bodies and heads were dissected and saliva harvested to determine infection, dissemination, and transmission rates, respectively. Infectious virus was measured by plaque assay and virus titers quantified by qPCR. Cx. pipiens (biotypes pipiens/molestus/hybrid) were identified by qPCR and other species by Sanger sequencing. Finally, the presence of the Wolbachia wsp gene was detected by PCR. We captured a total of 1,137 Cx. females. Those that blood-fed were mostly Cx. pipiens (n=140) followed by Cx. modestus (n=8), Cx. molestus (n=1), and a Cx. pipiens-molestus hybrid (n=1). In Cx. pipiens, infection rates were 11% for WNV (n=5), 13% (n=7) for USUV/EU, and 16% (n=6) for USUV/AF. Cx. pipiens dissemination was 40% (n=2) for WNV and 17% (n=1) for USUV/AF, but there was no USUV/EU dissemination. Surprisingly, Cx. modestus showed a 75% infection rate for USUV/AF (n=3) with 100% dissemination (n=3). The sample size of Cx. modestus was too low to measure vector competence for WNV or USUV/EU. Finally, there was no significant difference in USUV/AF titers in Cx. pipiens and modestus. The measure of transmission in saliva and detection of Wolbachia is currently ongoing. We hypothesize that Cx. modestus may be a more potent vector for USUV than Cx. pipiens in Belgium. We are planning targeted collections of Cx. modestus in 2023 for additional data supporting their vector competence to WNV and USUV.

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INVESTIGATION OF VIRUS-HOST INTERACTIONS IN SEVERE FEVER WITH THROMBOCYTOPENIA SYNDROME VIRUS INFECTION USING A LIPIDOMICS APPROACH

Hin Chu, Bingpeng Yan, Bingjie Hu, Terrence Tsz-Tai Yuen, Jasper Fuk-Woo Chan

The University of Hong Kong, Hong Kong, Hong Kong

Severe fever with thrombocytopenia syndrome virus (SFTSV) is an emerging tick-borne bunyavirus endemic in parts of Asia that causes severe disease in human. SFTSV is characterized by an acute febrile illness associated with high fever, thrombocytopenia, and hemorrhage. The pathogenesis of SFTSV remains poorly understood. Viruses often reprogram the host lipid metabolism machinery to facilitate their own replication, and host-targeting lipid modulators have been reported to be potential broad-spectrum antivirals. To provide insights into virus-host interactions in SFTSV infection, we investigated the lipidomics profile of SFTSV-infected Huh-7 cells. Our lipidomics analysis identified ~120 significantly changed lipids. These lipids belonged to 18 lipid classes, including cardiolipin (CL), ceramide (Cer), diacylglycerol (DG), ether-linked phosphorylcholine (etherPC), ether-linked phosphorylcholineetherlamelane (etherPE), lysocardiolipin (MLCL), n-acyl-lyso phosphatidyl-ethanolamine (LNAPE), lysophosphatidylcholine, ganglioside GM3 (GM3), hexosylceramide (HexCer), oxidized phosphorylcholine, phosphatic acid (PA), phosphorylcholine (PC), phosphorylcholineetherlamelane (PE), phosphorylcholineetherlanostols (Pi), phosphorylcholineetherlanostins (Pi), triacylglycerol (TG). Among them, TG,
PC, and Cer were the three most perturbed lipid classes. Around 20% of the perturbed lipids, including one CL, one MCL, most of PA, PI, and PS were downregulated. Around 80% of the perturbed lipids, including all GMS, HexCer, LNAPE, etherPC, etherPE, DG, PE, most of TGs, PCs, Cer, and a few of PA, PI, and PG were significantly upregulated. Pathway analysis revealed glycerophospholipid metabolism, sphingolipid metabolism, glycerolipid metabolism, and phenylalanine, tyrosine and tryptophan biosynthesis as the four dominantly perturbed pathways. In summary, we suggest that SFTSV-induced host lipidomics perturbations may facilitate the identification of lipid metabolism modulators as potential antivirals for SFTSV infection.

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APOLIPOPROTEIN-A1 MIMETIC PEPTIDE 4F BLOCKS FLAVIVIRUS NS1-INDUCED ENDOTHELIAL BARRIER DYSFUNCTION

Pedro H. Carneiro1, Scott B. Biering3, Ronaldo Mohana-Borges2, Eva Harris3

1Division of Infectious Diseases and Vaccinology, School of Public Health, University of California, Berkeley, Berkeley, CA, United States, 2Laboratório de Tecnologia e Biocenologia Estrutural, Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil

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DEVELOPING A MODEL OF PERSISTENT POWASSAN DISEASE IN C57BL/6 MICE

Stacey L.P. Scroggs1, Danielle K. Offerdahl2, Philip E. Stewart2, Carl Shaia1, Amanda J. Griffin3, Marshall E. Bloom2

1United States Department of Agriculture, Manhattan, KS, United States, 2National Institutes of Health, Hamilton, MT, United States

Powassan infection is caused by two closely related, tick-transmitted viruses of the genus Flavivirus (family Flaviviridae): Powassan virus (POWV) and II (known as deer tick virus (DTV)). Infection is typically asymptomatic or mild, but can progress to neuroinvasive disease. Approximately 10% of neuroinvasive cases are fatal and half of the survivors will experience long-term neurological sequelae. Understanding how these viruses cause long-term symptoms as well as the possible role of viral persistence is important for developing therapies. Animal models of acute Powassan infection have been developed, but models to study persistent infection are nonexistent. We hypothesized that a portion of DTV-infected C57BL/6 mice that survive the acute infection will become persistently infected. We intraperitoneal inoculated six-week-old C57BL/6 mice (50% female) with 103 FFU DTV and assayed for infectious virus, viral RNA, and inflammation during acute infection, and 21, 56, and 84 days post-infection (dpi). Although most mice (86%) were viremic 3 dpi, only 21% of the mice were symptomatic and 83% recovered. Infectious virus was only detected in the brains of mice sampled during the acute infection. Infectious virus was not detected in any other tissue. Viral RNA was detected in the brain until 84 dpi, but the magnitude decreased by time. Meningitis and encephalitis were visible in acute mice and from mice sampled at 21 dpi. Inflammation was observed until 56 dpi in the brain and 84 dpi in the spinal cord, albeit at low levels. These results suggest that the long-term neurological symptoms associated with Powassan disease are likely caused by lingering viral RNA and chronic inflammation in the central nervous system rather than by a persistent, active viral infection. The C57BL/6 model of persistent Powassan mimics illness in humans and can be used to study the mechanisms of chronic disease. This work was funded by the Intramural Research Program of the National Institutes of Allergy and Infectious Diseases of the National Institutes of Health.

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FACTORS ASSOCIATED WITH LASA FEVER FATLITY IN LIBERIA, 2016 - 2021: A SECONDARY DATA ANALYSIS

Emmanuel Dwalu1, Ralph W. Jetoh1, Bode I. Shobayo1, Irene P. Pewu1, Fahn M. Tarweh1, Julius S M Gilayeneh1, Himiede W. Wilson-Sesay2, Godwin E. Akpan2, Chukwuma D. Umeokonkwo2, Jane A. MaCauley1

1National Public Health Institute of Liberia, Monrovia, Liberia, 2Africa Field Epidemiology Network, Monrovia, Liberia

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Lassa fever (LF) is endemic in Liberia and is immediately reportable. Suspected cases are confirmed at the National Public Health Reference Laboratory. We described the epidemiological characteristics of LF cases and determined factors associated with mortality in Liberia from 2016 to 2021. We reviewed 867 case-based LF surveillance data from 2016 - 2021 obtained from the National Public Health Institute of Liberia (NPHIL). The cases that met the confirmed LF case definition were tested with RT-PCR and only the confirmed cases were included in the analysis in Epi Info version 7.2.5. We calculated the LF positivity rate, case fatality rate, and factors associated with LF mortality using chi-square statistics and logistic regression at a 5% level of significance. Eighty-five per cent (737/867) of the confirmed cases were tested and 26.0% (192/737) were confirmed LF positive. The median age of confirmed LF cases was 21 (IQR:12-34) years. Age 10-19 years accounted for 24.5% (47/192) and females 54.2% (104/192), Bong 33.9% (65/192), Grand Bassa 31.8% (61/192), and Nimba counties, 21.9% (42/192) accounted for most of the cases. The median duration from symptom onset to hospital admission was 6 (IQR:3-9) days. A majority, 66% (126/192) of the cases were reported during the dry season (October-March) and annual incidence was highest at 12 cases per 1,000,000 population in 2019 and 2020. The overall case fatality rate was 44.8%. Non-endemic counties, Margibi, 77.8% and Montserrado, 66.7% accounted for the highest CFR, while 2018, 66.7% and 2021, 60.0% recorded the highest CFR during the period. Age <30 years (aOR=2.195%; CI:1.08-4.11, p=0.027) and residing in Grand Bassa County (aOR=0.3, 95%CI:0.13-0.73, p=0.007) was associated with LF mortality. LF was endemic in three of the fifteen counties of Liberia, and the CFR remained generally high. The high fatality is currently being further investigated. There is a need to continuously train healthcare workers, especially in non-endemic counties to improve the LF treatment outcome. Lassa fever, endemic, Liberia, confirmed case, case fatality rate.
EEG PATHOLOGIES IN LASSA FEVER INDICATING CEREBRAL INVOLVEMENT: RESULTS FROM 53 PROSPECTIVELY FOLLOWED PATIENTS

Hannah Mueller1, Cyril Eramh2, Mathias Gelderblom3, Joseph Okegwu4, Osas Edawee2, Stephan Guenther5, Sylvanus Okogbenin4, Michael Ramharter4, Lisa Oestereich2, Till Omansen1
1Department of Tropical Medicine, Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany; 2Institute of Lassa Fever Research and Control & Department of Medicine, Irrua Specialist Teaching Hospital, Irrua, Nigeria; 3Department of Neurology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany; 4Institute of Lassa Fever Research and Control & Department of Obstetrics and Gynaecology, Irrua Specialist Teaching Hospital, Irrua, Nigeria; 5Department of Virology, Bernhard Nocht Institute for Tropical Medicine and German Center for Infection Research, Hamburg, Germany; 6Department of Tropical Medicine, Bernhard Nocht Institute for Tropical Medicine & I. Department of Medicine, University Medical Center Hamburg-Eppendorf; German Center for Infection Research, Hamburg, Germany; 7Department of Virology and Department of Tropical Medicine, Bernhard Nocht Institute for Tropical Medicine & I. Department of Medicine, University Medical Center Hamburg-Eppendorf; German Center for Infection Research, Hamburg, Germany

Patients severely affected by Lassa fever (LF) often exhibit neurological symptoms, like seizures or meningitis. These neurological complications are strongly correlated with fatal outcome. Though Lassa virus (LV) has been isolated from cerebrospinal fluid in two case reports, it remains unclear whether LV is truly causing meningoencephalitis. Proofing a possible neurotropism of LV is highly relevant to develop appropriate medical countermeasures. The aim of the study was to implement electroencephalography (EEG) on the LF isolation ward at Irrua Specialist Teaching Hospital, Nigeria, to assess the cerebral involvement of LF correlating EEG findings with laboratory and clinical parameters. From August 2021 to February 2022 we enrolled 53 consenting patients with RT-PCR confirmed LV in our prospective, observational cohort study. Study visits consisted of EEGs, clinical exams and laboratory analysis. One patient had to be excluded due to insufficient EEG recording quality. Of the 52 remaining participants 34 % were female and the mean age was 29 years (range 9 - 67 years). Two experienced, blinded neurologists used the American Neurophysiology Society’s Standardized Critical Care EEG terminology for EEG analysis. Thirty-six patients (69%) showed any neurological symptoms: meningitis occurred in 13 (25%), headache in 5 (10%) and seizure in 2 patients (4%). Metabolic derangement, most notably acute kidney injury did not occur in any of the enrolled patients. The most frequently observed EEG abnormality was a slowing of the EEG background activity in 8 patients (15%), closely followed by General Rhythmic Delta Activity (GRDA) and a focal slowing of the EEG activity seen in both 7 patients (14%). EEG abnormalities coincided with clinical neurological signs in a majority of cases pinpointing towards a possible neurotropism of LV. While slowing of the EEG background activity presents an unspecific EEG change indicating a generally “reduced” cerebral function, GRDA is a more specific pathology occurring in viral encephalitis. In summary our results pinpoint to a central nervous effect of LV in affected patients in our cohort.

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POTENTIAL FOR PREEXISTING IMMUNITY TO SARS-COV2 IN EASTERN SIERRA LEONE

Neil G. Bond1, Emily J. Engel1, Foday Al-Hasan2, Ibrahim M. Kanneh3, Lansana D. Kanneh4, Mambo Momoh4, Ibrahim Sumah4, Sruiti Chandra1, Robert F. Garry1, James E. Robinson1, Donald S. Grant4, Troy D. Moyn4, John S. Schieffelin1, Robert Samuels2
1Tulane University SOM, New Orleans, LA, United States; 2Kenema Government Hospital, Kenema, Sierra Leone; 3Tulane University School of Public Health, New Orleans, LA, United States

Since its emergence in December 2019, SARS-CoV-2 has swept the globe, infecting over 700 million and resulting in over 6 million deaths. While countries in the global north have been hard hit by the disease, Sub-Saharan Africa has been widely spared. There are multiple theories as to why Sub-Saharan African has not seen a large burden of SARS-CoV2 infection and disease including: younger age, fewer comorbidities, and pre-existing immunity to the disease. Our group has shown that approximately 30% of samples collected prior to the pandemic (n=120) as part of an ongoing cohort study in Sierra Leone tested positive for anti-SARS-CoV2 nucleoprotein (N) antibodies. In the current study, further this investigation in a cohort of 322 subjects with two pre-pandemic samples (collected at a median of July 2017 and March 2019) and one intra-pandemic sample collected in March 2022. We used the human-CoV V-plex panel 3 from Mesoscale Discovery to assess antibody reactivity to the spike protein from seasonal coronaviruses (229E, OC43, NL63, and HKU1), emerging coronaviruses (SARS-CoV1 and MERS), and to spike (S), nucleoprotein (N), and receptor binding domain (RBD) from SARS-CoV2. Our preliminary analyses show that similarly to previous studies (including our own) approximately 30% of study participants had reactivity to SARS-CoV2 N protein which is highly conserved in beta coronaviruses. We saw low, but notable, seropositivity to SARS-CoV2 (S) and (RBD). Surprisingly, despite the lack of recognized cases in Sierra Leone, we saw a large jump in seropositivity to all SARS-CoV2 proteins measured (80.1% N, 92.5% S, and 91.3% RBD) in by March 2022. Interestingly, we saw no neutralization in pre-pandemic samples tested but saw high levels of neutralization in the intra-pandemic samples confirming widespread SARS-CoV2 transmission despite the lack of recognized clinical disease. Next we will investigate the impact of pre-existing antibodies to seasonal and emerging coronaviruses on neutralizing titer and presumed protection from disease.

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FIRST STAGE GENOME-WIDE ASSOCIATION STUDY OF LYMPHATIC FILARIASIS PATHOLOGY IN AN AFRICAN POPULATION

Vera Serwa Opoku1, Sandeep Grover2, Linda Batsa Debrah2, Carlo Maj1, Jubin Osei-Mensah1, Derrick Adu Mensah1, Achim Hoerauf3, Alexander Yaw Debrah4, Johannes Schumacher2, Kenneth Pfarr2
1Kumasi Centre for Collaborative Research in Tropical Medicine, Kumasi, Ghana; 2Centre for Human Genetics, Philips University of Marburg, Marburg, Germany; 3Department of Clinical Microbiology, School of Medicine and Dentistry, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana; 4School of Veterinary Medicine, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana; 5Institute of Medical Microbiology, Immunology and Parasitology, Bonn, Germany; 6Department of Medical Diagnostics, Faculty of Allied Health Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

Human genetic factors that confer susceptibility or resistance to neglected infectious diseases remain unclear. One of such diseases is lymphatic filariasis (LF). LF results from infection with either of the three filarial nematode species: Wuchereria bancrofti, Brugia timori or B. malayi. An estimated 40 million individuals infected with the filarial nematodes present with the symptomatic LF manifestations of lymphedema (LE) and hydrocele. These symptoms develop in only a subgroup of infected people, and host genetics have been attributed to the disease heterogeneity. Studies that have sought associations between LF and host genetics have focused mainly on candidate genes. This study presents the first genome-wide association study (GWAS) to identify genetic markers involved in LF disease conducted in an African (Ghanian) population. Single nucleotide polymorphism (SNP) data from 3189 participants comprising 1508 LF cases and 1681 asymptomatic controls were analysed in the study. Cases were selected based on the presence of either LE and/or hydrocele while controls consisted of participants who had lived in the endemic community for at least 10 years prior to enrolment and had no LE and/or hydrocele. These unrelated participants were genotyped using the Illumina Global Screening array with multi-disease drop by Illumina®. Independent signals, rs2245413, rs2245710 and rs7742085 were observed on two loci at genome-wide significance (p<5x10^-8) to be associated with LF. Additionally, three HLA haplotypes (HLA- DPB1*04:02, -DRB1*03:02, and -C1*07:01) were found to be associated with LF. Other studies have associated these
SNPs with renal abnormalities, LE and hydrocele, respectively. The identified SNPs were mostly non-coding and located in the intergenic regions; and do not directly cause the symptomatic disease, but are rather proxy markers for nearby genes. This first stage GWAS in a Ghanaian population identified novel SNPs associated with LF risk and underscores the potential of GWAS to provide gene candidates for functional analyses as therapeutic targets towards the 2030 elimination by the World Health Organization.

GYPB GENE DELETIONS AFFECTS PLASMODIUM FALCIPARUM INVATION OFERYTHROCYTES AND GROWTH OF SIALIC ACID DEPENDENT PARASITES
Dominic SY Amuzu1, Lucas N. Amenga-Etego1, Collins M. Moranga1, Evelyn B. Quansah1, Henrietta Mensah-Brown1, Nancy K. Nyakoe1, Christina Hubbart2, Kate Rowlands2, Anna Jeffreys2, Kirk A. Rockett3, Dominic P. Kwiatkowski4, Gordon A. Awandare1, John Adams5, Oberstiller1, Thomas Otto2, julian Rayner3, Benoit Laleu4, Jeremy Burrows4, Du1, Alexandra Probst1, Flaminia Catteruccia3

1West African Centre for Cell Biology of Infectious Pathogens, University of Ghana, Accra, Ghana, 2Wellcome Centre for Human Genetics, University of Oxford, Oxford, United Kingdom, 3Welcome Sanger Institute, Wellcome Genome Campus, Cambridge, United Kingdom, 4Wellcome Sanger Institute, Wellcome Genome Campus, Oxford, United Kingdom

In malaria, invasion of erythrocyte by Plasmodium falciparum, mediated by the active interaction of parasite ligands and host receptors involves sialic acid (SA) dependent or SA independent invasion pathways. GYPB, GYPF and GYPC genes encode glycoporphins which serve as host receptors for the invasion of erythrocytes by the parasites. We determined effect of GYPB gene deletion on the invasion and growth of both SA dependent (Dd2) and independent (3D7) parasite strains of P. falciparum in vitro; and changes in the expression of other host erythrocyte surface proteins implicated in malaria pathogenesis. Growth and multipreference invasion assays were performed using erythrocytes genotyped by PCR-RFLP as wild type (GYPB non-DEL), heterozygous or homozygous for GYPB gene deletion. Flow cytometry analysis was used to determine relative expression of host erythrocyte surface proteins involved in malaria infection comparing erythrocytes with GYPB deletion and wild type. Erythrocytes heterozygous for GYPB deletion were preferentially invaded followed by the wildtype; homzygous were the least invaded. Dd2 strain had significantly (p<0.05) poor growth in erythrocytes homzygous for GYPB deletion compared to the wildtype. There was no significant (p>0.05) difference in growth of SA independent strain (3D7) in the different erythrocytes. Furthermore, expression of Band 3, transferrin, GYPB and GYPC significantly (p<0.05) increased in erythrocytes homzygous for GYPB gene deletion. Integrin was significantly increased in erythrocytes heterozygous GYPB gene deletion whereas there was significant (p<0.05) increases in expression of Complement Receptor 1 in erythrocytes homzygous and heterozygous for GYPB deletion. Basigin expression was relatively same in the three groups of erythrocytes. In conclusion, GYPB gene deletions affect parasite invasion and also protein expression on erythrocytes surfaces. Together, these finding suggests a mild protective nature of the GYPB gene deletion in malaria infection and may contribute to explanation for differences in malaria infectious outcomes in different individuals and populations.

OXIDATIVE STRESS AND ANTIMALARIAL RESPONSES OF BLOOD-STAGE PLASMODIUM FALCIPARUM
Camilla V. Pires1, Shulin Xu1, Min Zhang1, Chengqi Wang1, Jenna Oberstaller1, Thomas Otto2, Julian Rayner2, Benoit Laleu1, Jeremy Burrows1, John Adams1

1University of South Florida, Tampa, FL, United States, 2University of Glasgow, Glasgow, United Kingdom, 3University of Cambridge, Cambridge, United Kingdom, 4Medicines for Malaria Venture, Geneva, Switzerland, 5University of South Florida, TAMPA, FL, United States

Malaria caused by Plasmodium falciparum is one of the deadliest infectious diseases worldwide, responsible for more than 600,000 deaths in 2020. Rapidly evolving, drug-resistant parasites make the effort for finding new drug targets and understanding the cause of resistance a priority. Drugs treatment and fever condition, as well as infected sickle cells trigger oxidative stress in the asexual stages. Here, we hypothesize that oxidative stress is central to malaria parasite survival in blood stages. To test this, we performed large-scale forward genetic Plasmodium falciparum piggyBac transposon mutant screens to identify genes with altered sensitivity to oxidative stress. We observed that these genes are linked to lipid metabolism, exportome components, endocytosis, unfolded protein response and splicing machinery. Then, we compared the oxidative stress screen with two previous piggyBac genetic screens: dihydroartemisinin (DHA) and heat-shock screens. Mutants of interest growing in pre-induced oxidative stress were further exposed to selected MMV compounds as well as known antimalarials, such as DHA, Qinghaosu (QHS, artemisinin) and lumefantrine, to determine if the mutant's sensitivity to the drugs changes in the presence of elevated oxidative stress. Our data shows that exposure to environmental stress factors can alter the response of specific mutants to the inhibitory activity of some compounds. This shift seems to be due to overlap between the mechanism of action of the drug and the altered metabolic activity in the parasite due to the disrupted gene coupled to the commensurate metabolic changes induced by the parasite’s stress response. These results suggest that the disruption of artemisinin and oxidative stress sensitive genes likely enhanced the parasite’s capacity to mount a response to antimalarials, when the redox homeostasis is destabilized by the oxidative stress environment.
GENETICS OF THE INTERACTION BETWEEN PLASMODIUM FALCIPARUM AND ANOPHELES ALBIMANUS

Prince Chigozirim Ubiru, Sabyasachi Pradhan, Virginia Howick, Lisa Ranford-Cartwright
University of Glasgow, Glasgow, United Kingdom

Plasmodium falciparum isolates vary in their infectivity towards different species of Anopheles mosquitoes. This research aims to identify the genetic differences between P. falciparum parasite genotypes that might explain the variation in infectivity to the Central and South American mosquito vector, An. albimanus, and the African vector An. gambiae s.s. These two mosquito species varied in their refractoriness to two clones of P. falciparum (3D7 & HB3), which have been genetically crossed and many recombinant progeny produced. A linkage analysis approach (Quantitative Locus Analysis; QTL) was used to identify the parasite loci involved in the infection prevalence and oocyst intensity of P. falciparum in An. albimanus. Gametocytes of 3D7, HB3 and 17 recombinant progeny clones were grown in vitro, and were fed to mosquitoes using the membrane feeding method. The infected mosquitoes were dissected 10-11 days post-feeding and the midgut was examined for the presence of oocysts. QTL analysis identified three novel loci of which two loci are linked to the infection prevalence, named PfAlbip 1 and PfAlbip 2, spanning 79 kb & 183 kb, and containing 17 & 52 genes respectively. One locus was linked to oocysts intensity, named PfAlbipoi 1, spanning 46.5 kb, and containing 18 genes. These are the first loci linked to infection prevalence and oocysts intensity of Plasmodium falciparum in An. albimanus.

FIRST EVIDENCE OF EXPERIMENTAL GENETIC HYBRIDIZATION BETWEEN CUTANEOUS AND VISCERAL STRAINS OF LEISHMANIA DONOVANI WITHIN ITS NATURAL VECTOR PHLEBOTOMUS ARGENTIPES

Hasna Riyal1, Andrea Paun2, Tiago R. Ferreira2, Nilakshi Samaranayake1, David Sacks2, Nadira Karunaweera1
1Department of Parasitology, Faculty of Medicine, University of Colombo, Colombo 08, Sri Lanka. 2National Institute of Allergy & Infectious Diseases, National Institutes of Health, Bethesda, MD, United States

Leishmaniasis is a neglected tropical disease caused by the protozoan parasites of the genus Leishmania. More than 20 species of this genus are known to cause disease in humans and other animals. L. donovani species complex is known to have a vast diversity of clinical manifestations in humans but underlying mechanisms for such diversity are yet unknown. Even though these parasites are shown to have a strict asexual life cycle; inter and intra-species genetic exchange of Leishmania spp., through a meiosis-like process in their invertebrate stages of the life cycle have been shown. We investigated the ability of two distinct variants of L. donovani which are responsible to cause visceral and cutaneous forms of the disease, to undergo genetic exchange, inside its natural vector Phlebotomus argentipes sandfly species. Clinical isolates of L. donovani from a Sri Lankan patient with cutaneous leishmaniasis and an Indian patient with visceral leishmaniasis were used as parental strains. Parasites were genetically modified to have single drug-resistant markers along with a fluorescent tag and they were re-suspended in mice blood and fed to sandflies. After the 8th day of post-infection, sandflies were dissected and the midgut products were placed in double drug media to selectively grow hybrids. Results revealed two hybrid progenies out of 72 independent mating events that occurred in individual sandflies with a nearly 3% efficiency of hybridization. The intra-species genetic hybridization of L. donovani may explain the extensive phenotypic variations seen in patients in the Indian subcontinent. This provides the first evidence of the hybridization of L. donovani within its natural host Ph. argentipes and also confirms the existence of a sexual life cycle during its extracellular promastigote stages.

REDUCING MORTALITY DUE TO MALARIA THROUGH IMPROVED HEALTH WORKER PRACTICE, LESSONS FROM A DATA DRIVEN MENTORSHIP PROGRAM IN BUSOGA REGION IN EASTERN UGANDA

Patricia Mukose1, Chris Mugenyi1, Edward Mugwanya1, Susan Nabire1, Irene Ayaa1, Richard Opio Ongom1, Irene Ochola1, Angela Katee1, Myers Lugern1, Patrick Bukom1, Dorah Anika Talinde1, Amy Casella1, Aliza Hasham1, Benjamin Binagwa1, Natalia White1
1John Snow Inc, Kampala, Uganda, 2MCD Global Health, Kampala, Uganda, 3Ministry of Health Uganda, Kampala, Uganda, 4John Snow Inc, Boston, VA, United States

Maintaining adequate malaria surveillance systems is critical for any country to reduce malaria related deaths. According to the World Malaria Report 2022, Uganda contributed 5.1% to the global malaria cases and 3.2% to malaria-related deaths. Busoga Region, with a malaria prevalence of 21% in the Malaria Indictor Survey 2019, reported 390 malaria deaths in 2022 compared with the national average of 4,643 deaths. Malaria death audits, led by the Ministry of Health and health workers at facilities where the death occurred, aim to improve patient care, and avert future deaths. They are often not conducted. The PMI Uganda Malaria Reduction Activity, implemented by JSI, works with the MOH to strengthen malaria prevention and response efforts at all levels in the five highest burden regions of Uganda. In Busoga, a data driven mentorship approach was used to implement death reviews in 84%, 436 out of 517 health facilities. Between June and December 2022, patient records were reviewed to assess patient history, diagnosis, and treatment including complications, care and causes of death. Immediate feedback was provided and action plans developed to address gaps. Weekly malaria data bulletins were assembled, drawing from national HMIS surveillance data, and shared to monitor progress. Between April to December 2022, weekly surveillance data reporting by health facilities improved from 67% to 84% and the number of reported malaria deaths reduced from 103 to 56 deaths. Malaria in pregnancy deaths reduced from 38 to 6. Prompt identification of malaria deaths drove the formal introduction of malaria death audits and reviews in mentored hospitals and other high level health facilities. Better surveillance in Busoga region through targeted interventions with MOH may have led to improved reporting, early detection of epidemics and contributed to reduced malaria deaths. Improved surveillance coupled with targeted clinical interventions at health facilities can improve reporting, data for decision making, and management of severe malaria cases, leading to a reduction in malaria deaths reported at health facilities.
since policy changes can affect the selection of resistant strains across borders. GenFe-Mekong is project conducting genomic surveillance in partnership with National Malaria Control Programs in the Greater Mekong Subregion (GMS). Here, we present an analysis of epidemiological changes, based on 6,910 P. falciparum dried blood spot samples routinely collected from symptomatic patients in Lao PDR (2017 - 2021), Vietnam (2017 - 2021) and Cambodia (2017, 2020 - 2021). Samples were processed using the SpotMalara amplicon sequencing platform, which produces genotypes for markers of resistance to several antimalarials, and 101-SNP genetic barcodes. To facilitate translation of these data into actionable information for public health, we developed the grcMalaria R package, which produces intuitive geographical maps of prevalence, diversity and relatedness. This software library is also capable of identifying circulating strains, characterizing their drug resistance profile, and mapping their spread. Since 2020, a decline in case numbers was observed, coinciding with the decline of the dhfr/dhfrresistant KEL1/PLA1 lineages which dominated the GMS for years. Piperazine resistance reduced from 60% in 2017-2020 to 4% in 2021, after Cambodia, Thailand, and Vietnam switched from DHA-PQQ to other artemisinin-based combination therapies. Interestingly, this decline also occurred in Laos, where DHA-PQQ was not in use. Artemisinin resistance levels remained high, with an overall prevalence of 71%. Cluster analyses revealed that former KEL1/PLA1 lineages highly prevalent in Vietnam lost their pfcrt-375Thr amplification in the absence of piperazine pressure. In Cambodia, where artemether-mefloquine has been selected as front-line treatment, there is currently no indication of emerging resistance to mefloquine. Our results indicate that cross-border genetic surveillance is a strategic knowledge tool to inform elimination interventions.

**METHODOLOGY FOR MALARIA EPIDEMIOLOGICAL SURVEILLANCE USING FACILITY BASED DATA FROM SOUTHERN SENEGAL**

Daniel Zachary Hodson1, Médecine Diouf2, Mamadou Lamine Diouf3, Boutbacer Kandé4

1United States Peace Corps Senegal, Dakar, Senegal, 2National Malaria Control Program, Dakar, Senegal, 3Médina Yoro Foulah Health District, Médina Yoro Foulah, Senegal

Estimates of the heavy malaria burden in Senegal have varied. In 2013, the routine surveillance system calculated annual incidence by dividing the number of reported new malaria cases by the catchment area. This produced an average annual incidence of 380 cases per 1000 persons in the catchment area of 172 health facilities. In 2015, the routine surveillance system calculated annual incidence by dividing the number of confirmed new malaria cases by the catchment area. This produced an average annual incidence of 340 cases per 1000 persons in the catchment area of 120 health facilities. The updated data from the 2015 routine surveillance system was used to inform the national malaria control program. The updated data was used to inform the national malaria control program. The updated data was used to inform the national malaria control program.

**LOSSING SLEEP OVER DENOMINATORS: A NOVEL METHODOLOGY FOR MALARIA EPIDEMIOLOGICAL SURVEILLANCE USING FACILITY BASED DATA FROM SOUTHERN SENEGAL**

Daniel Zachary Hodson1, Médecine Diouf2, Mamadou Lamine Diouf3, Boutbacer Kandé4

1United States Peace Corps Senegal, Dakar, Senegal, 2National Malaria Control Program, Dakar, Senegal, 3Médina Yoro Foulah Health District, Médina Yoro Foulah, Senegal

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**QUANTIFYING THE VALUE OF ENTOMOLOGICAL SURVEILLANCE FOR PROGRAMMATIC DECISION-MAKING ON MALARIA CONTROL IN SUB-SAHARAN AFRICA**

Nora Schmit, Hillary M. Topazian, Matteo Pianella, Giovanni D. Charles, Peter Winskill, Penelope A. Hancock, Ellie Sherrard-Smith, Katharina Hauck, Thomas S. Churcher, Azra C. Ghani

Imperial College London, London, United Kingdom

The availability of many different tools for malaria control leads to complex decisions on the most cost-effective intervention package based on local characteristics in a setting. We aimed to quantify the monetary value of information provided by entomological surveillance for programmatic decision-making. We used a mathematical model of Plasmodium falciparum to simulate the 3-year impact and cost of various intervention packages in a range of transmission settings in sub-Saharan Africa. Interventions consisted of combinations of increasing insecticide-treated net (ITN) usage, switching from pyrethroid-only to next-generation PBO or pyrrolo ITNs,
and alternatives to vector control (increased treatment, seasonal malaria chemoprevention and/or the RTS,S vaccine). We compared their net monetary benefit at a threshold of US$250 per day averted and calculated the value of resolving uncertainty about the level of pyrethroid insecticide resistance, the associated effectiveness of ITNs, and biting behaviour in the local mosquito population. Across transmission settings, the most cost-effective intervention package on average was switching to pyrethroid ITNs, increasing ITN usage and increasing vector control alternatives, but there was uncertainty in the optimal intervention in each setting. The median expected value of perfect information on the entomological indicators was US$0.09 (range 0.01-0.72) per person at risk, corresponding to 0.17 (range 0.03-1.02) times the annual cost of distributing pyrethroid ITNs at baseline. This was highest in high-prevalence highly seasonal settings and lowest in low-prevalence perennial and seasonal settings. Resistance levels and ITN effectiveness influenced intervention choice most, but the value of data collection on a single indicator was 0 in 89% of settings. These results suggest that further investments in entomological surveillance are needed to facilitate decision-making on malaria interventions. Integrated programmes for data collection on a range of entomological factors are preferable, while prioritisation of areas based on seasonality and prevalence could be considered.

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MALARIA ATLAS PROJECT (MAP) HIGH BURDEN HIGH IMPACT (HBHI) GEOSPATIAL FRAMEWORK; LESSONS LEARNT FROM SUPPORTING THE GLOBAL FUND APPLICATIONS

Punam Amrati1, Samuel Oppong2, Tasmin Symons3, Susan Rumiha1, Beatriz Galatas3, James Colborn4, Balthazar Candrinho5, Celestin Danwang4, Mark Connell1, Abdisalan Noor3, Peter Gething1

Mozambique, Maputo, Mozambique

3World Health Organization, Geneva, Switzerland, 4Clinton Health Access Initiative, Boston, MA, United States, 5National Malaria Control Program Mozambique, Maputo, Mozambique

Since inception of High Burden High Impact (HBHI) Initiative in 2018, Malaria Atlas Project (MAP) have provided estimates of burden metrics - particularly prevalence to support namely the HBHI countries. Between the cycles, the MAP team have worked to build a novel modelling framework. Such framework leverages strengths from local surveillance data coming from multiple streams, for example, routine case incidence routinely collected from health systems (DHS2) and nationally representation cross-sectional surveys collecting information on malaria prevalence, fevers, treatment seeking and treatment (MIS/DHS). In turn, we are able to produce robust and accurate sub-national predictions of malaria prevalence and incidence at the finest temporal cadence available from the data (e.g. monthly), with intention that these maps would support evidence based decision making. This framework encapsulates many of the same conceptual thinking of malaria transmission from fever in the community, care seeking behavior, response to effective treatment, parasite clearance and testing rates. The framework builds on guidelines outlined by WHO when adjusting routine incidence data from health systems and takes advantage of several modelling frameworks at each stage to provide an accurate risk map. It further builds in a flexible and locally driven relationship learnt in space-time between prevalence and incidence. Here we present the outputs created for an HBHI country and review both metrics of incidence and prevalence against the previous iterations used in sub-national tailoring and discuss how use of different metrics can affect choices in the sub-national tailoring exercise. We explore how different analytical ways to categorize burden metrics can change perception of high, medium, and low risk in areas and provide recommendations to curb known biases.

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PROGRESS TOWARDS INTERRUPTING TRANSMISSION OF ONCHOCERCIASIS IN ETHIOPIA

Tekola Endeshaw1, Aderajew Mohammed1, Abebuul Yilak2, Yewondwosen Biteit2, Tewodros Seid1, Geremew Haileyesus1, Desalegn Jemberie1, Mohammed Hassan1, Fikreselassie Samuel1, Yohannes Eshetu1, Fanta Ngussie2, Fetene Mihretu1, Kadu Meribo2, Fikre Seife1, Emily Griswold2, Anley Haile1, Jenna E. Coalson3, Gregory S. Nolan4, Frank O. Richards4, Zerihun Tadesse1

1The Carter Center, Addis Ababa, Ethiopia, 2University of Tübingen, Tübingen, Germany, 3Federal Ministry of Health, Addis Ababa, Ethiopia, 4RTI International, Center for Disease Control and Prevention, Atlanta, GA, United States

Ethiopia aims to eliminate transmission of onchocerciasis by 2030. The implementation unit for treatment decisions in Ethiopia is the district, which usually has a population of <200,000. Districts qualify for impact assessments after at least 10 rounds of semi-annual ivermectin mass drug administration (MDA) with at least 80% coverage of the eligible population. If Ov16 antibody prevalence in samples in 300–500 children 5–9 years of age is <1%, the district may proceed to stop MDA serological and entomological surveys. For stop MDA surveys, 2–3 districts were clustered together into operational transmission zones (OTZ) according to geographical proximity, common river basins, and similar epidemiology. A combination of multi-stage random and purposive cluster sampling was used to select the study sites and population. From each OTZ, more than 3,000 dried blood spots (DBS) from children aged 5–10 years and at least 6,000 Simulium vectors were collected in accordance with WHO guidelines. Of the 96 districts undergoing impact assessment from 2016 to 2022, 73 (75%) had ≤ 1.0% Ov16 prevalence and became eligible for stop MDA evaluations. More than 58,500 DBS were then collected for these studies from Oromia, Southwest Ethiopia, and Amhara regions between 2017 and 2022. DBS were analyzed for Ov16 antibody prevalence by ELISA, while more than 40,000 black flies were collected and analyzed by O-150 PCR. Significant reductions in onchocerciasis prevalence were observed in many districts, with 28 (38%) satisfying WHO’s stop MDA criteria of Ov16 antibody prevalence below 0.1% and black fly infectivity significantly less than 1/2000. An additional 25 (34%) districts passed the serological criterion but need entomological evaluation. In contrast, 19 (26%) districts failed their stop MDA evaluation and need to continue MDA with improved vector control alternatives, but monitory benefit at a threshold of US$250 per day averted calculated to be expected value of perfect information on the entomological indicators was US$0.03-1.02) times the annual cost of distributing pyrethroid ITNs at baseline. US$0.09 (range 0.01-0.72) per person at risk, corresponding to 0.17 (range 0.03-1.02) times the annual cost of distributing pyrethroid ITNs at baseline. This was highest in high-prevalence highly seasonal settings and lowest in low-prevalence perennial and seasonal settings. Resistance levels and ITN effectiveness influenced intervention choice most, but the value of data collection on a single indicator was 0 in 89% of settings. These results suggest that further investments in entomological surveillance are needed to facilitate decision-making on malaria interventions. Integrated programmes for data collection on a range of entomological factors are preferable, while prioritisation of areas based on seasonality and prevalence could be considered.

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RESULTS FROM AN ENHANCED TRACHOMA IMPACT SURVEY IN FOUR LOW PREVALENCE DISTRICTS OF MOZAMBIQUE, 2022

Henis Sito1, Felizmina Zita1, Mawo Falli1, Tamimo Momade2, Rebecca Flueckiger1, Will Oswald1, Mabula Kasubi2, Molly Adams1, Thuy Doan3, Thomas Lietman6, Ben Arnold6, Sarah E. Gwyn7, Diana Martin1, Jeremiah M. Ngondi2

1Ministry of Health, Maputo, Mozambique, 2RTI International, Addis Ababa, Ethiopia, 3Federal Ministry of Health, Addis Ababa, Ethiopia, 4RTI International, Washington DC, DC, United States, 5Muhimbili University of Health and Allied Sciences, Dar es Salaam, Tanzania, United Republic of, 6Proctor Foundation, UCSF, San Francisco, CA, United States, 7Centers for Disease Control and Prevention, Atlanta, GA, United States

In Mozambique 47 out of 66 endemic districts have attained elimination of trachomatous Inflammation-Follicular (TF, prevalence <5%). However, persistent and recrudescence trachoma is a growing problem with districts reporting TF prevalences of 5–9.9%. It is not clear if this level of TF prevalence (5-9.9%) is due to ocular chlamydial infection or statistical sampling errors. We added testing of ocular Chlamydia trachomatis (Ct) infection and serology into routine trachoma impact surveys (TIS) to investigate: 1) if TF indicates conjunctival Ct infection in low prevalence settings; 2) population-level measures of trachoma transmission based on
antibody response; and 3) correlations of TF, Ct infection and serology. An enhanced TIS was done in four districts where 24 clusters were sampled in each district, and 35 households surveyed per cluster with the aim of including 1,164 children aged 1 to 9 years. The survey combined eye examination for trachoma with sampling of ocular swabs and dried blood spots (DBS). Ocular swabs were tested for Ct infection using iAMP CT detection Kit while DBS were tested for chlamydial pgp3 using lateral flow assay. We calculated yearly seroconversion rates using age-standardized district-level seroprevalence and examined correlation between cluster prevalences of each diagnostic result. TF prevalence ranged from 1.1% (95% confidence interval [CI]=0.3-2.2%) in Ilha Mozambique to 6.0% (95% CI=2.8-8.6%) in Inhassouga. Ct infection prevalence ranged from 1.1% (95% CI=0.2-2.5%) in Ilha de Moçambique to 4.8% (95% CI=2.2-7.4%) in Mossuril. PgP3 prevalence ranged from 8.4% (95% CI=5.1-14.3%) in Ilha Mozambique to 25.1% (95% CI=(22.5-27.9%) in Inhassouga. Yearly seroconversion rates ranged from 2.1 (95% CI=1.7-2.5) in Ilha de Moçambique to 6.6 (95% CI=5.8-7.4) in Inhassouga. Correlations of TF, Ct infection and pgp3 serology suggest ongoing transmission of ocular chlamydia in three out of four districts. Thus, despite having attained TF prevalence of <5%, further interventions or monitoring should be considered in Mossuril and Nacala-A-Velha districts.

**SOIL TRANSMITTED HELMINTH INFECTIONS FOLLOWING FOURTEEN YEARS OF MASS DRUG ADMINISTRATION IN SIERRA LEONE**

Ibrahim Kargbo-Labour1, Mohamed Bah2, Victoria Turay3, Abdulai Koroma3, Elisabeth Chop4, Patricia Houck3, Anna Phillips5, Angela Weaver6, Yaobi Zhang3

1Neglected Tropical Diseases Program, Ministry of Health and Sanitation, Freetown, Sierra Leone, 2Helen Keller International, Freetown, Sierra Leone, 3Helen Keller International, New York, NY, United States, 4Flinders University, Washington, DC, United States

Baseline mapping surveys conducted in 2008/09 in Sierra Leone found soil-transmitted helminth (STH) infections in all 16 districts, with moderate prevalence (≥20% to <50%) in eight districts and high prevalence (≥50%) in eight districts. Since 2008, STH mass drug administration (MDA) has been integrated with lymphatic filariasis (LF) MDA for all persons five years and above in all districts. In 2016, an impact assessment was conducted to assess the impact of five years of MDA. The results of this survey found three districts with low prevalence (<1% and <10%), five districts with prevalence >10% and <20%, and eight districts with moderate prevalence (≥20% and <50%). Current STH MDA is integrated with LF (1 district) or with onchocerciasis (targeting SAC only). A school-based impact assessment was conducted in October 2022, nine months after the last MDA, to determine the current prevalence and intensity of STH infections in children aged 5-14 years. The survey was carried out in 128 chiefdoms across nine districts (all identified as endemic for schistosomiasis in the 2016 assessment). A total of 201 communities were selected using probability proportional to population size of the chiefdoms, with specific sampling in large towns and small chiefdoms. Fresh stool samples were examined by Kato Katz (two slides per sample) from children aged 5-14 years. A total of 4,736 (male: 51%, female: 49%) children were examined by Kato Katz (two slides per sample) from children aged 5-14 years. The survey was carried out in 128 districts in Arm 1), from 23% in 2019/2020 to 5% in 2021/2022 (p<0.001) in Arm 2 (Bolosso Sore part of Arm 1), from 27.4% in 2019/2020 to 10.2% in 2021/2022 (p<0.001) in Arm 1 (remaining districts in Arm 1), from 23% in 2019/2020 to 5% in 2021/2022 (p<0.001) in Arm 2, from 49.6% in 2019/2020 to 43.4% in 2021/2022 (p<0.001) in Arm 3. The decrease in prevalence in Arm 3 was low compared to the other arms. The mean intensity of infection (based on Kato Katz egg count measures) for all parasite species decreased significantly in Arms 1 and 2, but not in Arm 3. The reduction in prevalence and intensity in Arms 1 and 2 revealed steady progress towards transmission interruption. More progress is required, through increasing MDA compliance (swallowing of treatment) in all villages. In the long term, continued improvements in WaSH are required to sustain the benefits achieved by cMDA.

**METHODS FOR TRACHOMA SURVEYS AMONG MOBILE AND MIGRANT POPULATIONS OF KWEEN AND BULAMBULI DISTRICTS, EASTERN UGANDA**

Stephen Begumisa1, Raphael Opor1, Joyce Achau1, Charles K. Kissaa2, Alfred Mubangizzi2, Sharone L. Backers1, Stella Agunyoa2, Edwin Mayoki2, Philip Kwembo2, Vincent Natega4, Clara Burgert5, Jeremiah M. Ngondi6

1RTI International, Kampala, Uganda, 2Ministry of Health, NTD Programme, Kampala, Uganda, 3Kween District Local Government, Kween, Uganda, 4Bulambuli District Local Government, Bulambuli, Uganda, 5RTI International, Washington DC, DC, United States

Uganda has made progress towards elimination of trachoma with 56 out of 61 endemic evaluation units having attained trachomatous inflammation-follicular (TF) prevalence of <5%. However, trachoma has remained persistent or recrudescent in Karamoja Region (Eastern Uganda) where mobile and migrant populations (MMP) comprising of nomadic pastoralists seasonally migrate across the porous Uganda/Kenya border. Kween and Bulambuli districts, both neighboring Karamoja region, were not suspected to be trachoma endemic. The two districts, seasonally, host MMP from neighboring Karamoja districts and West Pokot in Kenya where trachoma is still endemic. Given the high risk of trachoma where the nomadic pastoralists come from, there is need to determine the prevalence of trachoma among the pastoralists and to investigate the prevalence of trachoma among the host communities in Kween and Bulambuli districts. This trachoma baseline survey aimed to estimate prevalence of (TF) in children aged 1-9 years and trachomatous trichiasis (TT) in adults aged 15 years and above in Kween and Bulambuli districts and the MMP. Three evaluation units (EUs) were defined, and sampling frames developed comprising: 1) permanent settlements in Kween; 1) permanent settlements in Bulambuli and 3) temporary MMP cattle camps across both Kween
USE OF INVERSE DISTANCE WEIGHTING INTERPOLATION MODELLING AND GIS-BASED SPATIAL MAPPING TO ESTIMATE THE RISKS OF HOOKWORM AND INTESTINAL SCHISTOSOMIASIS INFECTIONS IN GHANA

Jeffrey G. Sumboh1, Yvonne Ashong1, Sedzro K. Mensah1, Jewelina Akorli1, Irene O. Donkor1, Elais A. Bempong2, Rahmat Yusuf1, Bright Idun2, Freda Kwartaing1, Frank T. Abogye1, Lisa Harrison3, Debbie Humphries1, Mike O. Atweneboana2, Michael Cappello2, Michael D. Wilson1

1Akros, Missoula, MT, United States, 2Akros, Lusaka, Zambia, 3Kenya Ministry of Health, Nairobi, Kenya, 4EndFund, New York, NY, United States

The global health community has established 2030 as the target year for the elimination of Neglected Tropical Diseases (NTDs), including hookworm and intestinal schistosomiasis. The current strategy to achieve this objective includes integrated implementation of mass drug administration of deworming drugs, along with WASH and health education interventions. Questions have been raised about the feasibility of achieving the goal, especially with the challenges of implementation in low-resourced settings and hard-to-reaching areas. To enable a focused targeting of resources to reduce transmission and achieve elimination in endemic communities, we aimed to identify the high-risk areas for hookworm infection and intestinal schistosomiasis in Ghana using Geographic Information Systems (GIS)-based spatial analysis with inverse distance weighting (IDW) interpolation.

Residents from 52 communities in 17 districts from 11 regions across Ghana were enrolled in a baseline survey. 47,753 individuals were surveyed, and stool samples were collected for the detection of intestinal parasites using the Kato-Katz method. The community-wide prevalences of hookworm infection (range: 1-37%) and intestinal schistosomiasis (range: 1-49%) were plotted and georeferenced on the spatial polygon of Ghana. The IDW mathematical interpolation model was used to estimate unknown values by specifying search distance, barriers, and closest points to produce country-wide risk maps. The predictive model revealed a 21% risk of hookworm in the middle belt of Ghana, and a 33% risk of intestinal schistosomiasis in northern and south-eastern Ghana, mostly in areas along the Volta Lake and irrigation schemes respectively. Our findings provide real-time data on drug stock levels used to make mid-campaign re-allocation decisions. Finally, microplanning resulted in better assessment of program performance, through improved supervision and accountability and faster and more accurate reporting. A total of 97% of respondents felt that developing a microplan increased their data use when planning the 2022 MDA campaign. 90% felt that Reveal resulted in more timely and accurate reporting. Discussions are underway about integrating the Reveal microplanning features into the improved eCHIS for scale in Kenya.
consistent and reliable P. vivax blood stage A. nancymaae model with well-characterized genomic data. This forms the foundation for the evaluation of emerging blood stage vaccines.

A HUMAN ANTIBODY EPITOPE MAP OF PFS230D1 DERIVED FROM ANALYSIS OF INDIVIDUALS VACCINATED WITH A MALARIA TRANSMISSION-BLOCKING VACCINE

Wai Kwan Tang1, Camila H. Coelho1, Kazutoyo Miura1, Bergeline C. Nguemwo Tentokam1, Nichole D. Salinas1, David L. Narum1, Sara A. Healy1, Issaka Sagara2, Long A. Carol1, Patrick E. Duffy1, Niraj H. Tolia1
1National Institutes of Health, Bethesda, MD, United States, 2University of Sciences, Techniques, and Technology, Bamako, Mali

Pfs230 domain 1 (Pfs230D1) is an advanced malaria transmission-blocking vaccine antigen demonstrating high functional activity in clinical trials. However, the structural and functional correlates of transmission-blocking activity are not defined. Here, we isolated sixty-three human monoclonal antibodies (hmAbs) with diverse transmission-reducing activity from vaccinees immunized with Pfs230D1. Epitope binning data from seventeen hmAbs showed they interact with distinct regions of Pfs230D1. We biophysically characterized the interaction of Pfs230D1 with nine individual hmAbs and showed they all have nanomolar affinities. Furthermore, we obtained atomic resolution structural definition of the binding epitopes of these complexes by X-ray crystallography. We compiled epitope-binning data and crystal structures of nine hmAbs complexes to construct a high-resolution epitope map and revealed the potent transmission-reducing hmAbs bound to one face of Pfs230D1 while the non-potent hmAbs bound to the opposing side. An additional structure of Pfs230D1D2 revealed that the non-potent transmission-reducing epitopes are occluded by the second domain. We further examined synergistic antibody combinations that may facilitate passive transfer of human antibodies as transmission blocking interventions. The hmAb epitope map identified binary hmAb combinations that synergized for extremely high-potency transmission-reducing activity. This work provides a high-resolution guide for structure-based design of enhanced immunogens, develops potent antibody combinations to combat transmission, and informs diagnostics that measure the transmission-reducing response.

TWO NOVEL PFS230 DOMAINS DISCOVERED AS TARGETS FOR MALARIA TRANSMISSION-BLOCKING ANTIBODIES

Matthijs M. Jore1, Maartje R. Inklaar1, Roos M. De Jong1, Ezra T. Bekkering1, Lisanne Hubregtse1, Maartje Meijer1, Hiroki Nagaoka2, Felix L. Fennemann3, Karina Teelen1, Rianne Stoter1, Marga van de Sande-Bolmer1, Geert-Jan van Gemert1, C. Richter King4, Nicholas I. Proellochs1, Teun Bousema1, Eizo Takashima2, Takafumi Tsuboi5
1Department of Medical Microbiology, Radboudumc, Nijmegen, Netherlands, 2Division of Malaria Research, Proteo-Science Center, Ehime University, Matsuyama, Japan, 3Division of Tumor Immunology, Radboudumc, Nijmegen, Netherlands, 4Path1s Center for Vaccine Innovation and Access, Washington, DC, DC, United States, 5Division of Cell-Free Sciences, Proteo-Science Center, Ehime University, Matsuyama, Japan

Malaria transmission-blocking vaccines (TBVs) aim to induce antibodies that block Plasmodium parasite development in the mosquito midgut, thus preventing formation of infectious mosquitoes. The clinically most advanced TBV candidate is Pfs230-D1M, which contains part of the Pro-domain (Pro) and first of fourteen 6-Cys domains (D1) of the gamete surface protein Pfs230. Whether other domains of Pfs230 contain epitopes that are targets for transmission-blocking antibodies is unknown. We identified a murine monoclonal antibody (mAb), 18F25.1, that targets an epitope outside Pfs230-ProD1. Using a panel of recombinant Pfs230 fragments produced in wheat germ cell-free system, we demonstrate that mAb 18F25.1 targets Pfs230-Domain 7 (D7). All functional Pfs230 mAbs described to date are complement-dependent, whereas 18F25.1 is a non-complement-fixing subclass mAb. We therefore generated a subclass-switched antibody, mAb 18F25.2a, using a CRISPR/Cas9-based hybridoma engineering method. mAb 18F25.2a potently lysed female gametes in vitro whereas the parental mAb 18F25.1 did not. Importantly, mAb 18F25.2a strongly, in a complement-dependent manner, reduced P. falciparum infection of Anopheles mosquitoes in standard membrane feeding assays (SMFAs). Inspired by this finding, we attempted expression of all fourteen individual Pfs230 domains in insect cells. Eight fragments were obtained in sufficient quantity for mouse immunizations. Sera raised against one of the non-ProD1 and non-D7 domains almost completely blocked parasite transmission in SMFA, with similar potency as sera raised against ProD1. The induced transmission-blocking antibodies were fully dependent on complement. We have raised a panel of 15 mAbs against this domain and are currently assessing epitope specificity and potency in SMFA. Results from these analyses will also be shared during the meeting. After 30 years of research on this leading TBV candidate, we provide the first conclusive evidence that Pfs230 domains D2-14 contain functional targets. Our study provides a strong incentive to further evaluate these identified Pfs230 domains as TBV candidates.

NOVEL BLOOD-STAGE VACCINE CANDIDATE RH5.1/ AS01B ELICITS A MIX OF NEUTRALIZING AND NON-NEUTRALIZING PLASMA IGG LINEAGES IN MALARIA-NAIVE UNITED KINGDOM ADULTS

Jeffrey Marchioni1, Allison Seeger2, Jordan R. Barrett3, Kirsty McHugh2, Jessica Kain1, Randall S. MacGill4, George Georgiou1, Simon J. Draper2, Jason J. Lavinder1, Gregory C. Ippolito1
1The University of Texas at Austin, Austin, TX, United States, 2Oxford University, Oxford, United Kingdom, 3University of Wisconsin, Madison, WI, United States, 4Center for Vaccine Innovation and Access, PATH, Washington, DC, United States

Malaria Plasmodium falciparum blood-stage infection, initiated by merozoites, is susceptible to vaccine-induced antibodies. Reticulocyte Binding Protein Homologue 5 (RH5) is a merozoite surface antigen that has low polymorphism frequencies and uses a non-redundant red blood cell (RBC) invasion pathway, overcoming bottlenecks of previous blood-stage vaccine antigen candidates. The Draper Lab (Oxford University) clinically tested RH5.1, an engineered variant of RH5, in AS01B adjuvant (GSK) designed to boost long-lasting, protective antibody titer. United Kingdom malaria-naive adult volunteers were vaccinated with RH5.1/AS01B and challenged with controlled human malaria infections. RH5-specific B cells were isolated from volunteers and their B cell receptors (BCRs) were sequenced, from which potent, neutralizing monoclonal antibodies (mAbs) were discovered able to inhibit merozoite invasion and growth in vitro. In contrast, the polyclonal plasma IgG of volunteers exhibited an average neutralizing potency over 10-fold greater than the average potency of individual mAbs cloned from B cells. This observation raised a question: what is the disconnect between the BCR and circulating IgG repertoires? To address this knowledge gap, we completed BCR sequencing coupled with plasma IgG proteomics to characterize in depth the polyclonal plasma antibody repertoires of individual volunteers. Plasma mAbs were identified that bind to previously described merozoite inhibitory epitopes, including those that block binding between RH5 and its RBC coreceptor, CD147 (Basigin), alongside a unique population of mAbs that synergize with neutralizing mAbs for increased potency. Notably, in the plasma repertoire of some donors, non-neutralizing mAbs targeting cryptic epitopes on the N- and C-terminus were found at much higher relative abundance than neutralizing mAbs; these mAbs are not well understood and suggest unknown functions. This dynamic between neutralizing and non-neutralizing plasma mAbs needs to be further explored in the context of blood-stage protection and future RH5 vaccine engineering efforts.

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PROTECTIVE IMMUNE MECHANISMS INDUCED BY THE RTS,S MALARIA VACCINE IN A PEDIATRIC PHASE IIb CLINICAL TRIAL

Lirije Kurtovic1, Gaoqian Feng1, Alessia Hysa1, Ali Haghir2, Katherine O’Flaherty1, Bruce D. Wines3, Rebeca Santano3, Heidi E. Drummer1, P. Mark Hogarth1, Jahit Sacarlal4, Freya J. I. Fowkes1, Julie A. Simpson2, Carlota Dobaño3, James G. Beeson1

1Burnet Institute, Melbourne, Australia, 2The University of Melbourne, Melbourne, Australia, 3ISGlobal, Hospital Clinic Universitat de Barcelona, Barcelona, Spain, 4Centro de Investigación en Salud de Manícha, Maputo, Mozambique

Plasmodium falciparum malaria is a major cause of global morbidity and mortality, especially in children. RTS,S is the only available malaria vaccine, recommended for young children in regions with moderate to high malaria transmission. RTS,S is a virus-like particle expressing the major P. falciparum sporozoite surface antigen, circumsporozoite protein (CSP), but only has modest efficacy in malaria endemic populations. Protection is primarily mediated by anti-CSP antibodies, but the immunological mechanisms involved are unclear, which hinders efforts to improve RTS,S and develop next-generation vaccines. We recently discovered that anti-CSP antibodies can interact with serum complement proteins leading to parasite lysis and interact with Fcy-receptors (FcyRs) to promote opsonic phagocytosis and NK cell activation. Here, we evaluated multiple functional antibodies that interact with complement and FcyRs in a phase IIb pediatric trial of the RTS,S vaccine in Mozambique (n=737) and machine learning methods to identify responses most strongly predictive of protection. RTS,S broadly induced antibodies (IgG, IgM, and IgA isotypes) that interact with complement and FcyRs (FcyRI, FcyRIIa, FcyRII). Functional antibodies recognized the immunodominant central-repeat and C-terminal regions of CSP, which were correlated with the induction of IgG1 and IgG3 subclasses. Responses were variable and negatively correlated with age and magnitude of exposure to malaria. We identified antibody Fc-dependent functional activities targeting specific CSP domains that were associated with protection against malaria over 1.5 years of follow-up and found differences in protective associations in boys and girls. Our findings suggest that antibody Fc-dependent functional activities are important in vaccine-induced immunity, and the variable induction of responses may explain why efficacy is modest overall. Knowledge of the functional activities and targets of protective antibodies, sex-based differences, and the impact of malaria exposure on vaccine responses, provides a basis for designing novel vaccines to achieve higher efficacy.

THE FINAL RESULTS OF A FIVE-YEAR, DOUBLE-BLIND, RANDOMISED CONTROLLED PHASE 3 TRIAL OF SEASONAL VACCINATION WITH RTS,S/AS01E VACCINE WITH OR WITHOUT SEASONAL MALARIA CHEMOPREVENTION IN YOUNG CHILDREN IN BURKINA FASO AND MALI

Alassanne Dicko1, Jean-Bosco Ouedraogo2, Issaka Zongo2, Issaka Sagara1, Matthew Cairns2, Serge Rakisvende Yerbangana3, Djibrilla Issiaka4, Charles Zoogunara5, Youssouffa Sidibe1, Amadou Tapily1, Frederic Nikiema6, Frederic Sompougoudi2, Koualy Sanogo2, Mahamadou Kaya3, Hama Yalcouye1, Oumar M Dicko1, Modibo Diarra1, Kalifa Diarra1, Ismalia Thera1, Alassane Haro2, Abdou Aziz Sienou2, Seydou Traore2, Almahamadou Mahamar1, Amagana Dolo1, Irene Kuepfer2, Paul Snell3, Jane Grant3, Jayne Webster3, Paul Milligan3, Cynthia Lee3, Christian Ockenhouse4, Opoku Ofori-Aninyam3, Halidou Tinto2, Abdoulajeh Djimde1, Daniel Chandramohan1, Brian Greenwood2

1MRCT, Bamako, Mali, 2INSTech/IRSS, Bobo-Dioulasso, Burkina Faso, 3LSHTM, London, United Kingdom, 4PATH, Seattle, WA, United States, 5GSK, Wavre, Belgium

Seasonal vaccination with the RTS,S/AS01E malaria vaccine combined with Seasonal Malaria Chemoprevention (SMC) prevented malaria in young children more effectively than either intervention given alone over a period of three years. The study objective was to determine whether this protection could be sustained when further doses of seasonal vaccination with RTS,S/AS01E were given until children reach the age of five years. Children 5-17 months of age were initially randomised to one of the three treatments groups, SMC plus control vaccines, RTS,S/AS01E plus placebo SMC, or SMC plus RTS,S/AS01E, and continued to receive the same interventions for two additional years. Over five year period of the trial, the incidence rate of clinical malaria per 1000 person-years at risk was 313 in the SMC alone group, 320 in the RTS,S/AS01E alone group, and 133 in the combined group. The combination of RTS,S/AS01E and SMC had a superior protective efficacy (PE) compared to both SMC (PE 57.7%, 95% CI 53.3, 61.7) and RTS,S/AS01E (PE 59.0%, 95% CI 54.7, 62.8) when given alone. RTS,S/AS01E remained non-inferior to SMC in preventing clinical malaria (hazard ratio 1.03 [95% CI 0.95, 1.12]). Over the five-year period, hospital admissions for WHO-defined severe malaria, malarial anaemia, blood transfusion, all-cause deaths, deaths excluding external causes/surgery, and deaths from malaria were reduced by 66.8% (95% CI 40.3, 81.5), 65.9% (95% CI: 34.1, 82.4), 68.1% (95% CI: 32.6, 84.9), 44.5% (95% CI: 2.77, 68.3), 41.1% (95% CI: -9.24, 68.3), and 66.8% (95% CI: -2.68, 89.3), respectively, in the combined intervention group compared to the SMC alone group. This study has shown the persistent high risk of malaria in children in Burkina Faso and Mali until they reach the age of five years and the need to provide these children with effective malaria control measures until they reach this age and perhaps beyond.

RESULTS FROM A PHASE III TRIAL EVALUATING THE R21/MATRIX-M MALARIA VACCINE

Alassanne Dicko1, Mainga Hamaluba2, Ally Olotu3, Jean Bosco Ouedraogo4, Halidou Tinto2, Mehreen S. Datoo5, Emma Beaumont7, John Bradley7, Katie J. Ewer6, Umesh Shaligram8, Adrian V.S Hill6, 1MRTC, Bamako, Mali, 2INSTech/IRSS, Bobo-Dioulasso, Burkina Faso, 3LSHTM, London, United Kingdom, 4PATH, Seattle, WA, United States, 5GSK, Wavre, Belgium

There is currently only one licensed malaria vaccine. Deployment is expected over 2023 but limited supply (6 million doses on average a
year for 3 years from 2023) may hinder efforts to substantially reduce the global malaria burden. Furthermore, its efficacy is below what is needed. Development of other malaria vaccines is required to achieve the WHO malaria goals by 2030. In 2021, 4800 participants, aged 5-56 months, were enrolled into a phase III double-blind, randomised controlled trial assessing the R21/MatFv-MTM™ adjuvant (R21/MM) malaria vaccine, at five African sites in Burkina Faso, Kenya, Mali, and Tanzania, with a range of transmission patterns (seasonal and perennial) and intensity. All participants were randomised 2:1, R21/MM: control rabbits vaccine. They received 3 doses, 4 weeks apart, followed by a booster dose a year later. At 12 months following the primary series of vaccinations, evaluation of time to first clinical malaria episode demonstrated vaccine efficacy (VE) of 75% [71-79] at the seasonal sites and 68% [60-74] at the perennial sites. When combining all sites, VE was 73% [69-76]. VE was significantly higher when comparing the 5-17-month age group (VE 78% [73-82]) with the 18-36-month age group (VE 69% [64-74]). At six months following a booster vaccination (one year after the primary series), VE was 75% [71-78] at the seasonal sites. VE did not change significantly when assessing single or multiple episodes of malaria. Six SAEs, all febrile convulsions, were assessed as definitely, probably or possibly related to study vaccination following approximately 19,000 doses of the malaria or rabies vaccine. Further safety and efficacy data from the second year of follow-up will also be presented. WHO prequalification and regulatory licensure applications are underway and initial deployment of R21/MM is expected in the coming months. The Serum Institute of India Pvt. Ltd have committed to large-scale manufacturing and supply. High-level efficacy of this vaccine, combined with the commitment to large-scale supply, at low cost, should have a significant impact now, and in the long term, on the lives of those in malaria endemic areas.

**EXAMINING THE EFFECT OF NEIGHBORHOOD LATRINE COVERAGE ON CHILDHOOD DIARRHEAL RISK IN RURAL BANGLADESH**

Hannah Van Wyk1, Andrew F. Brouwer1, Ayse Ercumen1, Jesse Contreras1, Matthew C. Freeman1, Joseph N. Eisenberg1

1University of Michigan School of Public Health, Ann Arbor, MI, United States, 2North Carolina State University Department of Forestry and Environmental Resources, Raleigh, NC, United States, 3Rollins School of Public Health Emory University, Atlanta, GA, United States

Sanitation in low-income settings may have surprisingly minimal direct health effect on children living in households with toilets; but evidence suggests that neighborhood latrine coverage may impact herd protection against diarrhea. The mechanisms of this herd protection, though crucial to the design of effective interventions, remain unknown. The WASH Benefits (WASH-B) Bangladesh trial, coupled with a nested environmental impact study among a subset of participants, provides a unique opportunity to lever the trial data to address this important question. Diarrheal prevalence was estimated among children enrolled in the sanitation and control arms of the parent trial. The nested study collected compound-level information on each neighboring compound (n=8,317) within 100 m of each study compound, including GPS coordinates, number of people in the compound, and number and quality of latrines. Using neighborhood data from the nested study, we developed a function to estimate total neighborhood fecal exposure within 100 m for each study compound, accounting for population density, reduction of fecal exposure from both hygienic and unhygienic latrines, and spatial distance between compounds. We then modeled the relationship between total neighborhood fecal exposure and diarrheal disease using logistic regression. We found the odds of diarrhea increased by 27% (OR = 1.27; 95% CI 0.97, 1.67; p = 0.08) for each tertile increase in total neighborhood fecal exposure, adjusting for child’s age and WASH-B treatment arm. Additional sensitivity analysis was used to explore the impact of sample size and parameter choice (e.g., risk ratio of hygienic versus unhygienic latrines and baseline risk) in the neighborhood fecal exposure function on the relative risk. This exposure function can be used in transmission models to investigate the indirect effect of community latrine coverage on diarrheal disease risk and can subsequently be used to inform site-specific coverage needs to achieve efficacious sanitation interventions.

**FIRST-EVER ENVIRONMENTAL SURVEILLANCE AT HUMANITARIAN SETTINGS IN COX’S BAZAR, BANGLADESH: DETECTION OF SARS-COV2 AND ENTERIC PATHOGENS**

Md Rezaul Hasan1, Md Ziaur Rahman1, Nuhu Amin1, Rehnuma Haque1, Md Shariful Islam2, Afroza Jannat Suchana1, Mohammad Ziaur Rahman1, Mohammad Enayet Hossain1, Mojinj Miah1, Suraja Raj1, Pengbo Liu2, Yuke Wang2, Marlene Wolfe2, Stephen Patrick Hilton2, Chloe Sveza2, Mahbubur Rahman3, Ahmed Nawsher Alam3, Zakir Hossain Habib3, Aninda Rahman3, David Otieno4, Feroz Hayat Khan4, Mahbubur Rahman1, Megan B. Diamond4, Tahmina Shirin5, Christine L. Moe6

1Environmental Interventions Unit, Infectious Diseases Division, International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), Dhaka 1212, Bangladesh, Dhaka, Bangladesh, 2School of Public Health, University of Queensland, Brisbane, Australia, Brisbane, Australia, 3One Health Laboratory, Infectious Diseases Division, International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), Dhaka 1212, Bangladesh, Dhaka, Bangladesh, 4The Center for Global Safe Water, Sanitation, and Hygiene at Emory University, USA, Atlanta, GA, United States, 5Institute of Epidemiology, Disease Control, and Research (IEDCR), Dhaka, Bangladesh, 6Communicable Disease Control (CDC) Program, Directorate General of Health Services (DGHS), Dhaka, Bangladesh, Dhaka, Bangladesh, 7World Health Organization (WHO) Sub-office, Cox’s Bazar, Bangladesh, Cox’s Bazar, Bangladesh, 8The Rockefeller Foundation, New York, NY, United States

Nearly one million forcibly displaced Rohingya refugees have been living in crowded camps of Cox’s Bazar, Bangladesh. The environment of camps is favorable for the spread of waterborne and respiratory diseases. Cox’s Bazar municipality has no centralized sewerage system, where household toilets are directly connected to community drains. We conducted environmental surveillance from October 2022 to February 2023 on three refugee camps (4 Ext, 9, 13) and Cox’s Bazar municipality to explore the spatial and temporal trend of four vaccine-preventable diseases causing pathogen-SARS-CoV-2, Group A Rotavirus, Salmonella typhi, and Vibrio cholerae. 192 wastewater samples (153 from refugee camps and 39 from the municipality) were collected from the end of the drain point and genetic markers were analyzed through multiplex qPCR. Rotavirus was positive in over 97% of the sample from both camps and the municipality. The positivity rate of S. typhi was much lower in both camps (5%) and municipality (18%). Positivity rates of SARS-CoV-2 were higher in the camps (68%) than in the municipality (31%), whereas positivity rates of V. cholerae were higher in the municipality (51%) than in the camps (29%). The highest log10 concentration of SARS-CoV-2 gene copies/Liter (gc/L) was detected in Camp 4 Extension [median=5.3 gc/L (range=4.2-6.9)], Rotavirus was in camp 9 [median=9.3 gc/L (range=6.9-10.8)], V. cholerae was in camp 15 [median=6.3 gc/L (range=5.4-6.9)] and S. typhi was in camp 15 [median=5.7 gc/L (range=4.9-6.4)]. Both in camps and municipality, positive detection of SARS-CoV-2 and V. cholerae declined from October 2022 to January 2023, but no temporal trends were observed for the other two pathogens. Environmental surveillance is a valuable tool for monitoring temporal and spatial trends of existing and emerging infectious diseases and provides guidance for public health measures especially in humanitarian settings.
ENTERIC PATHOGEN FLOWS AT CITYWIDE SCALES

Drew Capone1, Vanessa Monteiro2, Victoria Cumbane3, Edna Viegas4, Joe Brown3
1Indiana University, Bloomington, IN, United States, 2Centro de Investigação e Treino em Saúde da Polana Caniço, Maputo, Mozambique, 3University of North Carolina at Chapel Hill, Chapel Hill, NC, United States

Understanding the performance of extant sanitation infrastructure is a challenge for governments and development agencies in low-resource settings. A variety of tools have been developed to simplify the decision-making process. One tool that is being deployed widely is the "shit-flow diagram" (SFD), which pictorializes how safe and unsafe fecal flows occur throughout a city. However, the public health hazards of fecal waste streams are often not equal. Fecal wastes may vary substantially based on the localized disease burden and by the persistence of different pathogens. Our research aim was to overlay pathogen data onto the SFD to advance our understanding of how pathogens flow at citywide scales. First, we identified sampling locations across Maputo, Mozambique corresponding to the nodes on the city's SFD using satellite imagery and input from local government officials. Next, we collected 85 soil samples and 110 high-volume water samples. Sample collection locations included wastewater treatment plant influent and effluent, wastewater outfalls, surface waters, open drains, fecal sludges, and soils at solid waste disposal sites.

We cultured samples for fecal indicator bacteria, concentrated water samples, extracted nucleic acids, and quantified genes corresponding to 3gt;25 enteric pathogens using multiplex real-time PCR. We observed substantial variation in the pathogen profiles and concentrations between sampling locations. For example, median Giardia concentrations (gene copies per liter) were highest in fecal sludges (108.1), followed by wastewater influent (106.7), wastewater effluent (105.8), wastewater outfalls (105.5), flood waters (101.8), open drains (101.7), and surface water (101.3). We combined these quantitative data with the data from Maputo's SFD to generate novel pathogen flow tables, which visualize how pathogens move through the city. This approach may help prioritize investments in sanitation infrastructure to interrupt enteric pathogen transmission at citywide scales.

INEQUALITIES IN CHILD DIARRHEA AND EFFECT MODIFICATION OF WATER, SANITATION AND HANDWASHING INTERVENTIONS BY SOCIOECONOMIC POSITION AND MONSNO SEASON IN RURAL BANGLADESH: A SUBGROUP ANALYSIS OF A CLUSTER RANDOMIZED TRIAL

Pearl Anne Ante-Testard1, Francois Rerolle2, Anna Nguyên2, Sania Aslah3, Sarker Masud Parvez4, Abu Mohammed Naser5, Tarik Benmarnia6, Mahbubur Rahman7, Stephen P. Luby8, Jade Benjamin-Chung2, Benjamin F. Arnold2
1Francis I. Proctor Foundation, Department of Ophthalmology, University of California San Francisco, San Francisco, CA, United States, 2Department of Epidemiology and Population Health, Stanford University, Stanford, CA, United States, 3Department of Epidemiology and Health, Stanford University, Stanford, CA, United States, 4Environmental Interventions Unit, Infectious Diseases Division, International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh, 5Division of Epidemiology, Biostatistics, and Environmental Health, School of Public Health, University of Memphis, Memphis, TN, United States, 6Scripps Institution of Oceanography, University of California San Diego, San Diego, CA, United States, 7Division of Infectious Diseases and Geographic Medicine, Stanford University, Stanford, CA, United States

The benefits of water, sanitation and handwashing (WASH) interventions on child diarrhea could vary by household wealth and monsoon season. Identifying subgroups most likely to benefit can help inform equitable distribution of WASH programs. We conducted a secondary analysis of the WASH Benefits Bangladesh cluster randomized trial (8440 measurements, 360 clusters). We calculated Relative Index of Inequality (RII) and Slope Index of Inequality (SII) to measure relative and absolute inequalities in child diarrhea using an asset-based wealth index. We estimated effects of combined WASH by tertile of wealth index, and jointly by wealth tertile and monsoon season. We combined intervention trial effects estimated by wealth index with national surfaces of wealth and population to project diarrhea cases prevented by a combined WASH intervention throughout rural Bangladesh. Among the controls, we observed relative and absolute inequalities in child diarrhea favoring poorer households (RII-1 and SII-0, respectively) and that the WASH intervention reduced these inequalities. Reductions in diarrhea were largest in the poorest wealth tertile (interaction p-value<0.07) - with diarrhea prevalence of 8.1% in control versus 4.5% in WASH [difference=3.6% (95% Confidence Interval 1.4%, 5.7%)] and were larger during the monsoon season (interaction p-value < 0.001). There was no joint interaction between wealth and season, but reductions in diarrhea due to WASH were largest among the poorest tertile during rainy season (diarrhea prevalence of 10.3% in control versus 4.6% in WASH [difference=5.7% (2.7%, 8.6%)]). The projected diarrhea cases prevented by combined WASH across rural Bangladesh was 298 cases per 1000 children < 3 years per month, with marked heterogeneity by district. Our results show that the WASH Benefits intervention reduced the wealth disparity in diarrhea, with largest reductions in diarrhea amongst the poorest children during rainy season. The study provides a generalizable example of assessing equity of intervention effects and transporting effects from trials to help target programs to populations who would benefit most.
INFLUENCE OF TEMPERATURE AND PRECIPITATION ON THE EFFECTIVENESS OF WATER, SANITATION, AND HANDWASHING INTERVENTIONS ON CHILDHOOD DIARRHEAL DISEASE AND ENTERIC INFECTIONS IN BANGLADESH

Anna T. Nguyen1, Jessica A. Grembi1, Marie Riviere1, Gabriella Barratt Heitmann1, William D. Hutson2, Tejas S. Athni1, Arusha Raleigh, NC, United States, 4University of California, Berkeley, Berkeley, CA, United States, 5International Centre for Diarrhoeal Disease Research, Dhaka, Bangladesh, 67180

Diarhhea is a leading cause of child morbidity and mortality in Bangladesh. Water, sanitation, and hygiene (WaSH) interventions aim to reduce exposure to enteric pathogens, and weather and environment may impact effectiveness. Here, we investigated whether temperature and precipitation modified the effect of low-cost, household level WaSH interventions on diarrhea, Giardia, and soil-transmitted helminths (STH) infection. We merged remote sensing data on temperature and precipitation to households from a cluster-randomized trial in rural Bangladesh that measured the diarrhea, Giardia, and STH prevalence in children 0-2 years old from 2012-2016. To measure differences in WaSH effectiveness, we estimated prevalence ratios (PR) for WaSH interventions vs. control under different weather conditions using generative additive models and targeted maximum likelihood estimation. We found that WaSH interventions more effectively prevented diarrhea when there was heavy rain in the previous week (high rainfall PR = 0.38, 95% CI 0.23-0.62 vs. no heavy rainfall PR = 0.77, 95% CI 0.60-0.98) and during the rainy season, when effectiveness peaked at a PR of 0.35 (95% CI 0.19-0.65). WaSH interventions were also more effective against STH infections under heavy rain (PR 0.36, 95% CI 0.19-0.68 vs. 0.92, 95% CI 0.77-1.10), with the strongest effect modification for hookworm infections (PR 0.32, 95% CI 0.17, 0.64 vs. 0.82, 95% CI 0.64-1.05). There was moderate effect modification by weekly minimum temperature for Giardia infection, where the PR was 1.11 (95% CI 0.91-1.30) at 12.3°C and 0.81 (95% CI 0.64-0.99) at 21.0°C. Overall, WaSH interventions were more effective against childhood diarrhea, Giardia and STH infections under high precipitation and against Giardia infections under high temperatures. Our findings suggest that in settings similar to Bangladesh, WaSH interventions should be prioritized during the rainy season and in areas that are prone to extreme precipitation.

HUMAN FECAL CONTAMINATION OF HOUSEHOLD WATER AND SOIL AND ENTERIC PATHOGENS IN CHILD STOOL

David A. Holcomb1, Jackie Kneer2, Drew Capone2, Rassul Nalá3, Oliver Cumming2, Jill R. Stewart1, Joe Brown1

1University of North Carolina at Chapel Hill, Chapel Hill, NC, United States, 2London School of Hygiene and Tropical Medicine, London, United Kingdom, 3Indiana University Bloomington, Bloomington, IN, United States, 4Ministry of Health, Maputo, Mozambique

Recent household water, sanitation, and hygiene (WaSH) interventions have shown inconsistent effects on child health and modest impacts on fecal contamination, suggesting the household WaSH interventions did not prevent environmental exposure. Environmental exposure is often assessed using indicator organisms but relationships between fecal indicators and exposure to specific pathogens remain poorly characterized. We investigated whether E. coli and two human fecal markers (HF-183 and MnIIF) measured by quantitative polymerase chain reaction (qPCR) in urban Mozambican household soil and drinking water were associated with detection in child stool of eight bacteria, three viruses, and three protozoa measured by reverse transcription PCR and soil transmitted helminths (STH) assessed by microscopy. For each sample matrix and indicator, we used Bayesian multilevel logistic regression with pathogen-varying intercepts and slopes to obtain a pooled estimate of the overall indicator-pathogen relationship while simultaneously estimating pathogen-specific associations with adaptive shrinkage. At least one pathogen was detected in 86% (173/201) of child stools, most frequently Shigella (51%), Giardia (50%), and Trichuris (43%); norovirus GI/GII was the most common virus (14%). Increasing E. coli concentrations in drinking water were associated with elevated stool pathogen prevalence (pooled OR: 1.32, 95% CI: 0.99, 1.73), though significant only for Ascaris (OR: 1.7; 95% CI: 1.1, 3.1). The odds of detecting Shigella (OR: 0.46; 95% CI: 0.16, 0.91) or Giardia (OR: 0.53; 95% CI: 0.21, 0.99) in stool were lower when human marker HF-183 was detected in drinking water. No fecal indicator in soil was clearly associated with any pathogen. We did not find evidence to support human markers as reliable indicators of enteric pathogen carriage in a high-prevalence domestic setting. Future efforts to characterize environmental exposure pathways should prioritize direct pathogen assessment, which may offer richer, more relevant insights and be more responsive to WaSH conditions.

TOWARDS ARTIFICIAL INSEMINATION AND IN VITRO FERTILIZATION OF THE MALARIA ANOPHELES GAMBAE

Vincent O. Nyasembe, Claire Schregardus, Priscilla Bascunan, Mark Q. Benedict, Ellen M. Dotson

Center for Disease Control and Prevention, Atlanta, GA, United States

Malaria remains a leading cause of morbidity and mortality globally, hence there is a need for new control tools. The availability of the full genome of mosquito species that vector malaria and new molecular tools such as gene drive which target vector control through either population replacement or suppression. Consequently, numerous transgenic strains with potential for mosquito control have been generated by several research laboratories. However, continuous rearing of these transgenic lines can lead to the loss of unique genetic markers, genes and/or phenotypes due to genetic contamination and changes in genotype during passage of generations. Consequently, cryopreservation as a method that reduces the number of passages and therefore reduces genetic contamination has been proposed. Methods for mosquito embryos, larvae, and sperm cryopreservation are currently under investigation. Our collaborating partners at USDA in Fargo, North Dakota, have developed a procedure to cryopreserve Anopheles gambiae sperm and have achieved 70-95% sperm viability from cryopreserved testes. Nonetheless, a method for inseminating the A. gambiae females is needed. By combining a series of biochemical cell viability and sperm motility assays, we have formulated a medium for A. gambiae sperm harvesting and ex vivo capacitation. Molecular analysis of sperm incubated in this medium revealed an upregulation of three genes associated with flagella motility (AgSFP), cation influx channel (AgCatSper), and acrosomal reaction (AgSAP) during A. gambiae sperm capacitation. In addition, we employed immunodetection to map Protein Kinase A (PKA) and protein tyrosine phosphorylation patterns during sperm capacitation. Our findings highlight the biochemical signaling pathway during A. gambiae sperm capacitation and present a significant step towards achieving artificial insemination and in vitro fertilization of the malaria vector. We will also discuss our ongoing research on artificial insemination and in vitro fertilization of the malaria vector A. gambiae.

AEDES AEGYPTI ARGONAUTA 2 CONTROLS ARBOVIRUS-INDUCED MOSQUITO DEATH

Shengzhang Dong, George Dimopoulos

Johns Hopkins University, Baltimore, MD, United States

The yellow fever mosquito, Aedes aegypti, is the principal vector of numerous medically important human viral pathogens, including dengue virus (DENV), Zika virus (ZIKV), chikungunya virus (CHIKV), and Mayaro virus (Mayo).
virus (MAYV). These pathogens are transmitted between humans and mosquitoes and are a major public health and socioeconomic burden globally. To defend against virus infection, mosquitoes use their innate immune system, which includes the small interfering RNA (siRNA) pathway, Toll pathway, JAK/STAT pathway, and the arthropod-borne (arbo) viruses usually do not cause fitness cost to mosquitoes in nature. It has been demonstrated that the small RNA interfering (siRNA) pathway is the major antiviral defense system against arbovirus infections in Ae. aegypti and plays an important role in maintaining the mosquito-arbovirus balance. The siRNA pathway degrades exogenous viral RNA genome into virus-specific 21-nt siRNAs through an RNA-induced-silencing-complex (RISC), which consists of three core components: the RISCa, the siRNA pathway in the cytoplasm, this enzyme has been shown to regulate gene expression in the nucleus. Here, we generated Ago2-defective mosquitoes through CRISPR/Cas9 genome editing and reported a fundamental role for Ago2 in controlling arbovirus infection, as well as in protecting the mosquitoes from arbovirus-induced mortality by modulating DNA repair, apoptosis, and autophagy. Our study provides insights into the molecular interactions between mosquitoes and arboviruses that may facilitate the development of novel disease control strategies.

Increased virus infection in mosquitoes and the role of hemocytes in uninfected tissues. This study significantly advances our understanding of the virus infection and transport that infection through the hemolymph to study support a model for virus dissemination whereby hemocytes acquire virus replication and dissemination. Taken together, the results of this infection dynamics in those two mosquito organs and subjected the virus infects the fat body, the main immune and metabolic organ. We developed and optimized a semi-automatic image processing pipeline. We found that Anopheles sensitive head appendages contain ~3400 neurons. These include 1311 neurons on each antenna, 243 in each palp, and 153 in each labellar lobe, respectively. The terminal segment of each mosquito leg contains 42 neurons and there are 71 neurons in each ovipositor lobe. We compared these neuron numbers in our pan-neuronal mosquito line to those in a mosquito line with only odorant receptor neurons labeled, which contained 841, 151, 41 and 0 neurons in each antenna, palp, labellar lobe, and leg tips respectively. We found that 36%, 38%, 73%, and 100% of neurons in antennae, palps, labella, and legs, respectively, did not express any odorant receptors, suggesting that those additional neurons likely express ionotopic or gustatory gene families. Our pan-neuronal line allows genetic access and identification of new chemosensory gene targets for mutational and functional analyses of mosquito responses to important odors involved in host-seeking and blood-feeding, including attractants and repellents.

MOSQUITO RESISTANCE TO DENGUE VIRUS REVEALED BY SINGLE-CELL GENE EXPRESSION AND METABOLOMIC PROFILING OF MIDGUT AND FAT BODY

Thomas Vial, Louis Lambrechts, Sarah Merkling

Institut Pasteur, Université Paris Cité, CNRS UMR2000, Insect-Virus Interactions Unit, Paris, France

Viral pathogens transmitted by mosquitoes represent a growing threat to human health, due to global warming and globalization. For example, Aedes aegypti mosquitoes are the main vectors of dengue viruses (DENV) that infect 400 million people annually. Previous studies showed that ability of a given mosquito population to acquire and transmit a given virus is highly variable. The mechanisms underlying natural variation in susceptibility to viral infection between different mosquito populations remain mostly unknown. To explore this, we combined single-cell transcriptomics and metabolomics on Ae. aegypti populations showing contrasted infection phenotypes after DENV exposure. We identified Ae. aegypti mosquito populations, recently collected in the field, that are either resistant (<40% infected mosquitoes) or susceptible (>70%) to infection with DENV. When a mosquito bites a virus-infected human, the bloodmeal containing viral particles enters the mosquito’s digestive tract and is digested in the midgut, which is the entry gate for viruses into the mosquito’s body. Subsequently, the virus infects the fat body, the main immune and metabolic organ. We analyzed infection dynamics in both resistant and susceptible Ae. aegypti populations. We described the metabolic functions of different cell subpopulations of the mosquito midgut.

CHARACTERIZATION OF SENSORY NEURONS IN ANOPHELES MOSQUITO APPENDAGES

Joanna Konopka1, Darya Task1, Danny Poinapen2, Christopher Potter1

1Johns Hopkins University, Baltimore, MD, United States, 2Western University, London, ON, Canada

As vectors of Plasmodium parasites, Anopheles mosquitoes are a threat to global human health. To locate hosts for blood-feeding, female mosquitoes follow different long- and short-range cues in the environment. To detect those cues and find us, they use sensory receptor neurons of their chemosensory appendages. However, a comprehensive characterization of the neurons innervating appendages of Anopheles mosquitoes has been lacking. To gain genetic access to all Anopheles coluzzii mosquito neurons, we created a knock-in of the pan-neuronal gene bruchpilot (brp) using the Homology Assisted CRISPR Knock-in (HACK) approach. We validated this line by co-staining brains and nerve cords of adult mosquitoes for a knock-in reporter and the Brp protein. Using this new genetic reagent, we visualized and quantified all neurons in female sensory appendages of the head (antennae, maxillary palps, and labella) and body (tarsi and ovipositor). For consistent and efficient 3-dimensional neuron counting, we developed and optimized a semi-automatic image processing pipeline. We found that Anopheles sensory head appendages contain ~3400 neurons. These include 1311 neurons on each antenna, 243 in each palp, and 153 in each labellar lobe, respectively. The terminal segment of each mosquito leg contains 42 neurons and there are 71 neurons in each ovipositor lobe. We compared these neuron numbers in our pan-neuronal mosquito line to those in a mosquito line with only odorant receptor neurons labeled, which contained 841, 151, 41 and 0 neurons in each antenna, palp, labellar lobe, and leg tips respectively. We found that 36%, 38%, 73%, and 100% of neurons in antennae, palps, labella, and legs, respectively, did not express any odorant receptors, suggesting that those additional neurons likely express ionotopic or gustatory gene families. Our pan-neuronal line allows genetic access and identification of new chemosensory gene targets for mutational and functional analyses of mosquito responses to important odors involved in host-seeking and blood-feeding, including attractants and repellents.

INFLUENCE OF MOSQUITO IMMUNE CELLS IN ARBOVIRUS DISSEMINATION IN Aedes aegypti

David R. Hall1, Hyeogsun Kwon2, Rebecca Johnson3, Zannatul Ferdous3, S. Viridiana Laredo Laredo Tiscareño4, Bradley J. Blitvich4, Doug E. Brackney5, Ryan C. Smith5

1Interdepartmental Program in Genetics and Genomics, Iowa State University, Ames, IA, United States; 2Department of Plant Pathology, Entomology and Microbiology, Iowa State University, Ames, IA, United States; 3Center for Vector-Borne and Zoonotic Diseases, Department of Environmental Sciences, The Connecticut Agricultural Experiment Station, New Haven, CT, United States; 4Department of Veterinary Microbiology and Preventative Medicine, Iowa State University, Ames, IA, United States; 5Department of Plant Pathology, Entomology and Microbiology, Iowa State University, Ames, IA, United States

Mosquito-borne viruses are a rapidly increasing threat to public health, causing more than 400 million infections annually and placing over half of the world’s population at risk. Following the uptake of virus in an infectious blood meal, virus must traverse multiple physical barriers and evade immune defenses within the mosquito until it ultimately reaches the salivary glands where virus can then be transmitted to a new host upon blood-feeding. The mechanisms by which arboviruses elude these defenses and disseminate in the mosquito vector are a critical component of vector competence, yet these mechanisms are not well understood. In this study, we provide evidence for the importance of mosquito immune cells, known as hemocytes, in the dissemination of dengue virus (DENV) and Zika virus (ZIKV) in the mosquito Aedes aegypti. When phagocytic hemocytes are depleted prior to DENV or ZIKV infection, there is little effect on midgut infection, yet we demonstrate that the depletion of phagocytic hemocytes results in attenuated virus dissemination to the ovaries and salivary glands of mosquitoes, two tissues that are integral to virus transmission. Additional immunofluorescence experiments of virus-infected hemocytes demonstrate that phagocytic hemocytes (granulocytes) are a focal point for virus infection in the hemolymph and are capable of attaching to ovary and salivary gland tissues, establishing their role as a vehicle for virus dissemination in the mosquito host. To further dissect this mechanism, hemocytes from virus-infected mosquitoes were able to transfer infection to non-infected mosquitoes, demonstrating that hemocytes are an essential tropism for virus replication and dissemination. Taken together, the results of this study support a model for virus dissemination whereby hemocytes acquire a viral infection and transport that infection through the hemolymph to uninfected tissues. This study significantly advances our understanding of the dynamics of virus infection in mosquitoes and the role of hemocytes in mosquito vector competence for DENV and ZIKV.
and fat body upon DENV infection. We revealed metabolic pathways and gene expression patterns associated with susceptibility to virus infection at the organ and cellular levels. The functional role of candidate genes and metabolites in DENV susceptibility is currently being investigated in vivo by gene silencing and enzymatic inhibition assays. Results obtained from this study identify mosquito factors underlying natural variation to DENV infection and could lead to innovative tools for preventing arbovirus transmission.

**PLASMODIUM PARASITOPHOROUS VACUOLE MEMBRANE PROTEIN PFS16 PROMOTES MALARIA TRANSMISSION BY SILENCING MOSQUITO IMMUNITY**

Julian Ramelow, Yacob Keleta, Guodong Niu, Xiaohong Wang, Jun Li

Florida International University, Miami, FL, United States

With rising cases for the first time in years, malaria remains to be a significant public health burden. The sexual stage of the malaria parasite infects mosquitoes to transmit malaria from host to host. Hence, an infected mosquito plays an essential role in malaria transmission and disease prevalence. Plasmodium falciparum is the most dominant and dangerous malaria pathogen. Previous studies identified a sexual stage-specific protein 16 (Pfs16) localized to the parasitophorous vacuole membrane (PVM). Here we elucidate the function of Pfs16 during malaria transmission. Our structural analysis identified Pfs16 as an alpha-helical integral membrane protein with one transmembrane domain connecting to two regions across PVM. ELISA assays showed that insect cell-expressed recombinant Pfs16 (rPfs16) interacted with An. gambiae midguts. Transmission-blocking assays demonstrated that specific polyclonal antibodies against Pfs16 significantly reduced the number of oocysts in mosquito midguts. However, on the contrary, feeding rPfs16 significantly increased the number of oocysts. Further analysis revealed that Pfs16 reduced the activity of mosquito midgut caspase 3/7, a key enzyme in the mosquito Jun-N-terminal kinase (JNK) immune pathway. We conclude that Pfs16 facilitates parasites to invade mosquito epithelial cells by actively silencing the mosquito’s innate immunity through its interaction with the midgut.

**TEM Ephos RESISTANCE IS ASSOCIATED WITH REDUCED VECTOR COMPETENCE FOR ZIKA VIRUS IN Aedes Aegypti**

Grant A. Kay, Jennifer S. Lord, Grant L. Hughes, Lisa J. Reimer

Liverpool School of Tropical Medicine, Liverpool, United Kingdom

Arboviruses spread by mosquitoes present a major challenge to global health. The lack of effective vaccines and specific medical treatments means control efforts are heavily reliant on the use of insecticides to target the mosquito vectors. Insecticide selection and resistance cause profound alterations in the normal physiology of vectors. There are concerns that this may modify the vector competence of mosquitoes for arboviruses, and potentially alter transmission. The insecticide selection pressure was removed from a strain of Aedes aegypti with metabolic insecticide resistance to temephos. After 10 generations, the transcriptome of this unselected strain was compared to counterparts that had been maintained under temephos selection. After a further 10 generations, vector competence analysis was conducted using oral infections and intrathoracic injections. RNA sequencing revealed widespread transcriptomic changes within a small number of generations in response to the removal of insecticide. A number of genes potentially involved in the antiviral immune response were depleted in the unselected strain, including antimicrobial peptides and potential activators of the Toll pathway. Subsequent vector competence analysis showed an increase in the dissemination of ZIKV from the midgut in the unselected strain. Following intrathoracic injection, the prevalence of salivary infection was significantly lower in the temephos-selected strain (5.3%) than the unselected strain (41.9%) at 10-days post-infection. These data suggest that metabolic insecticide resistance to temephos is associated with reduced vector competence for ZIKV. The marked difference in salivary infection following intrathoracic injection suggests the presence of salivary gland infection and/or escape barriers in the temephos selected strain. Further research will establish the nature of these barriers and whether temephos selection is associated with the persistent overexpression of immune genes.
EFFECTIVENESS OF A CLINICAL DECISION SUPPORT ALGORITHM (ePOCT+) IN IMPROVING QUALITY OF CARE FOR SICK CHILDREN IN PRIMARY HEALTH FACILITIES IN TANZANIA (DYNAMIC PROJECT): RESULTS FROM A CLUSTER RANDOMIZED TRIAL

Godfrey A. Kavishe1, Alexandra V. Kulinkina2, Sabine Renggli3, Chacha D. Mangui1, Lameck Luwanda3, Peter Agrea1, Humphrey Mhagama1, Margaret Joram4, Ibrahim Mtebene5, Geoffrey I. Ashery1, Marie-Annick Le Pogam4, Honorati Masanja3, Nyanda E. Pradip Gyanwali1, George Korir2, Sabita Kandel1, Lisa Soti4, Phyu Hnin Hlaing5, Santa Kumar Das1, Kevin K.A. Ashery3, Marie-Annick Le Pogam4, Honorati Masanja3, Nyanda E. Pradip Gyanwali1, George Korir2, Sabina Dittrich2, Cassandra Kelly-Oxford, United Kingdom

The Integration of Childhood Illness (IMCI) booklets, a paper-based guideline for managing sick children under five years, has shown to improve quality of care and reduce child mortality. However, compliance to IMCI remains a challenge. Electronic clinical decision support algorithms (eCDSDAs) are a promising solution to improve compliance to IMCI. We performed a cluster randomized trial to evaluate whether the use of ePOCT+, a CDAS based on IMCI, increased quality of care compared to usual care among routine clinicians in primary health facilities in Tanzania. 18 sampled health facilities were randomized 1:1 (9 intervention and 9 control). Children aged 2-59 months presenting for the first time for an acute illness were enrolled and consultations were observed by an independent clinical research assistant. The intervention consists of the use of ePOCT+ with additional point of care tests (C-reactive protein, hemoglobin, pulse oximeter) and clinical mentorship. Primary outcomes measures were: percentage of children prescribed an antibiotic at initial consultation and mean score of major IMCI symptoms and signs assessed. A total of 450 consultations (225 in each arm) were observed between March and May 2022. The mean score of major IMCI symptoms and signs was 42% in intervention and 23% in control facilities (p<0.001). The use of ePOCT+ increased the proportion of consultations where history of convulsions (33% vs 7%) and mid upper arm circumference (60% vs 2%) were assessed compared to routine care clusters (p<0.001). However, there were no significant differences between the intervention and control arms for some measure as fever assessment (91% vs 87%; p=0.148) and height measurement (1.5% vs 0.4%; p=0.315). Proportion of consultations with an antibiotic prescribed was 37.3% in intervention facilities and 76.4% in control facilities (p=0.001). Using the ePOCT+, a clinical decision support algorithm based on IMCI, has significantly improved the quality of care for sick children in primary health facilities in Tanzania.

DEVELOPMENT AND EVALUATION OF A CLINICAL GUIDELINE FOR A PEDIATRIC TELEMEDICINE AND MEDICATION DELIVERY SERVICE: A PROSPECTIVE COHORT STUDY IN HAITI

Molly Klarmann1, Xiaofei Chi2, Youseline Cajusma1, Katelyn E. Flatherty1, Anne Carine Capcois1, Michel Daryl Vladimir Dofiné2, Libery Exantus2, Jason Fressent1, Valery M. Beau de Rochars2, Torben K. Becker1, Chantalé Baril2, Matthew J. Gurka1, Eric J. Nelson2
1University of Florida, Gressier, Haiti, 2University of Florida, Gainesville, FL, United States, 3Université d’État d’Haiti- Faculté de Médecine et de Pharmacie, Port au Prince, Haiti, 4Trek Medics International, Charlotte, NC, United States, 5Université d’État d’Haiti-Faculté de Médecine et de Pharmacie, Port au Prince, Haiti

Despite the emergence of telemedicine as an important model for healthcare delivery, there is a lack of evidence-based clinical guidelines, especially for resource-limited settings. Our objective was to develop and evaluate a clinical guideline for a pediatric telemedicine and medication delivery service (TMDS). The guideline was derived from the in-person World Health Organization (WHO) Integrated Management for Childhood Illness guidelines. To evaluate our guideline, a prospective cohort study was conducted at a TMDS in Haiti. Children 10 years and younger whose caregiver contacted the TMDS during operating hours (6pm-5am) were enrolled. Incoming calls were received by Haitian providers who triaged cases as mild, moderate, or severe. Severe cases were referred to the hospital. For non-severe cases, a ‘virtual’ exam was performed to formulate an assessment and plan. For cases within the delivery zone, a driver and provider were dispatched to the home to conduct a paired in-person exam. The primary outcome was the performance of the virtual exam compared to the in-person exam (reference standard). A total of 391 cases were enrolled. The most common chief complaints were fever (44%; 142) and ‘respiratory problem’ (17%; 54). Among 320 cases with paired exams, no general WHO danger signs were identified at the household; problem-specific danger signs were identified in 6 cases (2%). Cohen’s kappa for the designation of mild cases was 0.78 (95%CI 0.69-0.87). Among components of the virtual exam, the sensitivity and specificity of a reported fever was 91% (95%CI 87%-96%) and 69% (62%-76%); respectively, the sensitivity and specificity of ‘fast breathing’ was 47% (95%CI 21%-72%) and 89% (85%-94%), respectively. Kappa for ‘no’ and ‘moderate’ dehydration indicated moderate congruence (0.69; 95%CI 0.41-0.98). At 10 days, 95% (273) of the 287 cases contacted were better/recovered. This study represents a formative step towards an evidence-based pediatric guideline for telemedicine and medication delivery in Haiti.
IMPACT OF A DIGITAL CLINICAL DECISION SUPPORT ALGORITHM ON ANTIBIOTIC PRESCRIPTION IN RWANDA: PRELIMINARY RESULTS FROM A CLUSTER NON-RANDOMIZED CONTROLLED TRIAL

Alexandra V. Kulinkina1, Victor Rwandarwacu2, Joseph Habakurama3, Angelique Inagbire2, Ludovico Cobucci4, Emmanuel Kalisa5, Gilbert Rukundo6, Gillian Levine1, Martin Norris1, Rainer Tan1, Alan Vonlanthen2, Vincent Faivre1, Julien Thabard1, Marie-Annick Le Pogam2, Valerie D’Acremont3
1Swiss Tropical and Public Health Institute, Allschwil, Switzerland, 2Swiss Tropical and Public Health Institute, Kigali, Rwanda, 3Centre for Primary Care and Public Health, University of Lausanne (Unisants), Lausanne, Switzerland

Children account for most of the antibiotics consumed in low-resource settings. To address overprescription, we developed ePOCT+, a novel Clinical Decision Support Algorithm (CDSA) aimed to guide health workers in outpatient consultations for sick children under 15 years of age. To evaluate its impact on antibiotic prescription (primary outcome), we conducted a pragmatic parallel-group cluster non-randomized trial (NCT05108831) in 32 primary health centers in Rwanda. In 16 health centers allocated to the intervention arm, the algorithm, adapted to the Rwandan guidelines, was deployed on tablets together with point-of-care tests (C-reactive protein, hemoglobin, pulse oximeter), while 16 health centers allocated to the control arm continued providing routine care and completing an electronic case report form in tablets without decision support. Between December 2021 and October 2022, we included 18,843 first-time consultations of children visiting for an acute illness and for whom data were captured electronically. Antibiotics were prescribed in 24.5% of children managed with ePOCT+, versus 70.5% receiving routine care (OR = 0.14; 95% CI: 0.13, 0.14). Antibiotic prescription varied among intervention health centers from 6% to 67%. Average uptake of the intervention was 75%, also ranging between 46% and 96%. Clinical cure at day 7 (secondary outcome) was non-inferior in the intervention arm according to the upper limit of the odds ratio confidence interval being below 1.3 (adjusted OR = 1.14; 95% CI: 1.01, 1.23). We showed that the use of the ePOCT+ digital CDSA can safely reduce antibiotic prescriptions in sick children in outpatient settings. We are further analyzing which contextual factors contribute to variable effectiveness across time and health facilities.

ENVIRONMENTAL IMPACT AND MINERAL AND ENERGY REQUIREMENTS OF THE USE OF AN ELECTRONIC CLINICAL DECISION SUPPORT ALGORITHM TO MANAGE SICK CHILDREN IN TANZANIA: A LIFE CYCLE ASSESSMENT

Nina Emery1, Maxime Karlen1, Rainer Tan1, Godfrey Kavishe1, Peter Adege1, Sabine Renggli2, Alexandra Kulinkina3, Lameck Mwakinyo4, Chacha Lungwanda3, Pascale Schwab2, Xavier Bengoa3, Valerie D’Acremont3
1,3Unisants, Lausanne, Switzerland, 2Swiss Tropical and Public Health Institute, Basel, Switzerland, 4National Institute for Medical Research - Mbeya Medical Research Center, Mbeya, Tanzania, United Republic of

Digital health interventions are a promising way to improve quality of care in low resource settings, but their environmental impact is generally not considered. The objective of the present analysis was to measure the environmental impact of the DYNAMIC digital project, aimed at improving the rational use of diagnostics and medicines by clinicians at primary level in Tanzania using electronic clinical decision support algorithms. A Life Cycle Assessment (LCA) of the DYNAMIC project was carried out to calculate the greenhouse gas (GHG) emissions, the fossil energy and mineral resources use, and the damages on ecosystems and human health. At the time of data collection, the DYNAMIC project was implemented in 40 health facilities, allowing to treat 91’000 children per year. Its GHG emissions were 24.5 tons of CO2-eq per year, while 12.5 tons of CO2-eq were saved thanks to a decrease in antibiotics prescriptions. Medical supplies were the main source of GHG emissions (69%), followed by digital supplies and activities (20%), and finally logistics (11%, mainly transport for supervision visits). The fossil energy and mineral resources use of the project were 444 GJ and 86 kg per year. The damage on human health and ecosystems were 0.062 DALY and 12’000 PDF-m2 per year. This analysis highlights the environmental impact due to medical consumables like medicines and single use tests and demonstrates the environmental benefits of antibiotic stewardship initiatives. Digital tools had much lower impact on GHG emissions compared to medical consumables, as seen with other LCAs performed in high-income countries. Nonetheless digital tools account for a significant share of total mineral resource use. The 24.5 tons of CO2-eq emitted annually represents the annual emissions of 84 Tanzanians or 1.6 Americans. Our results should thus be interpreted while bearing in mind the principle of climate equity. The environmental impact of any health intervention should be considered along with other indicators like effectiveness or costs. Our results can help guide decision making for the implementation of other health projects, both in the global South and the global North.
REDUCING LOW BIRTH WEIGHT BY ADDING TWO DOSES OF AZITHROMYCIN TO THE INTERMITTENT PREVENTIVE TREATMENT OF MALARIA IN PREGNANCY WITH SULFADOXINE PYRIMETHAMINE: A RANDOMIZED CONTROLLED TRIAL IN BURKINA FASO

Moussa Lingani1, Serge Henri Zango2, Innocent Vala1, Sékou Samadoulougou2, Michèle Drame3, Halidou Tinto1, Philippe Donnen1, Annie Robert1
1Institut de Recherche en Sciences de la Santé/Direction Régionale du Centre Ouest (IRSS/DRDO), Nancoro, Burkina Faso, 2-Evaluation Platform on Obesity Prevention, Québec Heart and Lung Institute Research Center, Québec City, QC, 3-MICAP, Québec, QC, Canada. 1École de santé publique, Université Libre de Bruxelles, CP594, route de Lennik 808, 1070 Bruxelles, Bruxelles, Belgium. 2Epidemiology and Biostatistics Research Division, Institut de recherche expérimenterale et clinique, Université catholique de Louvain, Brussels B1.30.10, Clos Chapelle-aux-Champs 30, B-1200 Brussels, Bruxelles, Belgium.

Exposure during pregnancy to malaria and sexually transmitted infections is associated with adverse birth outcomes including low birth weight (LBW). Whether the addition of two doses of azithromycin to sulfadoxine-pyrimethamine during the intermittent preventive treatment of malaria in pregnancy would result in a reduction of LBW is unclear. We conducted a 2 parallel-groups, open-label randomized controlled trial involving pregnant women (16 to 35 years of age, and 12 to 24 weeks of gestation as confirmed by last menstrual period or fundal height) in rural Burkina Faso. Women were assigned in a 1:1 ratio either to receive azithromycin (1 g daily for 2 days) during the second and third trimesters of pregnancy plus monthly sulfadoxine-pyrimethaine (1,500/75 mg) (SPAZ) (intervention) or to continue receiving the routine monthly sulfadoxine pyrimethamine (1,500/75 mg) (SP) (control) under supervision. Primary outcome was a LBW (birth weight measured within 24 hours after birth < 2,500 g). Secondary outcomes including stillbirth, preterm birth or miscarriage are reported together with safety data. A total of 992 pregnant women underwent randomization (496 per group), and 986 (90.5%) valid birth weights were available (450 in SPAZ and 448 in SP). LBW incidence was 8.7% (39/450) in SPAZ and 9.4% (42/448) in controls (p-value = 0.79). Compared with controls, pregnant women with SPAZ showed a risk ratio (RR) of 1.16 (95% confidence interval (CI): 0.64-2.08) for preterm births, 0.75 (95% CI: 0.17-3.35) for miscarriage and 0.64 (95% CI: 0.25-1.64) for stillbirths. No treatment-related severe adverse events (SAEs) have been observed and there was no significant difference in the number of SAEs (13.5% [87/645] in SPAZ, 16.7% [83/496] in SP, p-value = 0.18) or AEs (17.1% [93/540] in SPAZ, 18.8% [93/496] in SP, p-value = 0.56). Adding azithromycin to the IPTp-SP regimen in malaria endemic areas does not reduce the risk of LBW, as far as women receive a malaria prevention regimen early enough during pregnancy.

THE UGANDA HOUSING MODIFICATION STUDY - A CLUSTER RANDOMIZED TRIAL EVALUATING THE IMPACT OF TWO TYPES OF HOUSING MODIFICATION ON MALARIA BURDEN IN UGANDA

Nelli Westercamp1, Samuel Gonahasa2, Agaba Katurebee2, Catherine Maiti-Setubuzi1, Joaunter Nankaborwa2, Jimmy Oggo1, Henry Maweje2, John E. Gimnig3, Peter Mutungi2, Katherine Snyman2, Walter Ochieng2, Susan Nyanzi3, Eleanor Hutchinson4, Seth R. Irish5, Jenny Carlson6, Mamie Niang7, Kassahun A. Belay8, Sarah G. Staelke9, Moses R. Kamya2
1Centers for Disease Control and Prevention, Atlanta, GA, United States, 2Infectious Diseases Research Collaboration, Kampala, Uganda, 3Ministry of Health (MOH/NMCP), Kampala, Uganda, 4London School of Hygiene & Tropical Medicine, London, United Kingdom, 5U.S. President’s Malaria Initiative, Centers for Disease Control and Prevention, Atlanta, GA, United States, 6U.S. President’s Malaria Initiative, USAID, Washington, DC, United States, 7U.S. President’s Malaria Initiative, Centers for Disease Control and Prevention, Kampala, Uganda, 8U.S. President’s Malaria Initiative, USAID, Kampala, Uganda

Once a key pillar of malaria control, housing modification remains underutilized in most endemic areas. A cluster-randomised trial to evaluate the impact of housing modification on malaria burden in Uganda is being conducted between October 2021 and July 2023. Sixty clusters in moderate to high malaria transmission areas in Jinja and Luuka districts were randomized 1:1:1 to three arms: 1) house screening: screening windows, eaves (if open), and ventilation openings, and patching holes in the walls; 2) eave tubes; and 3) control. These interventions were selected based on a pre-trial pilot of four different house modifications in 200 households. The trial is assessing impact on malaria incidence through a cohort of 0-5-year-old children from 1,500 households (25 per cluster) followed for 12 months; impact on malaria and anemia prevalence through three cross-sectional surveys; and changes in vector densities through repeated CDC light trap collections in each cohort household. Costing and qualitative evaluations addressed feasibility and acceptability. Housing modifications were installed in 2,042 houses in screening clusters and 1,964 houses in eave tube clusters, reaching 92.2% and 88.3% intervention coverage, respectively, and showing that both interventions were acceptable and feasible to implement. Parasite prevalence in the baseline survey was 31.4% by microscopy in 6-month-14-year-old children; improved housing quality was associated with lower odds of parasitemia (prevalence ratio: 0.80 [95%CI 0.71-0.90]). While trial data collection is still ongoing, the pre-trial pilot findings of 75% (density ratio [DR]: 0.25 [95% CI 0.12-0.53]) and 55% (DR: 0.45 [95% CI 0.21-0.99]) reductions in Anopheles gambiense (s.l.) density in the screening and eave tube arms, respectively, compared to the controls, are encouraging. Main trial results reporting the impact of house modifications on malaria incidence, prevalence, and vector density will be presented. The study will provide evidence about potentially valuable long term malaria interventions that could be important additions to the malaria prevention toolkit.

INSECTICIDE CHEMICAL CONTENT AND BIOEFFECTIVENESS OF INSECTICIDE-TREATED NETS CONTAINING CHLORFENAPRY OR PIPERONYL BUTOXIDE OVER 24 MONTHS OF FIELD USE IN BURKINA FASO, RWANDA, SIERRA LEONE, AND BURUNDI

Keith Esch1, Isabel Swaminidoss2, Jacky Raharinarivo3, Raymond Sudo4, Carla Mapp2, Denis Sinzinkayo5, Pierre Sinarizfi6, Mugisha Landrine7, Alikul Seyoum4, Beatus Cyubahiro8, Emmanuel Hakizimanana9, Aimable Mbituyumuremyi8, Elias Niyiturama6, Dennis H. Marke8,9, Frederick Yamba10, Stephen Poyer1

New insecticide-treated net (ITN) types have been distributed through mass campaigns since 2019.Given their limited deployment to date, published durability monitoring (DM) data on new ITN types is sparse and predominantly limited to field trials. The U.S. President’s Malaria Initiative (PMI) VectorLink project supported National Malaria Programs to gather empirical data on insecticide chemical content and bioefficacy of chlorfenapry (CFP) and piperonyl butoxide (PBO)-synergised ITNs under natural conditionsafter 24 months of field use in Burkina Faso, Rwanda, Rwanda, and Burundi.
Sierra Leone and Burundi. Chemical content was measured as the percentage reduction in active ingredients against the manufacturer's target dose and bioefficacy was measured with susceptible (sus.) and resistant (res.) mosquito strains using 24-hour mortality for PBO-ITNs with cone tests and 72-hour mortality for CFP-ITNs with funnel tests. In Burkina Faso, CFP and PBO content reduced by 67% and 84% in field samples, respectively, corresponding to sus. mosquito mortality of 93% and 94% and res. mortality of 51% and 26% after 24 months. In Rwanda, CFP and PBO content in field samples reduced by 29% and 54%, respectively, corresponding to sus. mortality of 82% for PBO samples (sus. mortality for CFP samples was not analyzed) and res. mortality of 53% and 51%, respectively, after 24 months. Over the same period in Sierra Leone, PBO content reduced by 57% and 66% in field samples of two PBO ITN brands, corresponding to sus. mortality of 47% and 80% and res. mortality of 4% and 9%, respectively. In Burundi, no chemical content testing was conducted but PBO sample sus. mortality was 100% and res. mortality was 95%, 24 months post-distribution. Insights into insecticidal effectiveness and chemical content of ITNs is necessary for stakeholders to determine which ITNs to procure, and how frequently. Mechanisms to capture this data should be included in the standard post-market monitoring guidance, which is currently under development, as part of the WHO ITNpre-qualification guidelines.

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THE RIPPLE EFFECT OF QUALITY IMPROVEMENT IN STRENGTHENING UPTAKE OF INTERTIMMENT PREVENTIVE TREATMENT FOR THE PREVENTION OF MALARIA IN PREGNANCY: A CASE STUDY OF KAKAMEGA COUNTY, KENYA

Agatha Kutoy Manu1, Francesca Nzuve1, Beth Barasa1, Faustina Sakari2, Linet Ilemenya3, Ahmeddin Omar3, Elvis Oyugi3, Peter Njiru3

1U.S. President’s Malaria Initiative, Impact Malaria Project, Nairobi, Kenya, 2Ministry of Health, Kakamega County, Kakamega, Kenya, 3Division of National Malaria Program, Ministry of Health, Nairobi, Kenya

Malaria during pregnancy is associated with an increased risk of premature birth, low birth weight, and maternal and infant deaths. The World Health Organization recommends the use of sulphadoxine-pyrimethamine (SP) as intermittent preventive treatment in pregnancy (IPTp) for the prevention of malaria in pregnancy (MIP) in regions of moderate to high malaria transmission. The uptake of three doses of IPTp (IPTp3) in Kenya has remained low at 43% (KMS 2020) compared to the 80% target. In Mumbia East and Ikolomani sub-counties, Kakamega county, IPTp3 uptake was 8% and 9% respectively, in 2020. Between 2020 and 2022, the President's Malaria Initiative Impact Malaria (IM) project and Kakamega county integrated quality improvement (QI) methods in routine service delivery to improve ANC attendance and IPTp3 uptake and established quality improvement teams (QITs). IM supported the orientation of 24 QIT members from 16 health facilities (HF), representing 55% of the HFs in the 2 sub-counties. QITs identified bottlenecks associated with low IPTp uptake, including late attending 1st ANC, poor IPTp documentation, poor default tracing mechanisms, and a weak facility-community linkage system. To strengthen facility-community linkage, the QITs mobilized community health volunteers (CHVs) to support the early identification of pregnant women and default tracing. QI coordinators mentored health workers on proper IPTp data capture in the ANC register and defaulting tracing. The QITs convened monthly monitoring meetings. QI coordinators held quarterly HF-monitoring visits to review interventions implemented and IPTp performance. The strengthened HF-community linkages led to an increase in pregnant women referred by CHVs to the HF from 3,537 in 2020 to 6,385 in 2022. Women attending 1st ANC before 12 weeks gestation increased from 4% to 15% in the same period. IPTp3 uptake improved from 9% in 2020 to 79% in 2022 in Ikolomani and 8% to 76% in Mumbia East. Integration of QI in routine service delivery can contribute to improved uptake of preventative services, reducing the risk of morbidity and mortality.

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REDUCING MALARIA TRANSMISSION IN FOREST-GOING MOBILE AND MIGRANT POPULATIONS IN LAO PDR AND CAMBODIA: A STEPPED-WEDGE CLUSTER-RANDOMISED CONTROLLED TRIAL

Win Han Oo1, Win Htike1, Thet Lynn2, Lun Sovandza2, Paul A. Agius3, May Chan Oo1, Galai Naw Hkwang1, Kaung Myat Thu1, Aung Khine Zaw1, El Phyu Htwe1, Julia Cutts1, Nick Scott1, Ellen Kearney1, Katherine O’Flatherty1, Wang Bangyuan1, Boualam Khamlome1, Phouthalong Vialay1, Siv Sovannaroth1, Freya Ji Fowkes1

1Burnet Institute, Melbourne, Australia, 2Health Poverty Action, Vientiane, Lao People’s Democratic Republic, 3Health Poverty Action, Phnom Penh, Cambodia, 4Deakin University, Melbourne, Australia, 5Health Poverty Action, London, United Kingdom, 6Center of Malariology Parasitology
Countries of the Greater Mekong Sub-region aim to achieve malaria elimination by 2030. In the region, malaria is concentrated in high-risk areas and populations such as forest-going mobile and migrant populations (MMPs). However, routine protective measures such as long-lasting insecticidal nets do not prevent all infectious bites in these high-risk populations. Evidence for the effectiveness of a personal protection package tailored to forest-going MMPs which is acceptable, feasible, and cost-effective for reducing malaria transmission is required to inform the malaria elimination toolkit in the region. A personal protection package consisting of long-lasting insecticidal hammock net, insect repellent and health communication pamphlet was developed in consultation with relevant implementing partners from Cambodia and Lao PDR. An open stepped-wedge cluster-randomized controlled trial was conducted from March 2022 to February 2023 in 488 villages to evaluate the effectiveness of the personal protection package. Villages were randomized into 11 blocks, with blocks transitioned in random order from control to intervention states at monthly intervals, following a one-month baseline period. The primary outcome of the trial was the prevalence of Plasmodium spp. infection diagnosed by rapid diagnostic test. Difference in prevalence of malaria infection was estimated across intervention and control periods using generalized linear mixed modelling. In this presentation the primary analyses of this recently completed trial will be presented together with a summary of the acceptability, feasibility, and cost-effectiveness of the personal protection package intervention. Results will inform national and regional policy on the use of personal protection packages for the prevention of malaria in high-risk populations.

**DO ATTRACTIVE TARGETED SUGAR BAITS REDUCE MALARIA BURDEN IN ZAMBIA? FIRST RESULTS FROM A PHASE III COMMUNITY-RANDOMIZED EFFICACY TRIAL OF ATSB IN WESTERN PROVINCE, ZAMBIA**

**Ruth Ashton**1, Kochelani Saili2, Chama Chishya2, Handrinah Bandia3, Annie Arzen2, Chanda Chitoshi2, John Chulu2, Frank Ministry of Health, Phnom Penh, Cambodia

2022 to June 2023 was 2.36 cases per person-year. The secondary

**MULTIPLE PLASMODIUM FALCIPARUM EARLY TRANSCRIBED Membrane Protein FAMILY MEMBERS ARE DIFFERENTIALLY EXPRESSED IN PEDIATRIC PATIENTS WITH SEVERE MALARIAL ANEMIA**

**Perez K. Olewe**1, Quying Cheng2, Clinton O. Onyango3, Samuel B. Anyora4, Ivy J. Hurwitz5, Sarah Kituyi6, Evans Raballah7, Beauty Kolade8, Philip D. Seidenberg9, Kristan A. Schneider8, Christophe G. Lamber2, Ananias A. Escalante9, Collins Ouma2, Benjamin H. McMahon10, Douglass J. Perkins11, Masinde Muliro University of Science and Technology, Bondo, Kenya, 2University of New Mexico, Center for Global Health, Department of Internal Medicine, Albuquerque, NM, United States, 3Maseno University, Kisumu, Kenya, 4University of Embu, Embu, Kenya, 5Masinde Muliro University of Science and Technology, Kakamega, Kenya, 6Theoretical Biology and Biophysics Group, Theoretical Division, Los Alamos National Laboratory, Los Alamos, NM, USA, 7Department of Emergency Medicine, Albuquerque, NM, United States, 8Department of Applied Computer and Biosciences, University of Applied Sciences Mittweida, Technikumplatz, Mittweida, Germany, 9Biology Department/Institute for Molecular Evolutionary Biology, Temple University, Philadelphia, PA, USA, Philadelphia, PA, United States, 10Theoretical Biology and Biophysics Group, Theoretical Division, Los Alamos National Laboratory, Los Alamos, NM, USA, 11Theoretical Biology and Biophysics Group, Theoretical Division, Los Alamos National Laboratory, Los Alamos, NM, USA, United States

Plasmodium early transcribed membrane proteins (ETRAMPs) are a group of parasite membrane proteins located at the parasite-host interface which have been evaluated as novel malaria vaccine candidates. Although P. vivax ETRAMP11.2 (pvETRAMP11.2) and pvETRAMP4 have been shown to be highly immunogenic in animal models, no studies have reported the impact of their P. falciparum homologs on stimulating immune responses. Moreover, it is currently unknown if pvETRAMPs gene expression profiles differ according to disease severity in children with malarial anemia. To address this gap in knowledge, we performed transcriptomic analysis on P. falciparum in samples collected from a pediatric cohort (3-36 months) in western Kenya. Children were stratified into either severe malarial anemia [SMA, hemoglobin (Hb)<6.0 g/dL, (n=20)] or non-SMA [(Hb)>6.0 g/dL, (n=40)]. P. falciparum gene expression profiles were determined by next-generation sequencing (NGS) on peripheral whole blood samples collected prior to antimalarial treatment. NGS was conducted at a depth of >20 million high-quality mappable reads using the Illumina NovoSeq platform with reads then mapped onto a Kenyan isolate reference genome (pFKE01) using HTSeq. This revealed ~2800 distinct P. falciparum transcripts for which children with SMA had upregulation of pfETRAMP4 (log2foldchange=-1.92, P=0.003) and downregulation of pfETRAMP10.1 (log2foldchange=-0.52, P=0.016), pfETRAMP11.2 (log2foldchange=-0.61, P=0.046) and pfETRAMP14 (log2foldchange=-0.50, P=0.042). Given the importance of the ETRAMP family in erythrocyte invasion, parasite development, and virulence, these findings support further exploration of their role as potential novel drug targets and vaccine candidates for the treatment and prevention of malaria, particularly in the context of severe disease. It remains to be determined if the differential expression of ETRAMPs in children with SMA is due to different immune responses and/or inherent properties of the infecting parasites.
PROTEOMICS ANALYSIS REVEALS ALTERED HOST PATHWAYS SPECIFIC TO SEVERE MALARIA IN BENINESE CHILDREN.

Jérémy Fraering1, Virginie Salnot2, Sam Ezinmegnon1, Nadine Fievé1, Jules Alao3, Katell Peoc'h4, Florence Migot-Nabias1, Nicolas Argy1, Emilie-Fleur Gautier5, Gwladys Bertin1, Claire Kaimalididi6

1IRD - UMR 261 - MERIT, Paris, France, 2Proteom'IC platform, Paris, France, 3Service de Réadaptation Chum Hospital, Cotonou, Benin, 4Bichat Hospital - Biochemistry department, Paris, France, 5Proteom'IC Platform, Paris, France, 6Cummng School of Medicine - University of Calgary, Calgary, AB, Canada

In 2020, 80% of malaria deaths occurred in children under five years old, vulnerable to severe forms of the disease like cerebral malaria (CM) and severe malaria anemia (SMA). Although specific parasite variants contribute to the disease progression, the host-parasite interaction leading to severe malaria remains poorly characterized. To study severe malaria’s pathogenesis, an untargeted proteomic analysis was conducted using clinical isolates of children attending health facilities in southern Benin during the malaria transmission peak. The study included 278 clinical samples as a secondary analysis of two cohorts, including 122 CM, 135 uncomplicated malaria (UM), 21 SMA, all infected by Plasmodium falciparum. In addition, 50 non-infected (Ni) children were included as a control group. The mass spectrometry analysis of a subset of samples revealed three plasma proteome clusters (Ni, UM, and SMA+CM) identified through a principal component analysis, and proteins differentially abundant were a signature of CM vs. UM. Transferrin Receptor protein was identified in infected mature erythrocytes (with a significant increase in CM samples, p=0.012). Furthermore, iron homeostasis (p<0.01) and ferroptosis signaling pathways (p=0.011) were over represented in CM vs. SMA differentially abundant proteins. In addition, CM and SMA samples showed an overrepresentation of ubiquitination and protein degradation by proteasome pathways compared with UM samples (p=0.033). The plasma protein analysis showed a higher abundance of seven subunit of the circulating 20S proteasome in CM samples compared with UM, and four of these units were also more abundant in SMA. Further confirmatory analyses are ongoing in the entire sample cohort (n=278) and will be presented at the conference. The results suggest an increase of erythroid precursors invasion by parasite causing CM, and that iron metabolism plays a role in the clinical presentation of pediatric malaria in endemic areas. Overall, this study provides an innovative approach to study and insights into the pathogenesis of severe malaria and highlights potential targets for therapeutic intervention.

ROLE OF PLASMODIUM FALCIPARUM HEMOZOIN-ASSOCIATED BIOMOLECULES IN THE PATHOGENESIS OF CEREBRAL MALARIA

Kelly A. Crotty, Ana Rodriguez
NYU School of Medicine, New York, NY, United States

One of the hallmarks of cerebral malaria, a severe complication of Plasmodium falciparum infection, is the adhesion of P. falciparum-infected red blood cells (iRBCs) to the microvasculature of the brain, which is frequently accompanied by the weakening of the junctions between endothelial cells lining the blood-brain barrier (BBB), resulting in vasogenic edema. Our lab is focused on identifying the mechanisms through which P. falciparum-infected RBCs are able to disrupt the BBB. We have observed that iRBCs (intact or lysed) disrupt intercellular junctions of human brain microvascular endothelial cells (HBMECs) in vitro. Removing hemozoin, a heme crystal that is formed during the blood stage of the parasite’s life cycle, eliminates the iRBC lysates’ ability to disrupt intercellular junctions in HBMECs. Furthermore, natural hemozoin isolated from P. falciparum-iRBCs is able to disrupt intercellular junctions in HBMECs, suggesting that hemozoin may carry the ability to induce the loss of barrier function in the brain endothelium. We also observed that, while natural hemozoin actively induces endothelial barrier disruption, commercially-available synthetic hemozoin does not have this effect. Since natural hemozoin is bound to a variety of biomolecules including proteins, lipids, and DNA released from P. falciparum-iRBCs, while synthetic hemozoin is not, we hypothesize that the biomolecules associated with natural hemozoin are required for the endothelial barrier disruption. We aim to determine the contributions that hemozoin-associated biomolecules make in the cell signaling events that lead to the disruption of endothelial junctions. Further elucidating the mechanism behind cerebral malaria may lead to the development of better therapeutics which could drastically drive down the high morbidity and mortality rates of this disease.

EXPRESSION PROFILES OF DBLA DOMAINS OF VAR GENES FROM PLASMODIUM FALCIPARUM PARASITES CAUSING CLINICAL MALARIA OR PROMOTING ASYMPTOMATIC INFECTIONS

Sukai Ceesay1, Martin Kampmann1, Helle Smedegaard Hansson2, Rasmus Weisel Jensen2, Manuela Carraçaquilla3, Safiatou Doumbo3, Usama Dabbas1, Hamidou Clisie2, Didier Doumtab2, Assisata Ongoba1, Kassoum Kayentao2, Boubacar Traore2, Peter D Crompton1, Thomas Lavstensen3, Silvia Portugal1

1Max Planck Institute for Infection Biology, Berlin, Germany, 2Centre for Medical Parasitology, University of Copenhagen, Copenhagen, Denmark, 3Mal International Center of Excellence in Research, University of Sciences, Techniques and Technologies of Bamako, Bamako, Mali, 4Laboratory of Immunogenetics, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States

In Mali, the vast majority of malaria cases occur during the wet season. During the dry season, mosquitoes are rare, thereby interrupting malaria transmission. Nevertheless, Plasmodium falciparum can persist in individuals without symptoms at low parasitaemias through the 6-month dry season. Our lab has described that dry season asymptomatic carriers harbour blood stage parasites that are further along in the erythrocytic cycle than parasites collected from individuals with clinical malaria. Longer circulation of infected erythrocytes promotes more effective splenic clearance of parasitized cells in the dry season, and highlights their less efficient cytoadhesion. How this is achieved seasonally remains unknown. Cytoadhesion is largely mediated by P. falciparum erythrocyte membrane protein 1 ( PfEMP1 ) expressed in a monoallelic fashion by the multigene family var. We aimed to explore var gene expression to better understand the dry season low cytoadhesion phenotype, characterize the PFEMP1s present on infected erythrocytes that become more easily cleared in the spleen, preventing high parasitaemias, and provide insight to the evolutionary selection process driving it. With parasites collected from subclinical and clinical infections of Malian donors, we sequenced RT-PCR amplified DLAB expression- tags located in the N-terminal of var genes, and used the Varia algorithm to predict the var genes’ domain composition, such as the Cysteine-rich Interdomain Region (CIDR). Our findings confirm previously published profiles from non-severe malaria cases dominated by DBOLa and CIDR domains of CD36- binding PFEMP1 found in group B/C var genes. The dry season parasite population appears to be a mixture of CD36- and EPCR- binding PFEMP1 and dominated by fewer var genes. Across all the timepoints ICAM-1 and CD36 var genes were most abundant. We are now investigating whether number and type of var genes expressed throughout the year correlate with number or particular parasite haplotypes, and whether var expression level varies during the year, to identify trends in the expression profiles of PFEMP1 in seasonal transmission setting.
ELEVATED URIC ACID PREDICTS MORTALITY AND COGNITIVE IMPAIRMENT IN UGANDAN CHILDREN WITH SEVERE MALARIA

Caitlin A. Bond1, Dibyaduthi Datta1, Ruth Namazzi2, Robert O. Opoku1, Anthony Batte1, Kavitha M. Udomula1, Deepali Balasubramanani1, Ana Rodrigues2, Tarek M. El-Achkar1, Chandy C. John1, Andrea L. Conroy1
1Indiana University School of Medicine, Indianapolis, IN, United States, 2Makerere University College of Health Sciences, Kampala, Uganda, 3New York University School of Medicine, New York, NY, United States

Elevated uric acid levels have been reported in children with severe malaria (SM), but the relationship of elevated uric acid levels with SM pathogenesis and outcomes is not well understood. We investigated the role of uric acid in SM pathogenesis in two populations of Ugandan children with SM using a test and validation approach. In the test cohort, platelet-free plasma uric acid was analyzed in 372 children aged 18 months to 12 years enrolled with SM (Cohort #1). In the validation cohort, serum uric acid was analyzed in 595 children aged 6 months to 4 years enrolled with SM (Cohort #2). Each cohort enrolled community children without SM (Cohort #1, n = 128; Cohort #2, n = 118). Uric acid levels at enrollment were significantly higher in children with SM compared to community children, with hyperuricemia (uric acid >7mg/dL) present in a quarter of all children with SM (15% in Cohort #1, 36% in Cohort #2) but 0% in community children. Hyperuricemia was more common in children who died vs. survived (Cohort #1, 36% vs. 13%, p < 0.01; Cohort #2, 81% vs. 32%, p < 0.001), and was associated with coma, acidosis, severe anemia, and severe acute kidney injury (AKI). Xanthine oxidase, the enzyme responsible for uric acid production, was positively correlated with uric acid in children with SM (rho: 0.41, p < 0.001) but not community children (0.04, p = 0.67). In children with severe AKI, uric acid levels remained elevated at 24 hours (p = 0.12), whereas in children without severe AKI, levels dropped significantly (p < 0.001). Mechanistically, elevated uric acid was strongly associated with markers of hemolysis, AKI, endothelial activation, and acidosis, but not parasite density or biomass. Among survivors, elevated uric acid was independently associated with worse long-term cognition in children <5 years of age across both cohorts. Elevated uric acid is associated with increased mortality and worse cognition in children with severe malaria. Clinical trials evaluating medications that reduce uric acid as adjunctive therapy for children with severe malaria should be considered to improve survival and protect neurodevelopment in survivors.

CONSERVED SUBGROUPS OF PLASMODIUM FALCIPARUM RIFIN ANTIGENS PREDOMINATE IN CEREBRAL MALARIA CASES FROM MALI AND MALAWI

Jonathan Lawton1, Albert E. Zhou1, Drissa Coulibaly2, Emily M. Stucke1, Rafał Sobota1, Savý M. Sebastian1, Bryan Cummings1, Ankit Dwivedi1, Antoine Dara1, James B. Munro1, Abdoulaye K. Koné2, Karim Traoré2, Bouréima Togola1, Bourama Tangara1, Amadou Niangaly1, Modibo Daou1, Lisa Diarra1, Yousouf Tolo2, Mody Sissoko2, Matthew B. Laurens1, Amed Ouattara1, Bourema Kouriba3, Ogobara K. Doumbo1, Shannon Takala-Harrison1, Christopher V. Plowe1, Don P. Mathanga1, Terrie E. Taylor1, Joana C. Silva1, Mahamadou A. Thera1, Karl B. Seydel4, Andrea L. Conroy1
1University of Maryland School of Medicine, Baltimore, MD, United States, 2University of Sciences, Techniques and Technologies, Bamako, Mali, 3Karazoo University College of Health Sciences, Blantyre, Malawi, 4Michigan State University, East Lansing, MI, United States

Cerebral malaria is the deadliest manifestation of Plasmodium falciparum infection. Central to its pathogenesis are parasite-derived antigens displayed on infected red blood cells that enable cytoadherence in the microvasculature. These antigens belong to diverse multi-gene families, the largest of which are the RIFINs. Our preliminary data showed that parasites expressed more RIFIN transcripts during cerebral malaria vs. uncomplicated malaria in Malian children. Here, we investigated RIFIN transcript diversity in cerebral malaria cases from two distinct African regions. We hypothesized that certain RIFIN transcripts from West African (Mali) and East African (Malawi) infections share high sequence identity, suggesting important roles in pathogenesis. We collected blood from Malian (n=22) and Malawian (n=10) children with retinopathy-confirmed cerebral malaria and matched uncomplicated controls. For each sample, we conducted RNA-sequencing, assembled de novo parasite transcriptomes, and identified mRNA transcripts encoding surface antigens. We performed a multiple sequence alignment of transcripts from cerebral infections and visualized relationships with a principal component analysis. The average amino acid identity shared between any two RIFIN transcripts was 44%. We identified two large clusters corresponding to the A-RIFIN and B-RIFIN groups, each with similar distributions of transcripts from Malian and Malawian cases. Highly expressed RIFIN transcripts formed two smaller, conserved subgroups. One subgroup included RIFINs expressed only in Mal and shared ~93% sequence identity. The other subgroup contained transcripts from both Mal and Malawi, sharing ~99% identity, but diverged significantly from the majority of sequences along the first principal component. Although parasites in cerebral malaria infections expressed a diversity of RIFIN variants, conserved subgroups predominated. These RIFINs may be promising candidates for vaccine development. Next, we will define additional RIFIN subgroups based on sequence similarity and compare expression levels in cerebral vs. uncomplicated malaria.

STOOL MICROBIOME IN UGANDAN CHILDREN IS ASSOCIATED WITH DIFFERENTIAL MALARIA OUTCOMES

Olivia J. Bednarski
IUSSM, Indianapolis, IN, United States

Gut microbiota in mice modulates the severity of malaria by regulating the humoral immune response to Plasmodium. It is presently unknown if gut microbiota also impacts the severity of malaria in humans. This study sought to assess if the composition of the gut microbiota differentially affects the development of severe malaria in Ugandan children infected with P. falciparum. We sequenced the bacterial 16S rRNA gene in over 500 stool samples from <5-year-old Ugandan children with five clinically distinct severe malaria presentations (prostration, severe malaria anemia, multiple seizures, respiratory distress, and cerebral malaria), and healthy community children (consisting of both P. falciparum negative and asymptomatic P. falciparum positive children). When assessing alpha diversity, asymptomatic and community children had greater richness and evenness compared to children that developed severe malaria. Beta diversity analysis demonstrated significant dissimilarity in stool bacteria communities between each severe malaria subgroup and asymptomatic children. Differential abundance testing revealed a single bacteria species as consistently enriched in all severe malaria subgroups. Additionally, longitudinal analysis over twelve months revealed specific bacteria were enriched in the community children that subsequently developed severe malaria. Ongoing studies include the investigation of bacterial biochemical pathways between groups and assessing the causative potential of the differential microbiome communities towards severe malaria using gut microbiome humanized mice. Our data provide the first demonstration that specific gut bacteria are associated with the severity of malaria in African children.

PROJECT “HEMOGLOW”: THE DEVELOPMENT OF GENETIC TOOLS FOR THE STUDY OF MOSQUITO HEMOCYTES

George-Rafael Samantsidis, Ryan C. Smith
Iowa State University, Ames, IA, United States

Immune cells, known as hemocytes, are central to mosquito cellular and humoral immune responses that include phagocytosis, the establishment of immune memory, and the recognition and killing of malaria parasites. Yet,
Despite their importance, studies of mosquito hemocytes have been limited by a lack of genetic resources that have restrained their characterization to morphological properties of size and shape. Recent single-cell studies have characterized mosquito immune cell populations with new and increased resolution, establishing a set of universal or subtype-specific marker genes that can define mosquito immune cell sub-populations. With the intent to develop genetic tools and resources to improve our understanding of mosquito immune cell function, we have now generated transgenic Anopheles gambiae expressing fluorescent markers under the regulation of promoters that drive universal hemocyte or granulocyte-specific gene expression. Additional oenocytoid-specific lines are still under development. Moreover, experiments are currently under way to use these promoters for the development of a binary Q system in mosquito hemocytes with promise for genetic ablation, overexpression studies, or hemocyte-specific gene-silencing. Together, our experiments will provide valuable new genetic resources for the mosquito hemocyte community that will significantly advance our understanding of Anopheles immune cell biology, while serving as the foundation for future studies to better define the contributions of hemocytes to malaria parasite killing.

**7210**

**SCALED PRODUCTION OF A FEMALE-SPECIFIC LARVICIDAL YEAST DIET TO FACILITATE MASS-REARING OF MALE MOSQUITOES**

*Teresa Njoroge*1, Corey Brizzee2, Jack Crawford2, Keshava Mysore1, Molly Duman-Scheel1

1Indiana University School of Medicine, South Bend, IN, United States, 2DelMeeta AgBio, Lexington, KY, United States

Sex-sorting prior to mass release of male mosquitoes is critical for several population-based mosquito control technologies, including the sterile insect technique. However, methods of sex-sorting that can be scaled and implemented globally in multiple species of mosquitoes have not been established. We demonstrated that yeast strains expressing shRNA targeting mosquito GGT genes facilitate sex sorting and generation of fit Aedes, Culex, and Anopheles gambiae males. Replacement of the nutritional yeast component of larval mass rearing diets with heat-inactivated dried yeast resulted in female-specific larval death, yielding production of fit males and 5 male:1 female ratios in adults. While these studies successfully demonstrated proof of concept, the global deployment of this strategy to mass rearing facilities worldwide requires scaled production of the yeast, which is not feasible with the laboratory yeast strains used in our pilot studies. To address this, Cas-CLOVER, an RNA-guided dimeric nuclease system, was used in combination with piggyBac transposase to generate a robust commercial-ready yeast strain with multiple integrated copies of an expression cassette for production of shRNA targeting the Culex spp. GGT gene. shRNA production levels in this robust yeast strain were confirmed to be several fold higher than that of the initial lab strain, facilitating efficient sex sorting with a fraction of the amount of dried yeast used in the initial studies. Large-scale fermentation facilitated kilogram-scale production of the yeast, which was heat killed and dried. shRNA levels were maintained during this process, which is being further optimized in preparation for global deployment of the yeast larvicides. The results of this study indicate that production of female-specific RNAi yeast larvicides can be scaled to facilitate global implementation of population-based control strategies that require releases of sterile or genetically modified adult males. Ongoing efforts to generate additional commercial-ready strains that facilitate sex-separation in other species of mosquitoes will be presented.

**7211**

**CHARACTERIZATION OF THE MOSQUITO SPOROZOITE-ASSOCIATED SALIVA PROTEINS: TWO POTENTIAL MALARIA PARASITE TRANSMISSION-BLOCKING TARGETS**

*Yuemei Dong*1, Emilia C. Cuccurullo1, Caire Barreto1, Yu-Min Chuang2, Erol Fikrig2, George Dimopolous1

1Johns Hopkins School of Public Health, Baltimore, MD, United States, 2Yale University School of Medicine, New Haven, CT, United States

Plasmodium relies on numerous mosquito-derived host factors when engaging its intimate interactions with the vector’s midgut, hemolymph, and salivary glands. Mosquito saliva primarily facilitates blood feeding but can also affect the malaria parasites transmission, yet mosquito salivary proteins’ function remains fully characterized. A general screen of the mosquito salivary proteins associated with Plasmodium sporozoites while moving out of the mosquitoes was established, and two specific sporozoite-associated mosquito saliva protein 1 (SAMSP1) and AgSAP were identified through this screen; SAMSP1 plays a role in facilitating Plasmodium infection in mice, and AgSAP has immunomodulatory activity. Using CRISPR/Cas9-mediated gene editing, we have generated knockout mosquitoes lacking SAMSP1 and AgSAP and used them for functional assays in the mosquito midgut and salivary gland stages. Both SAMSP1 and AgSAP knockout mosquitoes have yielded a significant reduction of P. falciparum oocysts and sporozoite loads. Using RNAi-mediated gene silencing, we are confirming the agonist function of SAMSP1 and AgSAP in the mosquito midgut and salivary glands. Co-feeding of the polyclonal antibodies of SAMSP1 has shown a significant reduction of P. falciparum oocysts loads, but no differences were observed when co-feeding with AgSAP IgGs. The effect of SAMSP1 on oocyst loads suggests that it may be ingested together with the gamaetocytes by the mosquito and there affects parasite infection of the midgut. Using these knockout mosquitoes we will elucidate this hypothetical mechanism of SAMSP1 host factor function.

**7212**

**CHROMOSOMAL INVERSIONS IN Aedes aegypti ARE ASSOCIATED WITH GEOGRAPHICAL ORIGIN, BEHAVIOR, AND RESISTANCE TO PATHOGENS**

Jiangtao Liang1, Ilya I. Brusentsov1, Varvara Lukyanchikova1, Noah Rose1, Dmitry A. Karagodin1, Zhijian Tu1, Carolyn McBride2, Maria V. Sharakhova2

1Virginia Tech, Blacksburg, VA, United States, 2University of California, San Diego, CA, United States, 3Institute of Cytology and Genetics, Novosibirsk, Russian Federation, 4Princeton University, Princeton, NJ, United States

Chromosomal inversions play a fundamental role in evolution and have been shown to be associated with the epidemiologically important traits in malaria mosquitoes. However, they have never been characterized in the major vector of arboviruses Ae. aegypti because of the poor structure of its polytene chromosomes. In this study, we applied a Hi-C proximity ligation approach to identify chromosomal inversions in 20 strains of Ae. aegypti including world-wide population from 13 countries across the tropics, two old laboratory colonies, and the closely related species Ae. mascarasia. The study identified 23 multi-megabase inversions with size variations between 5.2 and 55 Mb and uneven distribution along the chromosomes. Most of the inversions were located in the 1q and 3p chromosomal arms. The inversions were highly associated with the geographical origin of the strains and were more abundant in the African strains than in the non-African, 15 versus 3 inversions, respectively. Among African inversions, 2 inversions were found in multiple strains and spread all over the African continent, 4 inversions were discovered only in West Africa and other African inversions were rare, found in one or in two strains. All chromosomal inversions, including the one specific for Ae. mascarasia, were polymorphic. Some of the inversions were shown to be associated with genomic locations of chemoreceptor genes and quantitative trait loci...
to different infections. Thus, our results suggest existence of a large pool of structural variations in the Ae. aegypti genome potentially involved in the adaptation to humans and pathogenesis in this mosquito.

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TARGETING PLASMODIUM IN THE MOSQUITO VECTOR: VULNERABILITIES AND OPPORTUNITIES FOR MALARIA CONTROL

George Dimopoulos
Johns Hopkins University, Baltimore, MD, United States

Malaria, one of the world’s deadliest diseases, is caused by Plasmodium parasites that are vectored to humans by the bite of Anopheles female mosquitoes. Despite massive control efforts, malaria remains a major global public health burden. The emergence of parasite resistance to drugs and mosquito resistance to insecticides represent major hurdles for malaria control, stressing the need for the development of novel strategies to fight this deadly disease. In recent years, the development of novel Anopheles genetic tools has opened the path towards new strategies to control malaria by targeting the parasite in the vector. Plasmodium has to complete a complex infection cycle within the mosquito, involving multiple developmental transitions and interactions with mosquito tissues and host factors. Each one of these stages and interactions provide specific opportunities for transgenic interference with the parasite’s infection cycle that could result in transmission-blocking. While the invasive stages of Plasmodium infection in the mosquito midgut epithelium may be the most studied, the earlier stages in the blood bolus along with the later oocyst and sporozoite stages also present vulnerabilities that can be exploited to block infection. Here we present and discuss approaches, with their pros and cons, for targeting the various Plasmodium stages in the mosquito using genome editing and antibody-based approaches.

7214

FUNCTIONAL GENOMIC ANALYSIS OF TRANSCRIPTIONAL ENHANCERS IN THE MALARIA VECTOR ANOPEHLES COLUZZI

Natalia M. Zmurlak1, Karin Eiglemeier1, Inge Holm1, Kimani Njoya2, Cameron Anderson2, Michelle M. Riehle2, Kenneth D. Vernick1
1Institut Pasteur, Paris, France, 2Medical College of Wisconsin, Milwaukee, WI, United States

Transcriptional enhancers are noncoding regulatory elements that are responsible for almost all regulated gene expression in eukaryotes. The role of enhancers in vector phenotypes has barely been examined, but differential activity of enhancer likely plays a role in natural phenotypes such as insecticide resistance, parasite susceptibility, behavior, or adaptation to ecological conditions. We generated a genome-wide catalog of 3300 functional enhancers in the African malaria vector, Anopheles coluzzii, and filtered them genetically to identify a panel of candidate enhancers with the potential to influence susceptibility for Plasmodium falciparum infection. The activity of selected candidate enhancers was modified by CRISPR/Cas9 or by depletion of enhancer RNAs in Anopheles cells and whole mosquitoes. At least one enhancer identified in the screen regulates expression of a gene with large effect on P. falciparum infection rates. Results will be presented on enhancer function on gene expression and parasite infection.

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PREMONITION BARCODE: USING THE MITOGENOME FOR MOSQUITO SPECIES IDENTIFICATION AND SURVEILLANCE

Renee Ali1, Christian H. Gauthier2, Kevin Pritts3, Bret Nash3, Jeremy Verde1, Hengameh Rezaei1, Miguel A. Saldarriaga1, James Pipas3, Ethan Jackson3, Mike Reddy4, Douglas E. Norris1
1Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, 2University of Pittsburg, Pittsburg, PA, United States, 3Harris County Public Health, Houston, TX, United States, 4Microsoft, Redmond, WA, United States

Over the last few decades, mosquitoes vectors have expanded their geographic range; because of their medical importance a reliable and accurate strategy for species identification is necessary for implementation of control strategies for those associated with disease transmission. Despite advancements in molecular techniques, identification of taxa belonging to cryptic species complexes have remained problematic. DNA barcoding has been traditionally used to generate reference barcodes from the cytochrome oxidase unit I (COI) gene and the ribosomal DNA internal transcribed spacer 2 (ITS2) region, which have both shown to be limited in rectifying species classification. However, expansion of sequencing strategies, including capture of the complete mitogenome, have proven useful in providing more comprehensive phylogenetic information when compared to individual COI and ITS2 gene sequences. Here, we used genome skimming to recover the mitochondrial genomes of mosquitoes retrieved and identified from a Premonition’s Biological Weather Station (BWS) deployed in Harris County (Houston), Texas. Individual mosquito specimens were sequenced, and the raw reads were obtained using Illumina Miseq V2 chemistry; construction was executed using the NOVOPlasty assembler with related species. We generated mitogenomes for Aedes aegypti, Culex quinquefasciatus, Ae. albopictus and Anopheles crucians mosquito species which was also putatively identified by the Premonition BWS. The mitochondrial genomes produced in this study not only speciated but separated the mosquito specimens into individual clades. This study demonstrates that skimming for the mitogenome can be used for species identification and simultaneous taxonomic classification rectifying both mosquito and Plasmodium species, as well as provide a source of new useful genetic barcodes.

7216

AGE AND PARASITEMIA EXPLAIN MOST OF THE VARIATION IN HOST AND PARASITE GENE EXPRESSION AMONG MALIAN CHILDREN INFECTED WITH PLASMODIUM FALCIPARUM

Kieran Tebben1, Salif Yirampo2, Drissa Coulibaly3, Abdoulayye K. Koné4, Matthew B. Laurens1, Emily M. Stucke1, Ahmadou Dembélé1, Youssouf Tolo2, Karim Traoré2, Madoum Ngaly2, Andrea A. Berry1, Bourema Kouriba2, Christopher V. Plowe1, Ogobara K. Doumbo2, Kirsten E. Lyke1, Shannon Takala-Harrison1, Mahamadou A. Thera2, Mark A. Travassos1, David Serre1
1University of Maryland, Baltimore, Baltimore, MD, United States, 2University of Pittsburg, Pittsburg, PA, United States, 3Medical College of Wisconsin, Milwaukee, Milwaukee, WI, United States, 4Microsoft, Redmond, WA, United States

Plasmodium falciparum caused ~600,000 deaths in 2021, primarily in young children. In Bandiagara Mali, malaria transmission is seasonal; children experience 2 malaria episodes per season, on average. However, even in the same transmission area, the parasitemia and number of symptomatic infections vary between children, and the host and parasite factors contributing to this variation are incompletely understood. We characterized host and parasite transcriptomes from 136 blood samples from children infected with Plasmodium malaria and generated a total of 85 million reads per sample, on average, enabling analyses of 2484 Plasmodium and 9205 human genes. We used gene expression deconvolution to estimate the proportion of immune cells and parasite stages in each sample and corrected our differential gene expression analyses for differences between children. Parasitemia and host age

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explained most of the variation in host and parasite gene expression, while few genes were associated with the number of symptomatic infections in the study period, COI or sex. Gene expression differences associated with parasitaemia were driven by differences in cell composition. Higher parasitaemia infections had more neutrophils and ring-stage parasites and fewer T-cells and trophozoites, suggesting parasitaemia-dependent T-cell suppression and/or parasite sequestration. Similarly, parasite genes associated with the child’s age resulted from differences in stage composition, with older children having proportionally more male gametocytes. By contrast, the host gene expression associated with age was not completely explained by differences in immune cell composition, suggesting true transcriptional differences. In particular, many genes involved in innate response (TLR and NLR signaling) were more expressed in younger children while genes involved in adaptive immunity (TCR and BCR signaling) were higher in older children. These analyses contribute to our understanding of the pathogenesis of P. falciparum and can provide insight for targeted prevention and treatment strategies based on age and/or parasitemia of infected children.

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A FOURTH LOCUS IN THE PLASMODIUM FALCIPARUM GENOME ASSOCIATED WITH SICKLE HEMOGLOBIN

William L. Hamilton1, Gavin Band2, Eleanor Drury1, Christen Smith1, Sónia Gonçalves1, Kirk Rockett2, Dominic P. Kwiatkowski1, Lucas N. Amenga-Etego3

1Wellcome Sanger Institute, Cambridge, United Kingdom, 2Wellcome Centre for Human Genetics, Oxford, United Kingdom, 3West African Centre for Cell Biology of Infectious Pathogens (WACCBIP), Accra, Ghana

Malaria causes worldwide morbidity and mortality and has been a strong selective force on the human genome. Why some individuals develop life-threatening severe malaria while others experience mild or asymptomatic infection is incompletely understood. Heterozygosity for sickle haemoglobin (HbS) confers the strongest known genetic protection against severe malaria. A recent study identified genetic variants in three Plasmodium falciparum regions, termed Pfsa1-3, that were associated with HbS in severe malaria cases from Kenya and The Gambia. Here, we investigated parasite associations with haemoglobin variants in people with mild malaria in northern Ghana. Blood samples were collected for three years and underwent both human β-globin genotyping and P. falciparum whole genome sequencing. 1,368 samples were available for analysis after quality control filtering. HbS was found in approximately 3% of people, while the west African haemoglobin variant, HbC, was more common (approximately 23%). We replicated the previously identified associations with HbsA at Pfsa1 and Pfsa3. The Pfsa2+ allele was absent from this population. A fourth locus, which we term Pfsa4, was also associated with HbS. The Pfsa4 association was not apparent in the severe cases from Kenya and The Gambia, but replicated convincingly in a published sample of mild malaria from Mali. The Pfsa4 mutation lies in the gene FKK4.2, which encodes a serine/threonine kinase that is exported into the red blood cell (RBC) membrane and is thought to affect RBC rigidity and adhesion. The Pfsa1-4 loci vary widely in frequencies across Africa and are absent in Asia. Pfsa4 is generally absent or very low frequency in east Africa and much higher frequency in west Africa, while Pfsa2 shows the inverse pattern. The Pfsa1-4 loci are also highly correlated with each other to varying degrees in multiple populations in Africa and, unexpectedly, in Colombia. We did not identify significant parasite associations with HbC. These findings suggest new functional avenues for exploration and add new complexity to the emerging picture of association between human and co-evolving parasite genomes.

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MALARIA PARASITE RELATEDNESS IS UNDERESTIMATED WHEN USING SPARSE MARKER DATA FROM INBRED POPULATIONS

Somya Mehra1, Daniel E. Neafsey2, Aimée R. Taylor3

1University of Melbourne, Parkville, Australia, 2Harvard T.H. Chan School of Public Health, Boston, MA, United States, 3Institut Pasteur, Paris, France

Malaria genomic epidemiology is increasingly recognized as a tool for public health. Identity-by-descent (IBD), which captures likeness derived from common ancestors, is a useful concept for malaria parasites because they sexuality recombine. Analyses of malaria parasite relatedness based on IBD are important for generating results on spatiotemporal scales relevant to disease control. Relatedness estimates can be computed based on sparse genotypic data, including data on panels of SNPs (i.e., molecular barcodes) or microhaplotypes (a.k.a. amplicon sequencing panels), which can be generated at the scale of public health efforts. However, there are systematic differences between estimates based on sparse data compared to gold-standard whole genome sequencing (WGS) data, including the over-representation of zero-valued estimates. To better understand these differences, we mathematically interrogate models of relatedness and construct a tailored simulation framework. We show how sparse data can yield systematic underestimates of pairwise relatedness in settings with elevated relatedness across the malaria parasite population, due to the partial encoding of average population relatedness within sample allele frequencies. We propose a reinterpretation of maximum likelihood relatedness estimates based on sparse data, and practical diagnostics for identifying and correcting potentially problematic scenarios. Elevated relatedness across a parasite population is often found in pre-elimination settings. This is because elimination efforts often lead to fewer infectious mosquito bites, fewer multilocal infections, bottlenecks in parasite diversity, and thus fewer opportunities for effective parasite recombination. Our results call for immediate investigation of the practical consequences of systematic underestimation of relatedness in pre-elimination settings for all applications in malaria genomic epidemiology that use relatedness directly or indirectly, including molecular surveillance and genetic-based recrudescence classification in therapeutic efficacy studies.

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MODELING THE EFFECTIVENESS OF GENOMIC SURVEILLANCE AT DETECTING GENETICALLY DISTINCT MALARIA PARASITES POPULATIONS

Alex Ferris, Jessica Ribado, Albert Lee, Joshua Proctor

Institute for Disease Modeling, Seattle, WA, United States

Genomic surveillance of malaria is becoming increasingly common in sub-Saharan Africa and has the potential to enable tracking additional features of malaria parasite populations compared to using rapid diagnostic tests or microscopy on their own. Currently, genetic data is being used to monitor drug resistance markers, but it also has the potential to be used to differentiate between local and imported infections based on genetic similarity. We are using a diverse set of stochastic, agent-based transmission models with varying complexity and characteristics to explore the relationship between prevalence, importation intensity, sample size, and sequencing depth in a generalizable way. These simulated transmission trees are then processed using GENEPI, a software package that models the evolution of genetic diversity in the malaria parasite population. Our findings show that the model pipeline can help identify prevalence and importation regimes where it is more feasible to differentiate between local and imported infections. Additionally, the model can help us to find the limits of identifying genetically distinct parasite populations using variant panels or whole genome sequencing. Finally, this framework can also be used as a tool for identifying relevant genetic features and summary statistics that capture key transmission characteristics.
MEASURING CHANGES IN PLASMODIUM FALCIPARUM POPULATION SIZE AND STRUCTURE IN RESPONSE TO SEQUENTIAL MALARIA CONTROL INTERVENTIONS

Kathryn E. Tiedje1, Qi Zhan2, Shazia Ruybal-Pésantez2, Gerry Tonkin-Hill1, Qin He1, Mun Hua Tan1, Dionne C. Argyropoulos1, Samantha L. Deed1, Anita Ghanashii1, Abraham R. Oduro1, Kwadwo A. Koram2, Mercedes Pascual1, Karen P. Day2
1The University of Melbourne, Melbourne, Australia, 2The University of Chicago, Chicago, IL, United States, 3Noguchi Memorial Institute for Medical Research, Legon, Ghana, 4Navrongo Health Research Centre, Ghana Health Service, Navrongo, Ghana

The diversity of Plasmodium falciparum within human hosts requires parasite population size be defined in terms of parasite variation rather than the number of infected hosts. To calculate population size, we arrive at a definition of parasite variation known as multiplicity of infection (MOIvar), based on the hyper-diversity of the var multigene family to measure within-host diversity. Using this approach, we track changes in parasite population size and structure from baseline and through sequential malaria interventions by indoor residual spraying (IRS) and the seasonal malaria chemoprevention (SMC) in an area characterized by high-seasonal malaria transmission in northern Ghana. Deep sampling of the DBLε-encoding region of var genes by targeted amplicon sequencing was completed on asymptomatic P. falciparum isolates at baseline (2012), during IRS (2014), post-IRS (2015) and during SMC (2017) from ~2,000 individuals of all ages at each time point. Following IRS, which reduced transmission intensity by >80% and decreased parasite prevalence by ~40-50%, significant reductions in var diversity, MOIvar, and population size were observed across all ages. These changes, consistent with the loss of diverse parasite genomes, were short lived and 32-months after IRS was discontinued and SMC was introduced, var diversity and population size rebounded in all age groups except for the younger children (1-5 years) targeted by SMC. By measuring population size, we show that despite major perturbations, the parasite population remained very large and retained the var population genetic characteristics of a high-transmission system (high var diversity; low repertoire similarity) demonstrating the resilience of P. falciparum to short-term interventions in high burden countries of sub-Saharan Africa.

UNRAVELLING PLASMODIUM FALCIPARUM GENETIC DIVERSITY USING TARGETED AMPICLIN DEEP SEQUENCING TO GUIDE ELIMINATION INTERVENTIONS IN SOUTH AFRICA

Hazel Beverly Gwarinda1, Andres Aranda-Diaz2, Bryan Greenhouse2, Jaishree Raman1
1National Institute for Communicable Diseases, Johannesburg, South Africa, 2University of California San Francisco, San Francisco, CA, United States

The WHO recently acknowledged the progress made by South Africa towards halting local malaria transmission. However, persisting pockets of residual transmission have halted progress toward elimination. This study explored the feasibility and value of incorporating parasite genomic data into routine malaria surveillance data to provide evidence for decision-making by NMCPs. Dried blood spots and positive malaria rapid diagnostic tests were routinely collected from healthcare facilities in South Africa’s three malaria-endemic provinces, KwaZulu-Natal, Mpumalanga, and Limpopo, from 2020-2022. These samples were sequenced at the National Institute for Communicable Diseases, using a 274-target amplicon sequencing panel (Multiplex Amplicons for Drugs, Diagnostics, Diversity, and Differentiation Haplotypes using Targeted Resequencing, MAD4HatTeR). The panel includes antimalarial drug and diagnostic resistance targets, vaccine targets, and diverse microhaplotypes. During this pilot project, we successfully sequenced 500 samples predominately from the eliminating districts within KwaZulu-Natal and Mpumalanga. No evidence of drug or diagnostic resistance was detected, with limited genetic relatedness detected between pairs of infections of samples collected in KwaZulu-Natal and Mpumalanga. There was more clustering of parasite isolates collected in Limpopo, compared to KwaZulu-Natal and Mpumalanga, confirming local transmission in Limpopo, and limited local transmission in KwaZulu-Natal and Mpumalanga. This assisted the program with more accurate case classification and intervention selection to reduce residual transmission and emphasizes the need to strengthen malaria control efforts in Limpopo if South Africa is to eliminate malaria. This pilot study showed that it was feasible to incorporate genomic surveillance into routine malaria surveillance and generate data that is useful to NMCP in their evidence-based decision-making process. For sustainability, it is essential that there are adequate funding resources to support the sequencing platform and skilled genomic and bioinformatics specialists.

RISK AND SIZE OF AEDES-BORNE DISEASE OUTBREAKS ARE POORLY PREDICTED BY CLIMATE-BASED SUITABILITY INDICES

Alexander Dolnick Meyer1, Sandra Mendoza Guerrero2, Natalie E. Dean1, Kathryn B. Anderson1, Steven T. Stoddard2, T. Alex Perkins3
1University of Notre Dame, Notre Dame, IN, United States, 2Emergent BioSolutions, Gaithersburg, MD, United States, 3Emory University, Atlanta, GA, United States, 4SUNY Upstate Medical University, Syracuse, NY, United States

The recent geographical expansion of Aedes mosquito-borne diseases (ABD) is a global health threat. Quantifying these pathogens’ epidemiology and identifying at-risk populations are key steps toward preparing for future ABD outbreaks. Data from past outbreaks should be central to informing these efforts, but leveraging these data toward generalizable conclusions is often difficult. Outbreak data are context-dependent and take various forms (e.g., a time-series of cases or retrospective serology data), precluding straightforward comparisons. In this presentation, we approach this problem from two angles, using chikungunya virus (CHIKV) as an example. First, we show how outbreaks with different types of data can be compared directly through the framework of Bayesian inference and mathematical modeling. We use this approach to estimate several measurements of outbreak risk and potential size, such as the basic reproduction number (R0), for 87 CHIKV outbreaks. Second, we test whether these risk estimates can be predicted using local, pre-outbreak information, including demographic factors and previously published climate-based indices of suitability for ABD transmission. Our results suggest that climate-based indices may approximate where outbreaks can occur, but do not predict R0, outbreak risk, or potential outbreak size. More broadly, we illustrate the importance of combining a biologically realistic model with various data sources when quantifying the risk of ABD transmission.

REDUCED DENGUE INCIDENCE FOLLOWING LARGE-SCALE RELEASES AND ESTABLISHMENT OF WMEl WOLBACHIA IN AEDES AEGYPTI MOSQUITOES IN THREE COLOMBIAN CITIES

Ivan D. Velez1, Maria Patricia Arbelaez1, Simon C. Kutcher2, Alexander Uribe1, Luis Martinez1, Jai A. Denton2, Cameron P. Simmons2, Katherine L. Anders2, Peter A. Ryan2, Scott L. O’Neill2
1World Mosquito Program, Universidad de Antioquia, Medellin, Colombia, 2World Mosquito Program, Monash University, Melbourne, Australia

The introduction of the wMel strain of Wolbachia into Aedes aegypti mosquitoes reduces their capacity to transmit dengue and other arboviruses. Randomized and non-randomized studies in multiple countries have shown reductions in dengue incidence following field releases of WMel-infected Ae. aegypti. We present here the public health outcomes from phased, large-scale releases of wMel-Ae. aegypti mosquitoes throughout the contiguous cities of Bello, Medellin and Itagüí in the Aburra Valley, Colombia, with a combined population of 3.3 million people. Pilot wMel releases were conducted in Bello in 2015-2016, then staged city-wide adult releases throughout the three cities between May 2017
and December 2020, with supplementary egg releases in some areas. Prevalence of wMel in the Ae. aegypti population over time was monitored via adult mosquito trapping during and after releases. By the last monitoring in July 2021-April 2022, wMel was stably established at a high level (>80% prevalence) throughout Belo and Itagui and at a moderate-to-high level (>60%) in 11/18 release areas in Medellín. The public health impact of the wMel releases was evaluated to July 2022 using interrupted time series analysis of notified dengue cases in the three cities, and a prospective case-control study in outpatient clinics in 4 comunas in Medellín (2019-21). Stable introduction of wMel into local Ae. aegypti populations was associated with a significant reduction (94-97%) in notified dengue incidence in each city, compared to ten years pre-release, after adjusting for seasonal trends. A causal association between wMel deployments and reduced dengue incidence was supported by the results of the case-control study, which showed a 47% reduction in the incidence of virologically-confirmed and presumptive dengue cases wMel-treated versus untreated neighborhoods. These results from the largest contiguous implementation of Wolbachia mosquito releases to-date highlight the operational feasibility and real-world effectiveness of wMel deployment in large urban settings, and the reproducibility of the public health benefit across different ecological settings.

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THE IMPACT OF INTEGRATED VECTOR MANAGEMENT ON THE INCIDENCE OF DENGUE IN URBAN MALAYSIA: THE IDEM CLUSTER-RANDOMIZED CONTROLLED TRIAL

Nurulhusna Ab Hamid1, Neal Alexander2, Tim Möllmann3, Carole Langlois-Jacque4, Farah Diana Ariffin5, Mad-Hélénie Elsensohn6, Frederic Schmitt7, Jason H. Richardson8, Maxime Leduc9, Norazman Bin Mohd Rosli10, Nazni Wasi Ahmed11, Muriel Rabilloud12, Mitra Saadatan-Elahi13

1Medical Entomology Unit, Institute for Medical Research, WHO Collaborating Centre, Institute for Medical Research, Ministry of Health Malaysia, Kuala Lumpur, Malaysia, 2MPo Tropical Epidemiology Group, Department of Infectious Disease Epidemiology, London School of Hygiene and Tropical Medicine, United Kingdom, United Kingdom, 3In2Care, Wageningen, Netherlands, 4Hôpices Civils de Lyon, Lyon, France, 5Environmental Science France - Envu, Lyon, France, 6Innovative Vector Control Consortium (IVCC), Liverpool, United Kingdom, 7Bayer, Lyon, France, 8Vector Borne Disease Sector, Disease Control Division, Ministry of Health Malaysia, Kuala Lumpur, Malaysia

For dengue control, the World Health Organization recommends cost-effective and sustainable integrated vector management (IVM). Dengue is the communicable disease with the highest incidence in Malaysia. In the country’s capital, Kuala Lumpur and Putrajaya, we carried out a large cluster-randomised trial of an IVM strategy. Each cluster was a locality, i.e., an urban housing development with shared facilities and one or more medium- or high-rise apartment blocks. 280 localities were randomised either to control, i.e., routine control activities, or to an IVM strategy consisting of (1) targeted outdoor residual spraying with K-Othrine, (2) auto-dissemination devices with the active ingredients of Wolbachia mosquito releases to-date highlight the operational feasibility and real-world effectiveness of wMel deployment in large urban settings, and the reproducibility of the public health benefit across different ecological settings.

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ASSESSING AUTO-DISSEMINATION STATIONS AS A CONTROL TOOL FOR AEDES AEGYPTI IN THE RIO GRANDE VALLEY, TEXAS, USA

Nicole Scavo1, Jose Juarez1, Nadia Fernandez-Santos1, Ester Carbajal1, Luis Chavez1, Berlin Londono-Renteria1, Alyssa Branca1, John Bordens2, Mike Banfield2, Gabriel Hamer2

1Texas A&M University, College Station, TX, United States, 2Indiana University Bloomington, Bloomington, IN, United States, 3Tulane University, New Orleans, LA, United States, 4Banfield Bio, Woodville, WA, United States, 5JHB Consulting, Burnaby, BC, Canada

Novel mosquito control intervention methods are needed to reduce the transmissions of Aedes-borne viruses as only weak evidence exists for the effectiveness of current control practices. Autodissemination, in which the adult mosquitoes themselves spread a larvicide to aquatic environments, has been effective in lab and small-scale trials, but studies evaluating efficacy at larger spatial scales are lacking. We conducted a cluster randomized control trial using autodissemination stations (ADS) loaded with pyriproxyfen (PPF) in eight neighborhoods in the Lower Rio Grande Valley, Texas, USA. Adult Aedes aegypti surveillance was conducted before, during, and after the intervention using BG Sentinel 2 traps. Water was collected from the field to measure PPF concentration and for larval bioassays in the laboratory. Lastly, human exposure to Aedes bites was measured using a Bitemark assay, which is an ELISA-based test measuring human IgG antibodies to the A. aegypti Nteter-3kDa peptide. We achieved an average of 77% coverage (percent of homes with ADS) in our intervention neighborhoods. Our preliminary results suggest that the intervention was successful at reducing adult Aedes mosquito abundance by up to 70%. PPF concentration in field-collected water was generally below detectable limits and laboratory larval bioassays showed no difference in survival between reference and intervention arms. Differences in exposure to human Aedes bites between intervention and reference arms are under analysis. To our knowledge, our results are the second large scale field study to show evidence of ADS as an effective tool for Aedes mosquito population suppression.

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POOLING THE POOLS: REDUCING COSTS OF MOLECULAR ARBOVIRAL SURVEILLANCE WITHOUT LOSS OF SENSITIVITY IN DENGUE ENDEMIC AREAS

Joanelis Medina, Grayson Brown, Maria Garcia, Julienne Miranda

Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) assays are effective tools for detecting transmission of vector borne diseases. They are extremely useful for simultaneous detection of Zika, dengue and chikungunya viruses, to identify specific locations with ongoing transmission and enable health officials to target their efforts to control the spread of vector borne diseases when the mosquito was captured in surveillance traps. Molecular surveillance of mosquitoes can also complement human disease surveillance considering human clinical cases may take more complicated logistics and longer times to be reported. However, the excessive costs and low frequency of positive detections in mosquitoes is a limitation, particularly in lower income endemic areas. Positive mosquitoes are rare if compared to human positive detection; even in areas with high incidence, typically less than 1% of the collected mosquitoes test positive for arbovirus resulting in an extremely expensive cost/detection and mass trapping efforts, with too little actionable information obtained to make the effort cost effective. Normally, mosquitoes are grouped into “pools” from the same geo-coded trap. We developed an approach to evaluate 900 mosquito pool samples instead of 90 per PCR every week and reduce costs by developing a “super pool” with 10 individual pools. If negative, the cost of testing each individual pool is eliminated. If positive, then each pool must be individually evaluated. We demonstrate the super pooling technique developed for use in Puerto Rico to test 900 mosquito pools weekly with a cost reduction estimation of 98% without affecting results, astmh.org
maximizing the use of the procedure invested time, and minimizing reagents
consumption. It is shared how the technique was validated, how it is used
in practice, and how the “all-in” costs of this technique compared to single
pool testing is possible to be reduce. A large surveillance testing program is
not necessarily practical with the traditional approach, but it becomes more
practical with super pooling.

USE OF MACHINE LEARNING TO IDENTIFY NOVEL
MOSQUITO AND TICK REPELLENTS

Marnix Vlot*, Jennifer N. Wei†, Martijn Vos‡, Rob Henderson*, Benjamin Sanchez-Lengeling†‡, Luuk Berning†, Jessica Konijnenburg‡, Brian K. Lee‡, Wesley W. Qian‡, Richard C. Gerkin†, Alexander B. Wiltschko‡, Koen Decker§

†TropIQ Health Sciences, NLMEGEN, Netherlands, ‡Google Research, †Stanford University, Stanford, CA, United States, §Brain Team, Cambridge, MA, United States

Vector-borne diseases, such as malaria and dengue fever, kill over half
a million people annually. Currently available repellents for personal or
household protection are limited in their efficacy, applicability, and safety
profile. To address this issue, we developed a machine-learning-driven high-throughput method for the discovery of novel repellent molecules. Our approach involved digitizing a large, historic dataset containing ~19,000 mosquito repellency measurements. We then trained a graph neural network (GNN) to map molecular structure and repellency. The GNN was able to learn the complex relationships between molecular structure and repellency, allowing us to predict the efficacy of new molecules. To test our method, we selected ~400 candidate molecules to test in parallelizable behavioral assays. We quantified repellency in multiple species and conducted follow-up trials with human volunteers. Here we show that our method was able to identify novel molecules with high efficacy against multiple species. We further expanded the hits identified in our initial screening by testing analogous compounds and identified additional active molecules. Through this process, we discovered several novel repellents that exhibit greater potency than DEET. Our approach also led to the identification of several repellent molecules that demonstrate activity against mosquito species, ticks, and fleas, suggesting their broad-spectrum effectiveness. Additionally, we applied a similar machine learning-driven approach to identify tick repellents specifically. We trained ML models on tick repellency data, tested a selection of compounds, and achieved a hit rate of 57%, further demonstrating the utility and versatility of our high-throughput method for the discovery of novel repellent molecules. Our method has several advantages over traditional methods of discovering mosquito repellents. It is faster and more cost-effective than traditional methods, allows for the screening of large numbers of compounds simultaneously, and can be used to predict the efficacy of new molecules before they are synthesized or tested in vivo.

CHROMOBACTERIUM SPECIES PANAMA (CSP_P)
PELLET FORMULATIONS: A NOVEL BIO-LARVICIDE FOR
MOSQUITO VECTOR CONTROL

Vandana Vandana, George Dimopoulos
John Hopkins University, Baltimore, MD, United States

The development of novel biopesticide to control vector-borne diseases,
is urgently needed due to emerging resistance against currently used
chemical insecticides. Here, we have formulated a larvicide pellets
containing non-live Chromobacterium species Panama (Csp_P) which are
eco-friendly, cost-effective, with prolonged water-stability and residual
activity. Using Csp_P pellet formulation, significant larval mortality was
observed against Aedes aegypti, Anopheles gambiae, Anopheles stephensi
and Culex quinquefasciatus within 48 hrs post-exposure. Interestingly, upon
sub-lethal dose of Csp_P pellet exposure, significantly reduced and delayed
pupation was noticed compared to the control pellet exposed group.
Additionally, Csp_P exposed larvae reaching the pupal stage would not
fail to develop into adults. Notably, Csp_P pellets are irreversibly detrimental to the
larvae that fail to develop into adults. Altogether, our results show that our
Csp_P pellet formulation is effective at killing larvae of multiple mosquitoes’
species and could be developed into a new vector control tool.

DRONES AND DENGUE: PILOTING THE USE OF
UNMANNED AERIAL VEHICLES TO MAP TRASH
DISTRIBUTION AND DENGUE VIRUS RISK IN RURAL AND
URBAN KENYA

Joelle I. Rosser*, Juliet T. Bramante†, Andrew Chamberlin‡, Paul Sillaiii, Bryson Ndengaiv, Donal Bisanziio, Giulio DeLeo**, Francis Mutuku‡, A. Desiree LaBeaud†

*Stanford University, Stanford, CA, United States, †University of Washington, Seattle, WA, United States, ‡Technical University of Mombasa, Mombasa, Kenya, ¶Kenya Medical Research Institute, Nairobi, Kenya, **RTI International, Washington, DC, United States

The incidence of dengue and chikungunya, viruses transmitted by Aedes aegypti mosquitoes, has increased exponentially in the past 50 years and is projected to continue increasing with climate change. Trash, a favored breeding ground for Ae. aegypti, is a critical mediator of this increasing threat; however, research on trash is limited by inadequate techniques to quantify trash distribution and exposure. We hypothesized that high resolution aerial imaging from unmanned aerial vehicles (UAVs) could be used to map trash distribution, quantify trash exposure, and identify high-risk trash microclimates. We piloted the use of UAVs to map trash in rural and urban communities in Kenya and developed a trash classification scheme based on trash appearance and Ae. aegypti risk. We validated the trash identification by ground truthing and assessed the benefits and limitations of the use of UAV imaging for trash identification over ground methods. In our pilot UAV flights, conducted in two sites in Kenya, we identified over 1800 trash piles in the urban site and over 1300 piles in the rural site. The majority of trash sites were small household piles (41%) and the greatest proportion of trash by area consisted of trash scattered in the grass (43%) and trash collection centers (15%). The rural site had a significantly higher proportion of trash mixed with vegetation and deposited in partially completed building sites and decreased evidence of burning trash compared to the urban site. We generated a trash exposure score for individual households and identified trash microclimates in both communities, conferring differential risk for Ae. aegypti– vectored viruses, dengue, and chikungunya. As the global population expands alongside the consumption of non-biodegradable materials, solid waste, particularly plastics, create increasing environmental and human health problems. The validation of a reproducible, quantifiable measure of trash exposure opens the door for rigorously evaluating the relationship between trash and a variety of infectious diseases as well as designing and measuring much needed trash interventions.
In this cohort, 22.8% (1038) participants were seropositive for dengue and 21.4% (972) participants were seropositive for chikungunya based on IgG ELISA test results. Acute arboviral infection was rare; PCR results showed 0.06% (3) participants with an active chikungunya infection and 0.7% (31) participants with active dengue infection and no acute DENV-CHIKV co-infections. Malarial microscopy results showed 15.5% (707) malaria positive participants, three of whom were co-infected with DENV. Amongst the cohort, 0.6% (27) participants were co-exposed to all three infections with 1.5%-9% participants being exposed to more than one infection at the same time. Among the people having exposure to more than one infection, the majority were females, between 18-60 years of age residing in Kisumu. When analyzing the co-seroconversions, 2.2% (31/1379) participants co-seroconverted for DENV and CHIKV in 2020, followed by 2.3% (62/2660) participants in 2021 and 1.9% (7/370) in 2022. Preliminary analysis indicates that older age (OR: 1.04 [95% CI:1.02, 1.06]), presence of water containers and livestock in the house (OR: 2.87 [95% CI:1.22, 6.25]), (OR: 3.37 [95% CI:0.98, 8.87]), and going to school (OR: 0.27 [95% CI: 0.06, 0.78]) are significantly associated with co-exposure to all three infections. The results of the study suggest that Kenyans are highly likely to be exposed to arboviruses and malaria over the course of their lifetime and that the mosquito borne infections continue to represent a public health threat in Kenya.

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INCREASING MALARIA CASES IN THAILAND’S WESTERN BORDER PROVINCES

Prayuth Sudathip1, Sathapana Naowarat2, Suravadee Kitchakarn1, Deyer Gopinath3, Rungrawee Tipmontree1, Chantanta Padungtod1, Niparueradee Pinyajeerapatt1, David Sintasath1, Jui A. Shah2

1Division of Vector Borne Diseases, Department of Disease Control, Ministry of Public Health, Nonthaburi, Thailand, 2Inform Asia: USAID’s Health Research Program, RTI International, Bangkok, Thailand, 3World Health Organization, Nonthaburi, Thailand, 4U.S. President’s Malaria Initiative, United States Agency for International Development (USAID), Regional Development Mission for Asia, Bangkok, Thailand

In February 2020, Thailand became the second country, after China, to report a case of coronavirus disease 2019 (COVID-19). One year later, in February 2021, a political crisis erupted in neighboring Myanmar. In response to the local context, Thailand installed strict movement policies that gradually receded. Since March 2022, malaria cases have surged, contradicting years of progress and coinciding with the country’s biggest peak in COVID-19 cases. This study utilized a Seasonal Autoregressive Integrated Moving Average (SARIMA) time series analysis to assess malaria trends in this changing context, using fiscal year 2019 (FY19) as a baseline. The analysis included all malaria cases from FY19 to FY22 in the national database (n = 21,606). In FY22, 8,548 malaria cases were reported: a 95.6% increase since the previous year and 46.2% since FY19 (p < 0.05). Nearly all (94.4%) cases were reported in six border provinces, with 60.5% of FY22 cases in Tak and 17.2% in Mae Hong Son. Thailand’s seminal 1-3-7 surveillance strategy showed minimal drops in adherence over the study period: Day 1 (87.6% in FY19 vs. 85.3% in FY22), Day 3 (95.4% vs. 97.5%), Day 7 (87.3% vs. 84.4%); these drops were not statistically significant. SARIMA results of t-tests (paired by month) showed a significant increase in mean incidence during the high transmission season (March to September) in FY22 compared to the previous year; in Tak, FY22 mean incidence increased by 253.3 cases per month and in Mae Hong Son they increased by 39.5 cases per month. Thailand’s success in maintaining 1-3-7 surveillance is attributed to both a strong community health worker network and stringent domestic movement restrictions. Relaxations of international border restrictions began in January 2022 for in-demand workers; concurrent changes in population movement across the Myanmar border and behaviors could contribute to these changing epidemiological results. The June 2022 peak in malaria cases is limited to border provinces only, so malaria authorities are considering novel cross-border initiatives such as bilateral foci and active case detection to mitigate the outbreak.

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TRANSLATING CONTINUOUSLY COLLECTED ANTENATAL CARE MALARIA PREVALENCE INTO TRENDS OF COMMUNITY TRANSMISSION AND CLINICAL INCIDENCE — BURKINA FASO, MOZAMBIQUE, AND NIGERIA, 2020–2022

Joseph T. Hicks1, Anna Munsey2, Alexandre Hill1, Dele Babarinde3, Beltazar Candinho4, Siaka Debe5, Peder Digr6, Adama Gansane7, Christelle Gogue8, Chabu Kangal9, Hannah Koenker10, Julia Mwesigwa11, John Miller12, Oketu Oyale Okoko11, Ali Onoja13, Travis Porter14, Eleonore Sternberg15, Perpetua Uhomohi16, Molly Robertson17, Joseph Wagman18, Richard G. Fitzjohn19, Julie R. Gutman20, Patrick GT Walker20

1Imperial College London, London, United Kingdom, 2Centers for Disease Control and Prevention, Atlanta, GA, United States, 3Ibilda Health International, Abuja, Nigeria, 4National Malaria Control Program, Ministry of Health, Maputo, Mozambique, 5Centre National de Recherche et Formation sur le Paludisme, Ouagadougou, Burkina Faso, 6PATH, Seattle, WA, United States, 7PATH, Washington, DC, United States, 8PATH, Lusaka, Zambia, 9Tropical Health LLP, Baltimore, MD, United States, 10PATH, Kampala, Uganda, 11National Malaria Elimination Programme, Abuja, Nigeria, 12The Global Fund to Fight AIDS, Tuberculosis and Malaria, Geneva, Switzerland

Malaria surveillance data collected at antenatal care (ANC) are less prone to treatment-seeking bias compared to surveillance during outpatient visits. They also have advantages over data collected in demographic health surveys, as they are continuously collected and can achieve greater geographic granularity. While ANC malaria prevalence estimates malaria burden in pregnancy and correlates well with prevalence in children under 5 years old, estimation of general community transmission and incidence from this data source requires additional analytical methods. We sought to develop a tool to translate continuously collected prevalence data from ANC surveillance to trends in community malaria transmission. Malaria surveillance by rapid diagnostic test among pregnant women at first ANC visit was conducted as a sub-study of the New Nets Project in 82 health facilities from 10 districts across Burkina Faso (BF), Mozambique (M2), and Nigeria (NG) between September 2020 and December 2022. We incorporated particle Markov chain Monte Carlo (pMCMC) into an existing malaria model to infer trends in entomologic inoculation rate (EIR). Among 50,098 women tested over 28 months, 23.6% (n = 11,813; 95% CI: 23.2–24.0%) tested positive for malaria. Initial EIR ranged from 9 infectious bites per person per year (ibpppy; 95% CI: 5.7–12.9) in Changara, M2 to 2,637 ibpppy (95% CI: 561–4,756) in Asia, NG. Seasonal variation of estimated EIR patterns depended on location. For example, in Banfora, BF, following an EIR of 0.001 ibpppy (95% CI: 0.0–0.5) at the end of the dry season in June 2021, EIR peaked in July 2021 at the onset of rainy season (80 ibpppy; 95% CI: 22–212). In contrast in Ejigbo, NG where malaria burden is less seasonal, EIR did not fluctuate but rather declined steadily from 73 (95% CI: 35–278) to 11 ibpppy (95% CI: 2.5–29) over two years. Our pMCMC approach flexibly recaptured malaria dynamics from locations of varying transmission levels and seasonality patterns. With this framework, ANC surveillance data can help inform malaria intervention activities, such as monitoring chemoprevention impact, within the general community even at a sub-regional level.
ASSESSING HISTORY OF ACCEPTABILITY AND COVERAGE WITH MASS DRUG ADMINISTRATION (MDA) FOR LYMPHATIC FILARIASIS TO INFORM RE-START OF MDA 6 YEARS POST-ELIMINATION

Alison Krentel1, Cut Novianti Rachmi2, Hafizah Jusril2, Gusta Trisna Pratama2, Zahra Izza Arif3, Esther N. Tambunan2, Wiji Wahyuingsih2, Azka Aulia Fitr2, Suci Trisnaseri3, Peter U. Fischer4, Taniawati Supali4, Iwan Ariawan5

1University of Ottawa, Ottawa, ON, Canada, 2Reconstra, Jakarta, Indonesia, 3Washington University at St. Louis, St. Louis, MO, United States, 4Universitas Indonesia, Jakarta, Indonesia, 5Royal Museum for Central Africa, Tervuren, Belgium

Belitung District in Bangka-Belitung Province, Indonesia stopped mass drug administration for lymphatic filariasis (LF) in 2010. Following three rounds of transmission assessments, the district achieved LF elimination status in 2017. It was later revealed that LF transmission was ongoing, and Belitung qualified for the use of ivermectin, DEC and albendazole (IDA) as per WHO’s alternative treatment strategy for LF elimination. To inform the first round of IDA treatment in 2022, an acceptability study was carried out in June 2022 in 20 villages. 16 villages were identified via probability proportionate to size sampling and 4 LF-endemic villages were purposively included in the sample. Only individuals aged 18 years and older were included in the sample with one person per household. Within 444 households invited to participate, 44 individuals refused. A total of 400 participants were included in the final analysis. Results included: 5.5% had never heard about LF; 48.4% reported that they didn’t know the cause of LF (worms); 43.3% did not know the transmission route for LF (mosquitoes); 39.4% reported that they had never taken treatment for LF (never treated) and 37.6% reported taking treatment once. Recommended messages derived from the survey results included promoting: the safety and effectiveness of the MDA treatment; ancillary benefits of LF MDA (scabies, intestinal worms); importance of taking MDA for the benefit of the whole community. Results and suggested messages were presented to 73 district stakeholders in July ahead of the October 2022 MDA. Results from the consultations included: identify local resources to support MDA; revisit the occurrence of adverse events; reinforce directly observed treatment; recommend refresher training prior to MDA for health staff; consider urban characteristics to adjust MDA; work with community leaders to reduce number of households who may refuse MDA. Research revealed the importance of using evidence from a rapid and timely community assessment to inform socialization and implementation of MDA. Survey results can be used to create momentum within districts and communities towards an effective MDA.

BUILDING THE CAPACITY OF COMMUNITY INFLUENCERS TO INCREASE THE EQUITY AND IMPACT OF UGANDA’S TRACHOMA RESPONSE

Hilda Kyarisiima1, Alfred Mubangizi1, Emmanuel Ssegawa2, Palma Marwas2, Claire Karlsson3, Andrew Kyambadde3

1Uganda Ministry of Health, Kampala, Uganda, 2WI-HER, Vienna, VA, United States, 3RTI International, Washington, DC, United States

The Uganda MOH identifies and overcomes gender equity and social inclusion (GESI)-related barriers to the uptake of trachoma preventive medicine in Uganda through building the capacity of community influencers. The goal of the approach is to reach populations, like nomadic pastoralists, who have historically been missed by past mass drug administration (MDA), and by reaching them with MDA, accelerate trachoma elimination efforts. To improve medicine access and acceptance among these “hard to reach” groups, the Uganda MOH, with support from USAID’s Act to End NTDs | East (Act | East) program, has implemented targeted behavior change (BC) activities in villages with the highest burden of trachoma. The MOH trains district officials to supervise BC teams comprised of Village Health Teams (VHTs), local elders and religious leaders. Root cause analysis follows via focus group discussions and interviews with a selection of the community who missed the previous MDA to identify the key barriers to MDA uptake. The district officials validate the results of the root cause analysis with the influencers, and coach on addressing the root causes through concrete behavior change and social mobilization actions, tailored to the community’s needs and the influencers’ positions of trust and authority. The Uganda MCH and Act | East also provide training materials in the local language and coach MDA supervisors on how to make trachoma MDA more accessible and therefore more equitable. In 16 villages with some of the highest prevalence of trachoma, 68 (40 M; 28 F) community influencers have been trained on GESI-integrated BC and supported to implement interventions for improving MDA uptake. These efforts have contributed to village-level MDA coverage improvements from below 55% in 2020–21 to 80% and above in 2022, representing a total of 13,513 people in 2021 and 2022. (Source: MOH). Results from January 2023 implementation in 8 additional villages will be available in April 2023. This evidence-based methodology in Uganda can inform other health and development decision makers about the power of community influencers in increasing the equity of NTD health services.

CITIZENS CAN HELP TO MAP PUTATIVE TRANSMISSION SITES FOR SNAIL-BORNE DISEASES

Noelia del Carmen Valderrama Bhraunx1, Julius Tumusime2, Grace Kagoro Rugunda2, Daisy Namirembe2, Ronald Twongyirwe2, Christian Albrecht3, Casim Umba Tolo3, Liesbet Jacobs4, Tine Huysse5

1KU Leuven, Leuven, Belgium, 2Mbarara University of Science and Technology, Mbarara, Uganda, 3Justus Liebig University Giessen, Giessen, Germany, 4Institute for Biodiversity and Ecosystem Dynamics - University of Amsterdam, Amsterdam, Netherlands, 5Royal Museum for Central Africa, Tervuren, Belgium

Schistosomiasis is a snail-borne disease that affects over 200 million people globally, 90% living in Sub-Saharan Africa. However, the lack of malacologists presents a major challenge to implementing the WHO recommendations on snail control to supplement drug treatment towards disease elimination. Therefore, we adopted a citizen science approach to snail monitoring. A network of 25 trained citizen scientists (CSs) collected snail data at 73 water contact sites around southern Lake Albert (Uganda), on a weekly basis for 20 months. The quality of this data was assessed by comparing it to the data collected by an ‘expert’ malacologist that visited the same sites monthly. The binary agreement in the presence/absence of Biomphalaria, Bulinus and Radix snails reported by the expert and CSs ranged between 70% and 86% (900 reports) with an average of 17% false negatives (site wrongly defined as snail-free). The agreement for Biomphalaria and Radix increased with an increase in snail abundance, while false negatives decreased when considering the cumulative number of snails collected by citizens per month. Site type significantly predicted binary agreement, which was lowest at lake sites (55%) and highest at spring sites (99%) with variations across genera. The CS and expert data showed similar temporal trends in snail abundance and despite the expert recording higher snail abundance than the CSs, the relative abundance is consistent across site types. The match between the top 15 sites with the expert data showed similar temporal trends in snail abundance and despite the expert recording higher snail abundance than the CSs, the relative abundance is consistent across site types. 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PATTERNS OF HEALTH SEEKING BEHAVIOUR AND TREATMENT PRACTICES FOR FEBRILE CHILDREN FOLLOWING THE INTRODUCTION OF RTS,S/AS01 MALARIA VACCINE IN GHANA

Thomas Kwasi Gyana, Paul Welaga, Edwin Atari, Kwaku Poku Asante

1Kintampo Health Research Centre, Ghana Health Service, Kintampo, Ghana; 2C. K. Tedam University of Technology and Applied Sciences, Navrongo, Ghana; 3School of Public Health, University of Ghana, Accra, Ghana

Ghana has expanded RTS,S/AS01 malaria vaccine implementation programme. We assessed introduction of the vaccine on health-seeking behavior and treatment practices for febrile malaria children. Series of household surveys were conducted in 66 clusters in six regions of Ghana. A baseline survey was carried out prior to vaccine introduction in 2019, followed by midline and endline household surveys conducted November 2020 and March 2022 after vaccine introduction respectively. The survey data were summarized into frequencies and proportions. A total of 7795, 7915 and 8993 children were enrolled in the baseline, midline and endline household surveys respectively. Proportion of children with recent fever was comparable in the surveys, implementing areas at baseline 24.3%, midline 21.7% and 22.7% endline, and non-implementing areas; baseline 20.5% midline 18.4% and 21.9% at endline. A similar trend was noted among those who sought advice or treatment for fever, implementing areas at baseline 71.6%, midline 71.5% and 62.6% endline, and in non-implementing areas; baseline 71.6% midline 75.8% and 71.4% endline. Little differences was observed in those tested in the surveys, implementing areas; baseline 38.3%, midline 35.8% and 28.9% at endline and in non-implementing areas; baseline 42.1% midline 44.8% and 33.6% at endline. Proportion of children treated for fever was nearly same in the surveys, in implementing areas; baseline 84.7%, midline 81.7% and 82.9% at endline and in non-implementing areas; baseline 86.5% midline 84.4% and 84% at endline. Artemisinin-based combination was the most used drug for febrile children and it was almost same in the surveys, in implementing areas at baseline 13.4%, midline 25.1% and 9.7% at endline and non-implementing areas; baseline 10% midline 21.1% and 9.1% endline. There was little to no impact on health-seeking behavior or health worker provision of care following the introduction of the malaria vaccine.

OPERATIONAL PERFORMANCE AND ACCEPTABILITY OF A PROGRAMMATIC MASS DRUG ADMINISTRATION CAMPAIGN FOR MALARIA IN SOUTHERN MOZAMBIQUE: A CROSS-SECTIONAL SURVEY

Maria Tussel, Laura Fuente-Soro, Jacopo Vecchio, Amancio Nhanguve, Khalid Bapu, Christina Riley, Mericia Dimene, Samira Sibindy, Baltazar Candrinho, Pedro Aide, Caterina Guinovart

1Barcelona Institute for Global Health, Barcelona, Spain; 2Gaza Provincial Directorate of Health, Xai-Xai, Mozambique; 3Manhiça Health Research Centre, Manhiça, Mozambique; 4Akros, Lusaka, Zambia; 5National Malaria Control Program, Ministry of Health, Maputo, Mozambique

Mass Drug Administration (MDA) to reduce transmission of malaria in low transmission settings was recommended by the World Health Organization in 2022. Given that achieving high population coverage, of at least 80%, and good adherence to the antimalarials are critical aspects of MDA campaigns, National Malaria Control Programmes (NMCPs) need to develop programmatic delivery strategies and assess the real-world implementation challenges to optimize them. In December 2022 and January-February 2023, two rounds of programmatic MDA (pMDA) with dihydroartemisinin-piperaquine were conducted by the Mozambican NMCP in the administrative post of Chidenguene (district of Manjacaze, Gaza province), which has an estimated population of around 60,000 people. A cross-sectional community survey was then conducted to evaluate the operational feasibility, acceptability, and penetration of the pMDA delivery strategy. 770 individuals were selected through a multistage cluster sampling. After obtaining informed consent, a standardized questionnaire was administered. Preliminary data show that 61.6% (474/770) of households reported having been visited by a pMDA team during the first round, whereas in the household visitation coverage for the second round was 89.1% (686/770). For those that were visited during round 2, 82.9% (660/806) reported having taken the medication, and 96.1% (647/669) of those reported having completed the full treatment course. 23.2% (159/686) of participants reported drug-related side effects, of which 25.2% (40/159) resulted in the participant having to seek care at the nearest health facility. 91.3% (703/770) of participants thought that taking the medication regardless of malaria infection status was acceptable, and 84.2% thought that the MDA campaign could help reduce the malaria burden in the community. Final results will be available to be presented at the time of the ASTMH conference.
TRENDS IN ANTENATAL CARE VISITS AND INTERMITTENT PRESCRIPTIVE TREATMENT IN PREGNANCY IN SUB-SAHARAN AFRICA. IMPLICATIONS OF WHO POLICY

Bolanle Olapeju
Uniformed Services University of the Health Sciences, Bethesda, MD, United States

In 2016, The World Health Organization (WHO) recommended a minimum of eight antenatal care (ANC) visits during pregnancy with the first visit in the first trimester. However, the implementation and impact of this policy on malaria in pregnancy remain unclear. This study explores the relationship between ANC and intermittent presumptive treatment in pregnancy (IPTp) in sub-Saharan Africa from 2017-2022. The analysis included thirteen countries (Benin, Burundi, Cameroon, Côte d’Ivoire, Ethiopia, Ghana, Liberia, Madagascar, Mauritania, Mozambique, São Tomé & Príncipe, Sierra Leone, Uganda, Zambia) with a recent (2017-2022) demographic health survey (DHS) or malaria indicator survey (MIS). Bivariate associations explored sub-group trends in the percent of women with 1) 4+ ANC visits; 2) 5+ ANC visits; 3) Early ANC (first visit in less than four months of pregnancy); 4) IPTp 3+ (three or more doses of sulfadoxine/pyrimethamine (SP) or F syrup) during pregnancy. Meta-analysis explored correlations between these indicators across all countries. The national average of women with at least 4 ANC visits was 65% across all countries (ranging from 29% in Benin to 91% in Ghana). However, the national average of women with at least 8 ANC visits was 10% (well below 10% in ten countries). An average of 44% of women attended their first ANC visit in the first trimester while 36% of women received at least three doses of SP/F syrup (ranging from 10% in Mauritania to 61% in Ghana). There was a higher correlation between IPTp 3+ and 4+ ANC visits across the study countries: 0.58 compared to between IPTp 3+ and 5+ ANC visits (0.37). It is clear that the recommended 8+ ANC visits are far from being achieved and a review of its feasibility is needed as countries are still striving to achieve early ANC and 4+ ANC visits. Strategies to increase ANC visits in sub-Saharan Africa may include the use of home- and/or community-level ANC service delivery models, demand-generation interventions to increase early and frequent ANC visits as well as supply-side interventions to improve the quality of facility-based ANC. Commitment from both government and non-government stakeholders remains crucial.

SEASONAL MALARIA CHEMOPREVENTION EFFECTIVENESS IN NORTHERN MOZAMBIQUE, RESULTS FROM A CLUSTER RANDOMISED CONTROL TRIAL

Ivan Alejandro Pulido Tarquino1, Kevin Baker2, Sonia Maria Enosse3, Chuku Nnaji1, Albertino Unzaba1, Mericia Sitoe1, Baltazar Candrinho3, Maria Rodrigues1, Sol Richardson4
1Malaria Consortium, Maputo, Mozambique; 2Malaria Consortium, London, United Kingdom; 3National Malaria Control Programme, Maputo, Mozambique; 4Vanke School of Public Health, Tsinghua University, Beijing, China

Seasonal malaria chemoprevention (SMC) prevents malaria caused by Plasmodium falciparum in high-transmission areas. It involves administering antimalarial medicines intermittently during the transmission season. We conducted a cluster randomised control trial in Nampula province, Mozambique, from January to April 2022 to investigate the effectiveness of SMC in reducing malaria incidence in eligible children aged 3-59 months. The study was conducted in Nampula province, Mozambique, with one control and one intervention arm. 190 clusters were selected at the community level, with 76 in the intervention arm and 114 in the control arm. One eligible child was randomly selected from 15 households in each cluster, and a questionnaire and blood sample were collected after informed consent. Proportions of children with Rapid Diagnostic Test (RDT)-confirmed malaria were compared between control and intervention arms using Chi square tests and ORs. Survival analyses were performed for time to first RDT-confirmed malaria case, with random-effects models accounting for recurrent malaria events during follow-up.

The study included 1,338 eligible children aged 3-59 months, with 628 in the control arm and 710 in the intervention arm. Children in the control arm had more than double the odds of RDT-confirmed malaria fever compared to the intervention arm, OR 2.29 [95% CI 1.85-2.59 (p<0.001)]. The Cox proportional hazards models showed a significant reduction of 69%, HR 0.31 [95% CI 0.26-0.37 (p<0.001)], in the hazard of RDT-confirmed malaria cases in the intervention district compared to the control district. After covariates adjustment, the random-effects model showed an 83%, HR 0.17 [95% CI 0.13-0.21 (p<0.001)], reduction in rates of confirmed malaria cases in the intervention district. One year after the pilot implementation in Mozambique, this study found that administering SPAQ through SMC was effective, with up to 83% estimated protection. While effectiveness is high in Mozambique, we require chemoprevention efficacy results, due in June 2023, to define future effectiveness and therefore sustainability of the intervention in the future.

ADAPTING THE MASS ACTION AGAINST MALARIA APPROACH FOR MALARIA PREVENTION AND MANAGEMENT AT THE HOUSEHOLD LEVEL: IMPLEMENTATION LESSONS FROM FIVE HIGH BURDEN REGIONS IN UGANDA

Disan Ndaula Sempa1, Irene Ochola1, Dorah Anita Talantara1, Ekwado Mwugwanya1, Amanda Nagadya1, Rebecca Babirye2, Ambrose Okite3, Aaron Musimire3, Amy Casella4, Aliza Hasham5, Benjamin Binagwa6, Natalie Whitley7
1John Snow Inc, Kampala, Uganda; 2Program for Accessible Health Communication and Education, Kampala, Uganda; 3Another Option LLC, Kampala, Uganda; 4John Snow Inc, Boston, VA, United States; 5John Snow Inc, Dar es Salaam, Tanzania, United Republic of

Since 2019, Uganda has experienced a sustained increase in malaria cases, with 74 districts reporting upsurges above normal expected levels in 2022, 16% of which have reached epidemic level. The USAID Pmi Uganda Malaria Reduction Activity, implemented by JSI, works with the Ministry of Health to strengthen capacity of malaria prevention and ownership of health at community and household levels in five highest burden regions. The implementation framework is guided by the Government of Uganda’s Mass Action Against Malaria (MAAM) multisectoral approach for eliminating malaria at all levels. Effective implementation requires gaps in malaria prevention, management, and control strategies at community and household levels to be addressed. We adapted MAAM for implementation at household level by equipping them with the knowledge to own and put specific malaria prevention measures in place and achieve a malaria free status. In collaboration with local leaders and village health team members, we used national HMIS data and health facility outpatient registers to map most affected villages and assess households for specific malaria transmission drivers to address. Malaria champions were established in each household to reinforce accountability. From September 2022 to January 2023, 2,515 households in four upsurge districts were assessed. Only 35% of assessed households knew at least three ways to prevent malaria, 52% had one mosquito net for every 2 people, and 84% of pregnant women and children under five slept under mosquito nets. Only 30% of pregnant women reported receiving the recommended three doses of intermittent preventive treatment for malaria. First round follow up visits suggest improvements. 70% of households knew three or more ways to prevent malaria, 80% of pregnant women received three doses of IPTp and 80% reported sleeping under mosquito nets. Effective and sustainable malaria elimination strategies require active engagement by the affected individuals. Empowering households to own the process of preventing, managing, and controlling malaria in their communities can help the GOU realize MAAM’s vision of a malaria free Uganda.
EVALUATION OF NEW, INSECTICIDE-TREATED NET PRODUCTS, META-ANALYSIS OF OBSERVATIONAL STUDIES, AND ECONOMIC EVALUATIONS FROM FIVE SUB-SAHARAN AFRICAN SETTINGS

Joshua Yukich1, Riley E. Santiago1, Maya Schane1, Peder Digre2, Okefu Oyale Okoko3, Perpetua Uhomoibhi1, Emmanuel Obi4, Christelle Gogue5, Idrissa Cisse7, Giovanni Dusabe6, Zainab Baba Mai A11, Idrissi Cesse5, David Masiko6, Jimmy Opigo9, Medard Christelle Gogue5, Marcy Erskine6, Giovanni Dusabe6, Zainab Baba Mai A11, Idrissi Cesse5, David Masiko6, Jimmy Opigo9, Medard Rukaari9, Olivier Lemba Palata15, Albert Tuyishime11, Ines Juleca Antonio12, Guira Malitilou13, Joseph Wagman6, Molly Robertson14, Christen Femadè15

1Center for Applied Malaria Research and Evaluation, Department of Tropical Medicine, Tulane University School of Public Health and Tropical Medicine, New Orleans, LA, United States, 2PATH, Seattle, WA, United States, 3National Malaria Elimination Programme, Federal Ministry of Health, Abuja, Nigeria, 4Tropical Health LLP, Lagos, Nigeria, 5PATH, Washington, DC, United States, 6International Federation of the Red Cross and Red Crescent, Geneva, Switzerland, 7Programme National de Lutte Contre le Paludisme, Bamako, Mali, 8Batut Consulting and Advisory Services Ltd, Kampala, Uganda, 9National Malaria Control Division, Ministry of Health, Kampala, Uganda, 10Population Services International, Bamako, Mali, 11Rwanda Biomedical Centre, Kigali, Rwanda, 12Programma Nacional de Control de la Malária, Maputo, Mozambique, 13Programme d’Appui au Développement Sanitaire du Burkina Faso, Ouagadougou, Burkina Faso, 14The Global Fund to Fight AIDS, Tuberculosis and Malaria, Geneva, Switzerland, 15IVCC, Liverpool, United Kingdom

Standard insecticide-treated nets (ITNs) have been the main vector control tool for malaria in sub-Saharan Africa (SSA), but their effectiveness is currently limited by widespread pyrethroid resistance. New, dual active ingredient (AI) ITN products treated with multiple insecticides, synergists, or juvenile growth inhibitors have shown great effects in small entomological studies and clinical trials, but little is known about their effectiveness and cost-effectiveness at scale in field settings. We conducted observational studies and economic evaluations of dual AI ITN deployment in five settings in SSA (Burkina Faso, Nigeria, northern and western Mozambique, and Rwanda). Interceptor G2® ITNs (IG2; BASF AG), which combine alpha-cypermethrin and pyriproxyfen; Royal Guard® (RG; Disease Control Technologies, LLC), which combine alpha-cypermethrin and piperonyl butoxide (PBO) ITNs were deployed. Standard ITNs were also deployed in all settings as a comparator to dual AI ITNs. Observational studies followed district level difference in differences approaches for estimation of the average treatment effects. Incidence rate ratios (IRRs) and variance estimates were combined in a pooled random effects meta-analysis framework. Dual AI ITNs across all settings with an IRR of 0.60 (95% CI: 0.47-0.77), IG2 ITNs were more protective (IRR 0.57 in comparison to standard nets) than PBO ITNs (IRR 0.85 in comparison to standard nets). Effect models were used to calculate cases averted and costs per case, death, and disability-adjusted life (DALY) averted in each setting. IG2 ITNs were the most cost-effective intervention across the studies, though a switch from standard ITN to any dual AI ITN is likely to be highly cost-effective in most settings with a willingness to pay threshold of ≤1x gross domestic product per capita per DALY averted. Dual AI ITNs offer the potential to greatly improve the impact of malaria programs but will be more costly in the near term than standard ITN deployment.

PODOCONIOSIS: CLINICAL SPECTRUM AND MICROSCOPIC PRESENTATIONS

Wendemegen Enbiale Yeshan1, Almut Enbiale Boer Auer2, Bereket Amare1, Kristien Verdonck1, Gail Davey1, Johan Griensven3, Henry de Vries3
1Bahir Dar University, Bahir Dar, Ethiopia, 2Dermatologikum, Hamburg, Germany, 3Institute of tropical Medicine, Antwerp, Belgium, 4Brighton and Sussex Medical School, Falmer, Brighton, United Kingdom, 5University of Amsterdam, Department of Dermatology, Amsterdam, Netherlands

Podocoisosis is a skin Neglected Tropical Disease (skin NTD) that causes lymphoedema, and affects barefooted subsistence farmers in some tropical countries. The clinical presentation and histopathologic correlates of podocoisosis have been understudied. Here, we systematically document the clinical and histopathologic spectrum of podocoisosis. This is a cross-sectional study in Durbete, Ethiopia from February 2018 to October 2019. Dermatologists performed a patient history, physical examination, fibrin formation test, and skin biopsy for histopathologic examination. The results were summarised and a descriptive statistical analysis and Wilcoxon rank sum test with continuity correction was done. We recruited 289 patients for the study, 178 (61.6%) had stage 1 or 2 podocoisosis, and 111 (38.4%) stage 3 to 5 podocoisosis. 188 (64.1%) had a family history of podocoisosis. In 251 (86.9%) patients, both legs were affected by podocoisosis and in 38 (13.1%) only one leg was affected. 220 (77.5%) patients had warty lesions, 114 (39.4%) had nodules. The median number of episodes of Acute Dermato-Lymphangio-Adenitis (ADLA) reported by the patients in the last three months was 2 (interquartile range (IQR) 1–4). Increased episodes of ADLA were significantly associated with stage 3–5 podocoisosis (P = 0.002), while burning pain in the feet was more common in stage 1 or 2 podocoisosis. Stage 3–5 disease was histopathologically characterised by epidermal and dermal thickening, verrucous acanthosis, inflammatory cell infiltrates (predominantly lymphoplasmacytic), dilated anastomotic and a reduced number of lymphatic vessels, eccrine ductal hyperplasia, and sclerosis such as thickened collagen bundles. In conclusion, we provide a detailed description of the different clinical patterns, associated clinical findings and the histopathologic spectrum of podocoisosis at different stages of the disease. Our observations should serve as a guide to classifying patients with podocoisosis for prognostic assessment and treatment decision.

THROMBOSIS AND ORGAN DYSFUNCTION IN SNAKEBITE ENVENOMING: TIME TO REDEFINE VENOM INDUCED CONSUMPTION COAGULOPATHY?

Maya Gopalakrishnan, Akihlesh Kumar, Pankaj Sukhadiya, Divya Tanwar, Poornam Elhence, Mahendra K. Garg
All India Institute of Medical Sciences, Jodhpur, Jodhpur, India

The mechanism of venom-induced consumption coagulopathy (VICC) in viper envenoming is due to procoagulant venom toxins mediated consumption coagulopathy resulting in bleeding. Classical features of disseminated intravascular coagulation- systemic microthrombi and end-organ failure are absent. However, it remains unknown if this is uniformly so for different snake species. Here we report a series of 3 patients with Echis envenoming from desert regions of Rajasthan, India who presented with venom-induced consumption coagulopathy but had evidence of thrombosis leading to organ dysfunction along with bleeding. We report findings from an ongoing prospective study in patients with Echis envenoming after ethical approval. Clinical features, antivenom use, and coagulation parameters were collected. Post-mortem biopsies from the heart, lung, kidneys were collected after informed consent. Of 23 admitted with Echis envenoming in 2022, 19 had VICC, of whom three developed thrombosis and organ dysfunction. All 3 had coagulopathy, serious bleeding: hematuria in all, muscle hematoma in patients 1 and 3, and low serum fibrinogen. All needed intensive-care admission, transfusion support. All received 30 vials of Indian polyvalent antivenom with persistent bleeding suggesting
antivenom non-responsiveness. Patients 1 and 3 had acute kidney injury. Patient 3 had thrombotic microangiopathy. Patient 1 developed left arm deep venous thrombosis which resolved by day 12. Patient 2 developed intestinal obstruction and 3 had abdominal compartment syndrome with gangrenous bowel loops. Patient 1 was discharged on day 14 while the others expired. Post-mortem renal and lung histopathological samples from patients 2 and 3 and part of resected gangrenous intestines sent intraoperatively (patient 3) showed extensive fibrin microthrombi in vessel walls. Renal injury is frequently reported in the context of thrombotic microangiopathy in snakebites. To our knowledge, this is the first report of non-renal end-organ damage (lung and intestines) in snakebite envenoming suggesting that the currently accepted definition of VIHC needs to be reconsidered.

THE RELEVANCE OF REPORTING LEPROSY RELATED DISABILITY AT THE COMPLETION OF MULTI-DRUG THERAPY: A FIVE-YEAR RETROSPECTIVE ANALYSIS OF DISABILITY IN PERSONS AFFECTED BY LEPROSY AT ALERT HOSPITAL ETHIOPIA

Bereket A. Tegene¹, Thomas A. Atnafu², Saba M. Lambert¹, Stephen L. Walker³
¹London School of Hygiene and Tropical Medicine, London, United Kingdom, ²All Africa Leprosy TBI Rehabilitation Training Center, Addis Ababa, Ethiopia

Leprosy is one of the neglected tropical diseases associated with significant morbidity in endemic regions. Leprosy causes disability affecting the daily activities and social participation of affected individuals. Understanding the prevalence and trend of leprosy-related disability throughout the world and accuracy of disability data counted by WHO is crucial in guiding efforts to be made towards the targets set by WHO to be achieved by 2030. Our study aims to show the prevalence and trend of leprosy related disability and critique the reliability and usefulness of WHO Leprosy related disability data based on the data from ALERT hospital in Ethiopia. We did a mixed method study with a 5-year retrospective analysis of outcomes of newly diagnosed leprosy patients at ALERT Hospital in Ethiopia from 2016 to 2020. A comparative review and analysis of leprosy related G2D (Grade 2 Disability), globally, regionally, and in Ethiopia using WHO data was also done. In addition, we conducted semi-structured interview of health workers and professionals working in the field of leprosy at various organizations. The total number of newly diagnosed leprosy cases at ALERT hospital between January 2016 and December 2020 were 1032 and among those patients who had completed treatment the prevalence of G2D was 33% at diagnosis and 23% at completion. The trend of G2D among newly diagnosed leprosy patients shows no decline globally for the past 20 years, while it is increasing in Africa and stable in the Southeast Asian and American regions where majority of leprosy patients are found showing the gap in early case detection and effective patient management. The interview has also shown gaps in the completeness and quality of disability data reported to WHO and how disability is counted. Leprosy related G2D among newly diagnosed patients is not declining worldwide and even increasing in endemic regions like Ethiopia. More training should be given to health professionals in assessing disability. WHO shoulder consider counting disability at the end of MDT to know the impact of interventions and prospective studies are needed in assessing disability progression post treatment.

PATHOGENESIS OF ACUTE KIDNEY INJURY IN LASA FEVER

Matin Kohsar¹, Osas Edeawe², Christian Erohubie³, Benno Kreuels¹, Sylvanus Okogbenin⁴, Stephan Günther⁵, Michael Ramhardt¹, Lisa Österreich¹, Till Frederick Omansen¹, Cyril Erameh²
¹Department of Tropical Medicine, Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany, ²Department of Medicine, Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany, ³Department of Virology, Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany

Lassa fever (LF) is an endemic viral hemorrhagic fever in West Africa, with Nigeria being one of the main foci. The most common complications of severe LF are acute kidney injury (AKI) and neurological involvement. AKI often requires hemodialysis - which is often not available in resource-limited settings - and is an important cause of mortality in LF. The pathogenesis of AKI in LF is not well understood, both micro-bleeding and inflammation, as well as secondary causes have been discussed as potential mechanisms. Hence its management remains challenging. We are conducting a prospective, clinical, observational study with the aim to explore the underlying pathophysiological mechanisms of AKI in LF and to ultimately improve patient management. Adult patients with RT-PCR confirmed LF hospitalized at the Irrua Specialist Teaching Hospital in Edo State, Nigeria, with laboratory diagnosis of AKI according to KDIGO criteria were enrolled and followed prospectively. Secondary causes of AKI such as pre- and postrenal failure, as well as pre-existing chronic renal disease and pre-disposing conditions (history of diabetes mellitus, hypertension, among others) were assessed. Study visits included clinical examination, recording of clinical parameters such as vital signs and fluid input/output, laboratory testing (including HbA1c and Cystatin C) and point-of-care ultrasound examination. We here report the findings of the first 51 patients with AKI. The mean age of the case series was 41.7 years ±15.8 years. Most of our patients exhibited signs of severe LF. Ultrasound findings showed marked hyperechogenicity of kidneys in affected patients, similar to findings in other virus-associated nephropathies, such as HIV-associated nephropathy (HIVAN). Furthermore, we observed marked proteinuria and gross hematuria and elevated inflammatory markers in the cohort. Cystatin C based estimates of GFR correlated well with clinical course and serum creatinine. Pre- and postrenal failure were excluded, along with preexisting diabetes. The overall results indicate a possible inflammatory etiology of AKI in LF.

TRYING TO UNMASK THE HIDDEN CAUSES OF IMPORTED FEVER WITH NEW GENOME SEQUENCING: A MULTICENTER PROSPECTIVE COHORT STUDY

Daniel Camporubi Ferrer¹, Leire Balderi Sarasolea¹, Alex Tomazatos⁵, Ludovico Cobuccio⁶, Steven Van Den Broucke⁷, Blaise Genton⁸, Emmanuel Bottieau⁹, Natalia Rodriguez-Valero⁴⁵, Alex Almuedo-Riera⁵, Valerie d’Acremont⁷, Javier Gandasegui⁵, Carme Subirats⁷, Angelina Cruz¹, Daniel Cadar¹, Jose Muñoz¹
¹ISGlobal / Hospital Clinic Barcelona, Barcelona, Spain, ²Bernhard-Nocht-Institut für Tropenmedizin, Hamburg, Germany, ³Center for Primary Care and Public Health, University of Lausanne, Lausanne, Switzerland, ⁴Institute of Tropical Medicine, Antwerp, Belgium

Although acute undifferentiated febrile illnesses (AUF1) are the leading cause for hospitalization in returning travelers, up to 45% of them remain undiagnosed even at referral centers. Metagenomic next-generation sequencing (NGS) has been proposed as a promising tool, but evidence of its usefulness in AUIF is limited. Prospective multicenter cohort study of travelers with AUIF after international travel (November 2017- November 2019). NGS was performed in sera samples of travelers with AUIF and results were compared with those obtained by standard diagnostic methods (SDM). 507 returned travelers with AUIF were included, 165(32.5%) of them presented with severe disease and 133(26.2%) remained undiagnosed by SDM at the end of the follow-up. NGS allowed the identification of potentially pathogenic microorganisms in 172(33.9%) samples: 136 samples that tested positive by SDM and 36 additional samples. NGS results were negative in 222(43.8%) samples that resulted positive by SDM. 113(22.3%) samples resulted negative by SDM and NGS. NGS allowed the identification of microorganisms in 27/133(20.3%) undiagnosed cases and 5/26(19.2%) severe undiagnosed cases. The most common identifications obtained by NGS in patients undiagnosed by SDM were Pseudomonas aeruginosa (n=7), Enterovirus B (n=3), Aspergillus...
spp. (n=3), Hepatitis B virus (n=2), Bordetella spp. (n=2), Burkholderia spp. (n=2), primate erythroparvovirus 1 (n=2), Penicillium spp. (n=2), amongst others. NGS showed additional identifications in 29/374 (7.5%) cases already diagnosed by SDM. In conclusion, NGS from sputa might be useful for the diagnosis of selected cases of imported fever not-diagnosed by SDM. However, the interpretation of NGS results poses a great challenge from a clinical perspective. The evaluation of NGS in plasma and whole blood samples could improve the diagnostic performance of metagenomic NGS techniques in febrile travelers. 1

MALSCORE: AN INNOVATIVE TOOL TO PREDICT MALARIA IN PATIENTS WITH IMPORTED FEVER TO START EARLY TREATMENT.

Leire Balerdi-Sarasola1, Pedro Fleitas1, Ludovico Cobucci1, Steven Van Den Broucke2, Blaise Genton2, Emmanuel Botteau2, Valérie d’Acremont2, Natalia Rodriguez-Valero1, Alex Almudeno-Riera1, Carme Subirà1, Montserrat Roldán1, Claudio Parolo1, Jose Muñoz2, Daniel Camprubí-Ferrer3

1ISGlobal, Hospital Clinic - Universitat de Barcelona, Barcelona, Spain, 2Swiss Tropical and Public Health Institute, Basel, Switzerland, 3Department of Clinical Sciences, Institute of Tropical Medicine, Antwerp, Belgium

Fever after international travel is one of the most common conditions for seeking health care. Plasmodium falciparum malaria is the leading cause of fever and also the deadliest imported disease. Nonetheless, its presentation is commonly undistinguishable from other benign and self-limited conditions, and microbiological diagnosis can be challenging in non-referral centers. This can lead to delayed diagnosis of malaria and late initiation of treatment, which worsens patients’ clinical outcomes.

Therefore, new tools are needed in order to rapidly detect patients at risk of malaria. We conducted a nested study within a prospective multicenter cohort study of international returning travelers or recently arrived migrants with fever, attending three European Travel Clinics and/or Hospitals, from November 2017 to November 2019. The objective of this study is to build a predictive model to identify suspected malaria cases that should be treated promptly. For this purpose, a machine learning approach was used, using demographic characteristics, clinical and laboratory variables as features of the model. A total of 765 patients with fever were recruited and 95 (12.4%) malaria cases were diagnosed. The median age was 36 years (IQR: 28-47), and 133 (17.4%) went visiting friends and relatives. Out of a total of 97 features, we built an xgboost model with only six features to predict suspected cases of malaria: visiting friend and relatives, Africa as a travel destination, platelet value, C-reactive protein, bilirubin value and respiratory symptoms. The model showed an AUROC of 0.94 [95% CI 0.91-0.97] in the cross-validation with the training set (80%), and an AUROC =0.97 with the test set (20%). Also, a sensitivity of 100% (95% CI 96.15-100) and specificity of 80% (95% CI 77.05-83.08) was obtained. With only 6 variables that are easy to obtain through anamnesis or basic laboratory results, the predictive model is an accurate resource to detect malaria cases in patients with imported fever. This tool could be easily scalable to a digital application and could help clinicians without access to microbiologic tests to start empiric antimalarial treatment.

IDENTIFICATION OF TARGETS OF PROTECTIVE ANTIBODY RESPONSES AGAINST PLASMODIUM VIVAX MALARIA USING A MULTIFUNCTIONAL ANTIBODY PROFILING APPROACH

D. Herbert Opi1, Rhea Longley1, Linda Reiling1, Kael Schoffer2, Yanie Taytopy1, Ali Haghiri3, Jessica Brewster4, Damien Drew5, Gaoqian Feng6, Bruce Wines7, Daniëlle Stansic7, Mathias Harbers8, Takafumi Tsuboi9, Eizo Takashima10, P. Mark Hogarth11, Benson Kinoboro12, Leanne Robinson13, Julie Simpson13, Ivo Mueller12, James G. Beeson12

1Burnet Institute, Melbourne, Australia, 2Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia, 3University of Melbourne, Melbourne, Australia, 4Griffith University, Gold Coast, Australia, 5Cell Freece Sciences Co Ltd, Yokohama, Japan, 6Ehime University, Matsuyama, Japan, 7Papua New Guinea Institute of Medical Research, Port Moresby, Papua New Guinea

A need for highly effective malaria vaccines has been made more urgent following stalled progress in reducing the global burden of malaria since 2015. While advances in Plasmodium falciparum malaria vaccine development have seen the recent approval of the RTS,S vaccine, very limited progress has been made towards development of a P. vivax vaccine. No vaccines for P. vivax have completed testing for efficacy in malaria-endemic settings and limited candidates are in the discovery pipeline. P. vivax is the most widespread Plasmodium species and a major cause of malaria outside Africa, with over 3 billion people at risk of infection with. A major challenge to developing a P. vivax vaccine is a limited knowledge of the targets of protective immune responses. Antibodies play a major role in protective immunity against P. vivax. In humans and baboons, protective antibodies target the circumsporozoite protein (CSP) and anti-CSP antibodies are associated with protective immunity. Such antibodies are produced following natural infection and are present in naturally infected individuals. P. vivax infections in non-immune humans are highly variable, presenting with manifestations varying from asymptomatic infection to severe malaria. CSP is a highly conserved antigen and the CPSV2 region is the most highly conserved region of the CSP.

Antimicrobial resistance (AMR) is a growing public health threat. Strategies are needed to reduce unnecessary use of antibiotics, a known driver of AMR. We hypothesized that point-of-care tests and diagnostic algorithms could reduce antibiotic prescriptions without compromising clinical outcomes in patients with fever. We conducted a two-arm, open-label, randomized controlled trial in three African and two Asian countries. The intervention included a diagnostic algorithm to guide antibiotic prescription, based on Selected Rapid Diagnostic Tests (RDTs). The control arm used standard care. Primary outcomes were antibiotic prescribing on the day of presentation, and clinical status after seven days. From September 2021 to September 2022, we enrolled 11,664 participants. 30% were children aged <5 years, 47% were female, and 47% presented with respiratory symptoms. Antibiotics were prescribed for 29.4% of patients.

The intervention significantly reduced antibiotic prescriptions for patients with: negative malaria RDT at African sites (absolute risk difference (ARD) [95%CI] -32.5% [-38.6%, -26.4%], P-Value <0.001), respiratory Symptoms (-9.3% [-12.0%, -6.5%], P-Value: <0.001), and no vaccination against COVID-19 (-28.1% [-32.4%, -23.9%], P-Value: <0.001). Conversely, there were significantly more antibiotic prescriptions for patients with positive malaria RDT (ARD: 11.2% [6.3%, 16.0%], p-value <0.001), non-respiratory symptoms (7.2% [4.9%, 9.4%], p-value: <0.001), and unknown COVID-19 vaccination status (14.8% [12.5%, 17.1%], p-value: <0.001). African and Asian settings varied in disease epidemiology and antibiotic prescribing. The intervention significantly reduced antibiotic prescribing in Burkina Faso (-24.9% [-30.6%,-19.2%], p-value <0.001) but significantly increased it in Uganda and three regions of India. Overall, 11,243 (99%) had favourable outcomes at day 7 with no significant differences among sites. Diagnostic tests and algorithms have the potential to safely reduce antibiotic prescribing for sub-populations with fever. However, their use must be tailored to local epidemiology.
role in acquired immunity to malaria and likely act through three major mechanisms: direct inhibition of host cell invasion, recruitment and activation of complement, and interactions with Fcγ-receptors to promote phagocytosis or killing by immune cells. However, knowledge of functional antibody mechanisms in P. vivax immunity is very limited. To address this, we developed novel high throughput multiplex assays to identify the targets of functional antibodies against P. vivax that interact with complement and Fcγ-receptors. In a longitudinal cohort study of 1-3-year-old children from PNG, we measured antibody magnitude (IgG, IgG subclasses and IgM) and functions (complement fixation, FcγR binding and opsonic phagocytosis) to 30 P. vivax antigens. Using these approaches, we identified known and novel antibody targets and specific functions associated with protection against clinical P. vivax malaria. Using statistical modelling approaches we identify important combinations of antigen-specific functional antibodies that may provide maximal protection against P. vivax malaria. Our findings identify promising antigens for prioritisation in P. vivax vaccine development, and a knowledge of target-specific functional immune responses that are most important for protective P. vivax immunity.

**7251**

**ANTIBODY AND CELLULAR RESPONSES TO IN UTERO MALARIA EXPOSURE IN INFANTS**

Felistas Namirimu Nankya1, Florian Bach2, Kathleen Press3, Odorizzi Pamela3, Kenneth Musinguzi4, Kate Naluwu4, Isaac Ssewanyana2, Abel Kakur3, Moses Kamya1, Grant Dorsey1, Feeney Margaret1, Prasanna Jagannathan2

1Infectious Diseases Research Collaboration, Kampala, Uganda, 2Stanford University, Stanford, CA, United States, 3Gilead Sciences, California, CA, United States, 4University of California San Francisco, San Francisco, CA, United States

Antibody-producing B cells and CD4+ T cells are key in control of malaria infection. Transplacental transfer of maternal-origin antibodies is essential for protecting the infant from pathogen encounters during the initial months of life. CD4+ T follicular helper cells (Tfh) help generate protective B cell and antibody responses. Placental malaria and antimarial chemoprevention may affect this response by altering exposure to malaria antigens. We assessed antibody responses to 25 malaria antigens from Ugandan infants born to mothers with and without placental malaria using a Luminex multiplex bead array. We also assessed the frequency and phenotype of circulating follicular helper T cells and B cells for these infants. Antibody concentrations decrease significantly from birth to six months for all malarial antigens and appear to increase from 6 months to 12 months for all except CSP, tetanus toxoid and AMA1. Antibody responses to EBA181, HSP40 and SEA were associated with a lower incidence of malaria in the first six months of life. Etramp5, GLURP, HYP2 and SBP1 emerged as markers of exposure. At one year of age, Th1 were the most abundant Tfh subset while naïve B cells were the most abundant B cell subset. Symptomatic malaria in pregnancy was associated with lower Th2/Th17-like Tfh at one year of age, and there was a strong positive correlation between Th2-like Tfh, and Th2/Th17-like Tfh, with antibody levels to all tested malaria surface antigens. We also found an inverse relationship between the frequency of atypical memory B and follicular helper T cells of infants at age one year. These data suggest important relationships between in utero malaria exposure and infant Tfh and B cell responses.

**7252**

**SEX HORMONES, CD8+ T CELLS, AND THE LIVER: HOW THE ENDOCRINE-IMMUNE INTERFACE ALTERS MALARIA LIVER-STAGE VACCINE OUTCOMES**

Caroline J. Duncombe, Felicia Watson, Kenneth Boey, Anya Kalata, Melanie Shears, Maniko Seille, Shruthi Raman, Sean C. Murphy

University of Washington, Seattle, WA, United States

Sex bias in parasitic infections is a well-documented phenomenon. Males experience higher severity and/or prevalence to almost all known parasitic infections compared to females. Despite such strong sex-specific phenotypic differences, the influence of host sex on immune responses to parasites is understudied. This is the case for malaria, where the impact of host sex may modulate the host response to each stage of the Plasmodium life cycle. This is especially relevant during liver-stage infection since the liver is one of the most sexually-dimorphic organs in vertebrates. By studying Plasmodium yoelii (Py) rodent malaria parasites in both male and female mice, and through the use of in vivo sex hormone manipulation models, we show sex and sex hormones are dramatic mediators of immune responses to both wild-type malaria sporozoite challenge and vaccination with live-attenuated sporozoites. We demonstrate that female mice experience heightened type I and II interferon signaling in response to liver stage infection after wild-type Py sporozoite challenge. We further demonstrate that androgens suppress and estrogens promote host inflammation and innate immune responses to Py infection. This same trend is also recapitulated following immunization with live-attenuated Py sporozoite vaccines. Finally, we demonstrate that sex hormones skew CD8+ T cell frequencies following live-attenuated Py sporozoite vaccination, resulting in sex-divergent protection outcomes. This result demonstrates sex-specific effects on liver resident memory CD8+ T cell, a critical correlate for protective liver stage vaccines. Sex hormone modulation on host response to sporozoites adds an endocrine perspective to our understanding of innate and adaptive immune responses to Plasmodium in the liver. This work further emphasizes the importance of considering biological sex and the endocrine-immune axis when studying parasite infection and immunity.

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**INDUCTION OF LIVER-RESIDENT MEMORY CD8+ T CELLS AND PROTECTION AGAINST MALARIA AT EXOERYTHROCYTIC STAGE BY MRNA-CONTAINING LIPID NANOPARTICLES**

Sayuri Nakamae1, Satoshi Miyagawa2, Koki Ogawa3, Jyun-Yu Jian4, Mayumi Taniguchi1, Takeshi Annoura3, Katsuyuki Yui2, Kenji Hirayama2, Shigeru Kawakami3, Shusaku Mizukami3

1Institute of Tropical Medicine, Nagasaki university, Nagasaki, Japan, 2Graduate School of Biomedical Sciences, Nagasaki University, Nagasaki, Japan, 3National Institute of Infectious Diseases, Tokyo, Japan, 4School of Tropical Medicine and Global Health, Nagasaki university, Nagasaki, Japan, 5Graduate School of Biomedical Sciences, Nagasaki university, Nagasaki, Japan

Malaria is a febrile disease caused by Plasmodium parasites, and one of the important life-threatening infectious diseases in the world. Right after entering the human body by mosquito bite, Plasmodium sporozoites invade hepatocytes and proliferate (liver-stage), followed by the development of symptomatic blood-stage. Recent studies showed that resident memory T cells (TRM) in liver play a critical role in liver-stage malaria protection. Although RTS,S was recommended by WHO as a malaria vaccine in 2021, its efficacy is moderate. Therefore, more effective vaccine is required. Recently, mRNA contained lipid nanoparticles (LNPs) was approved as a vaccine platform. In this study, we aimed to develop liver TRM inducing malaria vaccine based on mRNA contained LNPs (mRNA-LNPs). We utilized pH-sensitive lipid LNPs, which contains third generation SS-cleavable pH-Activated Lipid-like Material (ssPalm) that enhances endosome disruption and mRNA release to the cytosol and promotes efficient protein production. Single dose intravenous injection of ovalbumin (OVA) mRNA LNPs induced antigen-specific cytotoxic T lymphocytes (CTLs) efficiently in a dose-dependent manner in the liver. Furthermore, five weeks after the immunization, TRM were generated in the liver. Finally, we immunized mice intramuscularly twice with LNPs containing mRNA encoding P. berghei ANKA (PbA) circumsporozoite protein (CSP), and examined protection against PbA sporozoite. Sterile immunity was induced in 70% of the immunized mice. These results demonstrate that mRNA-LNPs is a promising liver-stage malaria vaccine platform.
ONCE YOU’VE HAD MALARIA YOU’LL NEVER FORGET (OR WILL YOU?): THE MEMORY B CELL AND PLASMA CELL RESPONSE TO PLASMODIUM REINFECTION

Elizabeth M. Fusco, Nathan W. Schmidt
Indiana University School of Medicine, Indianapolis, IN, United States

Malaria is caused by parasites in the genus Plasmodium, with symptoms caused by rounds of parasite growth and rupture inside red blood cells. Clearance of blood stage parasites is driven by a germinal center response that yields plasma cells (PCs) capable of producing high affinity antibodies and memory B cells (MBCs), which can respond during subsequent infections. After years of repeated infection some people develop clinical immunity to malaria, but why this varies between individuals is poorly understood. An emerging modulator of the germinal center response against Plasmodium is the gut microbiome. Curiously, mice that are genetically identical but with differing gut microbiomes (Taconic (Tac), and Charles River (CR)) show drastic differences in their susceptibility to non-lethal P. yoelii 17XNL. Tac mice, which are protected from severe disease, generate a stronger germinal center response against P. yoelii compared to CR. When challenged with a lethal secondary P. berghei ANKA infection, Tac mice demonstrate heightened survival, suggesting that the gut microbiome also influences immune memory. Characterization of immune memory demonstrated that Tac mice generate more MBCs than CR mice during P. yoelii infection, but there is no difference in the number of PCs produced. We next investigated whether Tac mice generate more P. yoelii-specific PCs, however no differences were observed, suggesting that memory PCs do not contribute to the heightened survival against P. berghei seen in Tac mice. To test this further, we depleted individual memory cell subsets from P. yoelii-immune Tac mice and then challenged them with P. berghei. We discovered that depleting MBCs made previously protected Tac mice susceptible to P. berghesi challenge, but depleting PCs did not. This indicates that MBCs are necessary for protection against reinfection with Plasmodium, while PCs and circulating antibodies play a lesser role.

NEUTRALIZING AND INTERFERING HUMAN ANTIBODIES DEFINE THE STRUCTURAL AND MECHANISTIC BASIS FOR ANTIGENIC DIVERSION

National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States

Defining the protective modes of antibody protection and the methods of pathogen immune evasion are crucial to the development of protective and durable vaccines. Merozoite Surface Protein-1 (MSP-1) is a key malaria vaccine antigen that has achieved limited clinical success due to poor understanding of antibody-mediated neutralization of this protein. In addition, MSP-1 is a prototypical example of an antigen that displays “antigenic diversion”, an immune evasion phenomenon wherein the action of neutralizing antibodies is prevented by non-neutralizing antibodies that enable parasite survival. However, the structural and mechanistic bases for antigenic diversion have not yet been defined. We investigated a panel of MSP-1-specific naturally acquired human monoclonal antibodies through a combination of structural biology and biophysics tools, and parasite neutralization to study the complex interplay between neutralizing and non-neutralizing antibodies during natural infection. One of these antibodies potently neutralized parasites, whereas the others had minimal or no effect. We therefore determined the co-crystal structures of MSP-1 with neutralizing and non-neutralizing antibodies. This human antibody epitope map revealed a novel potent and strain-transcending epitope that overlaps with the epitopes of non-neutralizing interfering antibodies. Strikingly, the non-neutralizing interfering antibodies outcompete the neutralizing antibodies, which facilitates parasite survival. Overall, these findings demonstrate the structural and mechanistic basis for a parasite immune evasion mechanism through naturally acquired neutralizing and interfering human antibodies to MSP-1 elicited by antigenic diversion. The findings are highly relevant to malaria vaccine and therapeutic antibody development. This work is currently being leveraged to design potent and durable malaria interventions through structure-guided vaccine design.

STRUCTURE GUIDED MIMICRY OF AN ESSENTIAL PLASMODIUM FALCIPARUM RECEPTOR-LIGAND COMPLEX ENHANCES CROSS NEUTRALIZING ANTIBODIES

Sean Yanik1, Varsha Venkatesh1, Bianca Loveless2, Deepthi Sarkar1, Kazutoyo Miura2, Carole Long3, Martin Boulanger2, Prakash Srinivasan4
1Johns Hopkins, Baltimore, MD, United States, 2University of Victoria, Saanich, BC, Canada, 3NIAID, Rockville, MD, United States

Invasion of human red blood cells (RBCs) by Plasmodium falciparum (Pf) merozoites relies on the interaction between two parasite proteins, apical membrane antigen 1 (AMA1) and rhoptry neck protein 2 (RON2), making it an attractive vaccine target. However, clinical trials with recombinant AMA1 alone (apoAMA1) did not provide protection, likely due to inadequate levels of functional antibodies. Stabilizing AMA1 in the receptor-ligand bound conformation using RON2L, a 49 amino acid peptide from RON2, conferred superior protection against P. falciparum malaria by enhancing the proportion of neutralizing antibodies. Here, we engineered chimeric antigens by strategically replacing the AMA1 DII loop that is displaced upon ligand binding with RON2L. The structure of one of the fusion chimeras (Fusion-FD12) was determined to 1.55 Å resolution and found to mimic the receptor-ligand complex. Interestingly, Fusion-FD12 immune sera neutralized parasites more efficiently than apoAMA1 immune sera despite having an overall lower anti-AMA1 titer, suggesting improvement in antibody quality. Furthermore, immunization with the fusion chimera enhanced antibodies targeting conserved epitopes on AMA1 resulting in greater neutralization of non-vaccine type parasites. Identifying epitopes of such cross-neutralizing antibodies will help in the development of an effective, strain-transcending malaria vaccine. Our fusion protein design can be expanded to cover polymorphisms in AMA1 to effectively neutralize all P. falciparum parasites.
of Microsporidia MB. Microsporidia MB-infected adults have a higher mating rate compared to uninfected mosquitoes. In general, Microsporidia MB-infection has a positive effect on the development of An. arabiensis mosquitoes. Microsporidia MB-infection is influenced by diet type and quantity. Diet can, therefore, be manipulated to rear highly infected mosquitoes. Microsporidia MB is inherently able to spread in mosquito population due to higher mating rate making it a promising candidate for malaria transmission blocking strategy.

**CHARACTERIZING ANTIBODY RESPONSES TO MOSQUITO SALIVARY ANTIGENS OF THE SOUTHEAST ASIAN MALARIA VECTORS WITH A HUMAN CHALLENGE MODEL OF CONTROLLED EXPOSURE**

Victor Chaumeau1, Ellen Kearney2, Paul A. Agius3, Sunisa Sawasdichai4, Katherine O’Flaherty2, Praphan Wasisakun1, Daniela Da Silva Goncalves2, Laaongsri Niwetphongprai5, Thaw Htwe Min1, James Beeson1, Julie A. Simpson1, François Nosten1, Freya Fowkes2

1Shoklo Malaria Research Unit, Mae Ramat, Thailand, 2Burnet Institute, Melbourne University, Melbourne, Australia, 3Deakin University, Melbourne, Australia, 4University of Melbourne, Melbourne, Australia

Measurement of antibody titers directed against mosquito salivary antigens in blood samples has been proposed as an outcome measure to assess human exposure to vector bites. However, only a handful of antigens have been identified and the specificity and longitudinal dynamics of antibody responses are not well known. Therefore, we conducted a world-first clinical trial of controlled exposure to mosquito bites striving to identify and validate biomarkers of exposure to bites of Southeast Asian malaria mosquito vectors. This trial was an exploratory factorial randomized controlled trial of longitudinal modeling. Preliminary analyses indicate that antibody titers against candidate peptides were determined in participants with antigen prediction algorithms using mosquito DNA sequence data. Candidate peptides were identified from the published literature and with antigen prediction algorithms using mosquito DNA sequence data. Antibody titers against candidate peptides were determined in participants with high-throughput ELISA. Quantification of the antibody response profile over time (including an estimate of the decay rate) and the effect of biting level and species on the antibody response was estimated using longitudinal modeling. Preliminary analyses indicate that antibody levels against Anopheles salivary peptides were boosted during and after mosquito biting challenges, but decayed overall over the course of the study. This research generates important knowledge on species-specific antigens for vector sero-surveillance and evaluation of vector-control interventions in the Greater Mekong Subregion.

**WIDE AND SUSTAINED LONG-TERM TARGET EFFICACY OF AN INJECTABLE LONG-ACTING IVERMECTIN FORMULATION AGAINST PLASMODIUM VECTORS IN THE FIGHT AGAINST MALARIA**

Cheick O. Ouédraogo1, Lamidi Zela2, Angélique Porcianni1, Diedou Dama Somi1, Sié H. Poda2, Fabrice A. Somé1, Joel Tarning2, Kevin Kobylinski2, Malik A. Bandaogo3, Ali Quain3, Thibaut Deramoudt4, André B. Sagna2, Sophie Le Lamer-Déchamps2, Christophe Robepe1, Cédric Penntier2, Nicolas Moiroux2, Guigugbaza-Kossignan Dayo5, Roch K. Dabiré6, Karine Moulène3

1Institut de Recherche en Sciences de la Santé - IRSS-DRO, Bobo Dioulasso, Burkina Faso, 2Centre International de Recherche Développement pour l’Elevage en zones Sub-humides - CIRDES, Bobo Dioulasso, Burkina Faso, 3Institut de Recherche pour le Développement -IRD, Montpellier, France, 4Université Nazé Boni, Bobo Dioulasso, Burkina Faso, 5Université de Dédougou, Dédougou, Burkina Faso, 6Mahidol Oxford Tropical Medicine Research Unit, Mahidol, Thailand, 7Centre de Recherche Développement pour l’élevage en zones Sub-humides - CIRDES, Bobo Dioulasso, Burkina Faso

Recent biological and behavioral changes of Anopheles result in impaired effectiveness of insecticidal nets in the prevention of malaria. Mass drug administration of ivermectin to humans as a systemic insecticide could help the vectors control. However, the efficacy of this strategy is hampered by the short duration of efficacy as approved ivermectin remains at mosquitocidal plasma concentrations only for few days after administration, and thus requires repeated dosing to achieve full coverage of transmission period. Long-Acting Ivermectin Formulations (LAIFs) that deliver ivermectin to Anopheles salivary and parasite transmission-stage proteins followed seasonal trends in vector abundance and malaria transmission and varied over small spatial scales. Joint modelling of anti-Anopheles and parasite antibody seroprevalence identified potential foci of ongoing transmission. These findings suggest antibodies to Mosquito salivary proteins could be an informative vector-control trial outcome measure and support their use as a serosurveillance tool to measure vector exposure and identify foci of malaria transmission.
ASSESSING THE STATISTICAL POWER OF A SEMI-FIELD EXPERIMENT: TESTING SINGLE AND COMBINED INTERVENTIONS AGAINST MALARIA VECTOR ANOPHELES ARABIENSIS

Andrea M. Kipingua1, Dick son W. Lwatoejejera2, Samson S. Kiware1, Mafalda Viana3, Paul C.D. Johnson1
1University of Glasgow, Glasgow, United Kingdom, 2Ifakara Health Institute, Dar Es Salaam, Tanzania, United Republic of

Vector control remains one of the most efficient strategies against malaria. Semi-field experiments are a very good first way of understanding the impacts of potential new vector controls before going to the field. However, the design of the semi-field experiment is critically important to ensure the outcomes are measurable and robust. One of the best ways to assess this is by power analysis. Assessing a study’s statistical power can help avoid wastage of resources, ethical concerns or promising control methods being prematurely dismissed. We developed a power analysis framework to assess how many semi-field chambers, the frequency of sampling and sampling size that a semi-field setting would provide enough power to determine the impact of the interaction between two tools, here pyriproxyfen (PPF) and the widespread long-lasting insecticidal net (LLIN) against malaria vector Anopheles arabiensis. We estimated power across a range of semi-field experimental design objectives and scenarios including testing LLIN alone and the interaction between LLIN and PPF by analysing more than 1000 simulated data sets per scenario using generalized linear mixed-effects models. Power was estimated as a proportion of the simulated data sets in which the null hypothesis was rejected. Although power increased with the increasing number of chambers, sampling frequency and mosquitoes to be sampled, the number of chambers was the dominant factor determining power relative to all other design choices. We also noted that the target effect size has a large impact on power, highlighting that careful and rational choice of this parameter value is critical to achieving realistic power estimates. The higher the effect sizes, the higher the power to quantify the interaction between LLIN and PPF with minimal resources. In our case study, a minimum of 4 chambers per treatment for high interaction effect size between LLIN and PPF should be considered. However, high variance among chambers was noted to contribute to the decreases in power, highlighting the importance of making conditions similar among chambers, e.g., by rotating treatments and samplers among chambers.

SEMI-FIELD EVALUATIONS OF THE IMPACT OF NOVEL BITE PREVENTION INTERVENTIONS ON ANOPHELES MINIMUS LANDING AND KEY LIFE HISTORY TRAITS IN THAILAND

Elodie Vajda1, Amanda Ross2, Manop Saeung3, Arissara Pongsiri4, David McIlver2, Allison Tatatsky4, Nakul Chitnis, Jeffrey Hill5, Jason Richardson6, Michael Macdonald1, Sarah J. Moore7, Neil F. Lobo8, Theeraphap Charoenviriyaphap9, Alongkot Ponlawat4
1University of Califonia, San Francisco/University of Basel/Swiss Tropical and Public Health Institute, San Francisco, CA, United States, 2University of Basel/Swiss Tropical and Public Health Institute, Basel, Switzerland, 3Kasetsart University, Bangkok, Thailand, 4Army Forces Research Institute of Medical Sciences, Bangkok, Thailand, 5University of California, San Francisco, San Francisco, CA, United States, 6Innovative Vector Control Consortium, Liverpool, United Kingdom, 7Ifakara Health Institute, Bagamoyo, Tanzania, United Republic of, 8University of Notre Dame/University of California, San Francisco, Notre Dame, IN, United States

The downward trend in the global malaria burden has stalled. Malaria vector control largely relies on indoor residual spraying (IRS) and insecticide treated nets (ITNs), which target mosquitoes resting and feeding indoors. As outdoor biting is increasingly prominent, interventions that target outdoor resting/biting mosquitoes and complement IRS and ITNs are needed. This semi-field study evaluated three transfluthrin- and one metofluthrin-based volatile pyrethroid spatial repellents (VPSRs), as well as etofenprox-treated
A high-resolution analysis of translational regulation during life cycle transitions in Toxoplasma gondii

Michelle Peters, Aditi Shukla, Kehui Xiang, Dylan McCormick, David Bartel, Sebastian Lourido
Whitehead Institute/Massachusetts Institute of Technology, Cambridge, MA, United States

Protein expression can be regulated through cis and trans factors acting on mRNA and translation machinery. This regulation allows cells to fine-tune expression of specific factors in response to environmental cues. The transcriptome of the Apicomplexan parasite Toxoplasma gondii represents a challenge to canonical models of translational regulation in eukaryotes as its 5' UTRs are several times longer than most other characterized species and the vast majority of 5' UTRs harbor at least one, if not several, upstream AUGs. These unusual features may be maintained in Toxoplasma because they confer regulatory information important for gene expression across the parasite life cycle. To understand how the parasite utilizes translational regulation across both the tachyzoite and bradyzoite stages, we performed the parasite life cycle. To understand how the parasite utilizes translational regulation in Toxoplasma as well as determinants of translation that shape the gene expression program of chronic stages.

Cryptosporidium remodels host microvilli through an exported virulence factor

Elena Rodrigues, Tapoka T. Mkandawire, Kat Sala, Silvia Haase, Adam Sateriale
The Francis Crick Institute, London, United Kingdom

The intestinal parasite Cryptosporidium is a leading cause of diarrhoeal disease, contributing to early childhood morbidity and mortality. Like other members of the phylum Apicomplexa, Cryptosporidium has secretory organelles containing proteins that are exported into host cells following parasite invasion. For Cryptosporidium, the identity and function of the vast majority of these proteins is unknown. Using a bioinformatics approach, we first identified a putative host-exported protein with serine repeats, which we epitope tagged at the endogenous locus. With a combination of super-resolution and expansion microscopy we discovered that this protein localises to the parasite's secretory dense granule organelles prior to host-cell invasion, and then within the host microvilli following invasion. To determine the function of this MicroVilli Protein (MVP) we used yeast-2-hybrid screening, detecting interacting partner EBP50; a scaffold protein known to facilitate F-actin recruitment and control microvilli dynamics. Microvilli elongation is commonly seen in Cryptosporidium infected epithelial cells, but the mechanism for this was previously unknown. Parasites deficient in MVP have moderately attenuated growth yet show a complete lack of elongated host microvilli during infection. It is known that the Escherichia coli virulence factor MAP also interacts with EB50, driving cell surface membrane protrusions and displacement of the NH3 sodium transporter contributing to diarrhoeal symptoms. While MVP has C-terminal homology with MAP, there does not appear to be evidence of a horizontal transfer event. This suggests a convergent evolution between bacteria and parasite that may contribute to diarrhoeal symptoms during infection.

Dynamics and signaling of mitochondrial mRNA U-indel editing during T. brucei differentiation

Joseph T. Smith Jr.¹, Brianna Tylec¹, Arunasalam Naguleswaran², Isabel Roditi², Laurie K. Read³
¹University at Buffalo, Buffalo, NY, United States, ²University of Bern, Bern, Switzerland

Twelve of the 18 mitochondrially encoded mRNAs in T. brucei must be post-transcriptionally modified by insertion/deletion of uridine residues (U-indel editing) to establish an open reading frame that is competent for translation on mitochondrial ribosomes. Here, we investigated changes in total and edited mRNAs in pleomorphic T. brucei at various times during the differentiation from long slender bloodstream form (BSF) to procyclic form (PCF). We found that, for most mRNAs, the abundances of total
and edited mRNAs increase modestly in stumpy forms and return to near slender BSF levels in PCF. In contrast, when stumpy BSF parasites are transitioned to PCF medium, the four cytochrome mRNAs (COI, COII, COIII, and CYb) are upregulated. Using droplet digital PCR, we quantified precise numbers of total and edited molecules per cell, allowing us to calculate editing efficiencies. Interestingly, while COII and CYb mRNAs exhibit increased editing efficiencies during differentiation, COI and COIII mRNAs are upregulated primarily by changes in total abundance. These increases in cytochrome mRNAs were also reflected in vivo during tsetse fly midgut infections, thus demonstrating this is a physiologically relevant event. We also found that a decrease in temperature is a key differentiation cue that sensitizes the parasites to other biomolecular triggers such as RDK1 depletion. We propose a working model in which temperature serves as a sensitizing trigger for the pathways governed by RDK1 to upregulate the abundance of the edited and never-edited cytochrome mRNAs during BSF-to-PCF differentiation.