6000

HIGHLY SENSITIVE TARGETS FOR DIAGNOSIS AND SPECIATION OF HUMAN LEISHMANIASIS

Nicholas Ray Duncan[1], Elise O'Connell[1], Janitzio Guzmán[1], Joshua R. Lacsina[2], Thalia Pacheco-Fernandez[3], Sreenivas Gannavaram[3], Andrea Paun[1], Thomas Nutman[1], Sasisekhar Sasisekhar Bennuru[1]

[1]Laboratory of Parasitic Diseases, NIAID, National Institutes of Health, Bethesda, MD, United States, [2]Laboratory of Malaria & Vector Research, NIAID, National Institutes of Health, Bethesda, MD, United States, [3]Center for Biologics Evaluation and Research, FDA, Silver Spring, MD, United States

Leishmaniasis, caused by various species of the *Leishmania* parasite, remains a significant public health concern in many parts of the world. Accurate and efficient diagnostic methods are crucial for timely intervention and management. The limitations of current diagnostic approaches necessitated the exploration of novel targets and tools. Through bioinformatic analysis of highly repeated elements in satellite regions of all Leishmania spp. genomes followed by PCR validation, CL3 and CL179 were identified as novel qPCR targets that were predicted to amplify all known Leishmania spp. Pathogenic to humans. The limits of detection for CL3 and CL179 qPCRs were consistently below 150 fg of gDNA - equivalent to fewer than ~5 copies of the haploid genome across all available Leishmania species known to infect humans (*L. aethiopica, L. amazonensis, L. braziliensis, L. chagasi, L. donovani, L. guyanensis, L. infantum, L. major, L. mexicana, L. panamensis* and *L. tropica*). The CL3 and CL179 qPCRs each reliably detected a single infected macrophage, using in vitro infected murine macrophages. Both CL3 and CL179 demonstrated high specificity, showing no cross-reactivity with other related parasitic intracellular protists. When tested with DNA extracted from skin biopsies from patients confirmed positive for leishmania infection by 18S qPCR (n=8), both CL3 and CL179 exhibited 100% sensitivity. Swabs and/or microbiopsies from a subset of these patients were also qPCR positive. Subsequently, we adapted the CL179 qPCR for use in recombinase polymerase amplification (RPA). The RPA amplicons were analyzed by Nanopore™ sequencing, resulting in the identification of species-specific SNPs and indels that enabled accurate parasite speciation in patients confirmed to have leishmaniasis (n=6). Our methodology provides an ultrasensitive one step approach to the rapid diagnosis and subsequent speciation of all known human Leishmania species.

6001

THE ROLE OF LIPIDS AS POTENTIAL BIOMARKERS OF DISEASE PROGRESSION AND THERAPEUTIC RESPONSE IN PATIENTS WITH CHRONIC *TRYPANOSOMA CRUZI* INFECTION

Juan C. Gabaldón-Figueira[1], Albert Ros-Lucas[1], Nieves Martínez-Peinado[1], Gavin Blackburn[2], Irene Losada-Galván[1], Elizabeth Posada[1], Cristina Ballart[3], Elisa Escabia[1], Jordi Capellades[4], Oscar Yanes[4], Maria J. Pinazo[5], Joaquim Gascón[1], Julio Alonso-Padilla[1]

[1]Barcelona Institute for Global Health, Barcelona, Spain, [2]Glasgow Polyomics, Glasgow, United Kingdom, [3]Universitat de Barcelona, Barcelona, Spain, [4]Universitat Rovira i Virgili, Tarragona, Spain, [5]Drugs for Neglected Diseases Initiative (DNDi), Rio de Janeiro, Brazil

Chagas disease, caused by the parasite *Trypanosoma cruzi*, is a zoonosis that affects more than 7 million people, mostly in Latin America, and represents an increasingly serious public health concern in the United States, Western Europe, Australia and Japan. Gaps in the understanding of the disease pathogenesis prevent the development of novel tools for diagnosis, prognosis and evaluation of treatment response, making the study of practical outcomes in clinical trials challenging; and hindering the approval of new therapies. The infection is known to disrupt several host metabolic pathways, and lipid metabolism is considered essential for parasite survival, providing an opportunity for the identification of biomarkers. The metabolomic and lipidomic profile of a cohort of eight symptomatic and 20 asymptomatic patients with *T cruzi* infection and a group of 15 uninfected controls was studied using liquid chromatography/mass spectrometry. All infected participants were tested for detectable parasitaemia using qPCR before and after receiving treatment. Differences between all groups were analyzed using a covariate-adjusted multiple linear regression, and changes before and after receiving treatment with benznidazole were evaluated using paired t-tests. Significance values were adjusted for multiple comparisons using the Benjamini-Hochberg's method. We studied the abundance of over 2,600 metabolites, identifying two phosphatidylethanolamines and one saturated fatty acid able to discriminate between symptomatic and asymptomatic participants. Additionally, three closely-related and possibly parasite-derived sphingolipids showed significant reductions in their abundance following treatment with benznidazole, reaching levels similar to those observed in uninfected controls, and among patients with no evidence of treatment failure. Pending further validation, these molecules represent potentially useful biomarkers to monitor cardiovascular damage and therapeutic response in patients with chronic *T. cruzi* infection.

6002

DEVELOPMENT OF A CRISPR-LAMP BASED BIOSENSOR WITH A LATERAL FLOW READOUT FOR THE DETECTION OF CUTANEOUS LEISHMANIASIS

Laud Anthony Wihibeturo Basing[1], Belinda O. Antwi[1], Anthony Y. Dziworshie[1], Francisca Adomako[2], Christabel Aning Boateng[3], Yaw Adu-Sarkodie[4]

[1]Department of Medical Diagnostics, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, [2]R&D Department, Incas Diagnostics, Kumasi, Ghana, [3]School of Medicine and Dentistry, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, [4]Department of Clinical Microbiology, School of Medicine and Dentistry, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

Cutaneous Leishmaniasis (CL) constitutes a significant public health challenge, particularly in resource-limited regions where it is endemic and where the lack of rapid and accurate diagnostic tools often leads to delayed treatment and increased morbidity. Conventional diagnostic methods for these NTDs are often time-consuming, expensive, and require specialized equipment, which limits their utility in remote areas. Therefore, there is an urgent need to develop an efficient, cost-effective, and user-friendly diagnostic tool for the early detection of CL. This study was aimed at the development of a Crispr-Lamp based diagnostic platform with a lateral flow readout for the detection of CL. A Loop-mediated isothermal amplification (LAMP) assay with primers targeting the unique CL conserved A2 gene were developed and optimized. The limit of detection and sensitivity were evaluated using 108 PCR PCR confirmed clinical samples (88 positives and 22 negative samples). A CRISPR-Cas12a system assay for molecular detection of Leishmania spp targeting the small subunit ribosomal ribonucleic acid (SSU rRNA) was then developed. The limit of detection and sensitivity were also evaluated using the 108 clinical samples. The two assays were then integrated with visual detection by lateral flow test strip. The limit of detection for both the Lamp test and the Crispr-cas test as well as the combined platform was found to be $1.0x10^3$ parasites/ml. The LAMP test had a sensitivity of 81.4% (70/86) and a specificity of 100% (22/22) whilst the Crispr Cas 12 assay had a sensitivity of 86.0% (74/86) and a specificity of 100% (22/22). Combined, the Crispr Lamp assay had a sensitivity of 98.8% (85/86) and a specificity of 100% (22/22). We show the development of a Crispr-Lamp platform with high sensitivity and specificity for the detection of cutaneous Leishmaniasis. With an integration of a heating platform, this could be adapted for use in remote endemic communities for the detection of CL.

6003

DEVELOPMENT AND CLINICAL VALIDATION OF LEISHID, A LAMP-BASED SPECIES-SPECIFIC *LEISHMANIA* DETECTION TOOL FOR THE MOLECULAR DIAGNOSIS OF LEISHMANIASES

Alessandra Mara de Sousa[1], Raphael França Oliveira[1], Stephanie Megale Ferreira[1], Ellen Gonçalves de Oliveira[1], Ana Caroline de Castro Nascimento Sousa[2], Rafael Luiz da Silva Neves[1], Eduardo Martinez[3], Célia Maria Ferreira Gontijo[1], Rodrigo Pedro Soares[1], Vinicius Maracajá Coutinho[3], Nilmar Silvio Moretti[4], **Rubens Lima do Monte-Neto**[1]

[1]Instituto René Rachou - Fundação Oswaldo Cruz - IRR/Fiocruz Minas, Belo Horizonte, Brazil, [2]Laboratório de Biologia Molecular de Patógenos (LBMP) - Departamento de Microbiologia Imunologia e Parasitologia - Universidade Federal de São Paulo - Unifesp, São Paulo, Brazil, [3]Integrative Bioinformatics, Universidad de Chile, Santiago, Chile, Santiago, Chile, [4]Department of Pathology and Microbiology, Faculty of Veterinary Medicine, Université de Montréal, Saint-Hyacinthe, QC, Canada

Point-of-care (PoC) molecular differential diagnostic tests for the detection and identification of *Leishmania* species are urgently needed, influencing effective treatment and avoiding misdiagnosis of leishmaniases, a neglected tropical disease threatening 1 billion people living at risk of infection. Recent reports reveal the presence of dermotropic-associated species, causing visceral leishmaniasis (VL) and vice-versa. Thus, a test able to concomitantly identify the *Leishmania* species is important to better understand the epidemiology and physiopathology of leishmaniases. In this regard, we developed the LeishID, a LAMP-based molecular tool able to detect small quantities of parasite DNA and differentiate among *L. amazonensis*, *L. infantum*, and *L. braziliensis*, the main species occurring in Brazil. The probes were designed based on a *Leishmania* pangenome approach, where species-specific DNA sequences were filtered from the accessory genome. The loop-mediated isothermal DNA amplification (LAMP) reaction result is detected by the naked eye using a pH-sensitive colorimetric sensor. Depending on the target, positive reactions can be detected as soon as 15 min at 65 ºC. The LAMP test was able to detect as low as 1 pg of extracted Leishmania DNA for all tested species. Species-specific sets of primers were able to detect the species they were designed for without cross-reactivity among them, neither on mammalian DNA. Clinical validation using spleen biopsies of dogs with VL, samples derived from skin lesions of cutaneous leishmaniasis from human patients, and Phlebotominae sandflies, revealed sensitivity varying from 93-98% and specificity of 90-100%. To meet PoC requirements, we selected a boiling/spin DNA extraction method able to amplify L. infantum DNA derived from skin biopsies. Additionally, we are developing a CRISPR/Cas-based PoC diagnostic tool for one-pot reaction with high sensitivity and specificity for *Leishmania* DNA detection. In this sense, LeishID is a PoC-compatible solution for rapid, accurate, and sensitive detection tool to differentiate Leishmania species in clinically relevant concentrations.

6004

NEW STRATEGY FOR THE OPTIMIZATION OF TAQMAN QPCR FOR *ENTAMOEBA HISTOLYTICA* BY DROPLET DIGITAL PCR

Akira Kawashima[1], Yanagawa Yasuaki[2], Rieko Shimogawara[3], Kenji Yagita[4], Hiroyuki Gatanaga[1], Koji Watanabe[5]

[1]AIDS Clinical Center, National Center for Global Health and Medicine, Tokyo, Japan, [2]Department of Microbiology and Immunology, Stanford University School of Medicine, Stardford, CA, United States, [3]Department of Parasitology, National Institute of Infectious Disease, Tokyo, Japan, [4]Department of Parasitology, National Institute of Infectious Diseases, Tokyo, Japan, [5]Division of Host Defense Mechanism, Tokai University School of Medicine, Kanagawa, Japan

Taqman-probed quantitative PCR (Taq-qPCR) is considered as high diagnostic value for amebiasis. However, there are variations in methodology among institutions. Furthermore, the absence of a clear cutoff value for cycle threshold (Ct) leads to numerous low-titer positives, complicating result interpretation. Here, we developed a new strategy evaluating amplification efficacy of primer-probe set of Taq-qPCR using droplet digital PCR (ddPCR). Twenty one primer-probe sets, which targeting small subunit ribosomal RNA gene region (X64142), were designed according to the previous reports. Amplification efficacy of primer-probe set was evaluated by absolute positive droplets (APD) counts and average amplitude of APDs in ddPCR. Firstly, we compared the amplification efficacy using laboratory strain of *Entamoeba histolytica* (HM1:IMSS strain) and ddPCR with low annealing temperature (AT) (59℃). Amplification efficacy is the same extent by different primer-probe sets at high PCR cycles (35-50 cycles). However, it was clearly differentiated by lower cycles (minimum 25 cycles), in which seven primer-probe sets showed high amplification efficacy. Among them, only two primer-probe sets maintained amplification efficiency on the higher AT (62℃). Thereafter, the correlation curve between APD counts (ddPCR by 50 cycles) and Ct value (Taq-qPCR) were made by the titration of HM1:IMSS templates, which determined cut-off Ct value as 35 cycles in Taq-qPCR. Thereafter, we performed ddPCR (50 cycles) and Taq-qPCR using clinical samples. Interestingly, in some cases, we found that unexpectedly higher number of APD counts with low average amplitude of APDs from the sample with high Ct value (>35 cycles by Taqman qPCR), which indicating that higher Ct value than cutoff was caused by the non-specific amplification in the templates extracted from fecal samples. The causative agent producing non-specific amplification is currently under investigation. Droplet digital PCR (ddPCR) visually and quantitatively evaluates and optimizes primer-probe sets for Taq-qPCR, ensuring accurate *E. histolytica* detection and highlighting false-positive risks.

6005

CHARACTERIZATION OF THE LEISHMANIN SKIN TEST ANTIGEN AS A BIOMARKER OF VACCINE EFFICACY AND DISEASE SURVEILLANCE

Laura Klenow[1], Ranadhir Dey[1], Hannah Markle[1], Nazli Azodi[1], Lais Da Silva Pereira[1], Thalia Pacheco-Fernandez[1], Patrick Lypaczewski[2], Greg Matlashewski[2], Abhay Satoskar[3], Sreenivas Gannavaram[1], Hira Nakhasi[1]

[1]FDA, Silver Spring, MD, United States, [2]McGill University, Montreal, QC, Canada, [3]Ohio State University, Columbus, OH, United States

Leishmaniasis is a neglected tropical disease transmitted through sand fly vectors carrying protozoan parasites from the genus *Leishmania*. Currently, there is no vaccine available for human use to prevent the spread of leishmaniasis. The leishmanin skin test (LST) has been used for almost a century in endemic countries to determine the exposure status and immunity of the local population. Lack of availability of a well-characterized *Leishmania* antigen manufactured under Current Good Manufacturing Practice (cGMP) conditions has hindered its use as a diagnostic and surveillance tool in the field. Therefore, the development of an effective, shelf-stable *Leishmania* antigen for the LST is needed for its use in endemic countries where consistent cold chains might not be available. Our lab has established a well-characterized leishmanin soluble antigen from *Leishmania donovani* (Indian strain) and produced it under Good Laboratory Practice (GLP) conditions. Using both a mouse model of cutaneous leishmaniasis and a hamster model of visceral leishmaniasis, the GLP-LST antigen induces a delayed-type hypersensitivity (DTH) response following both Leishmanization and vaccination with a live-attenuated vaccine comprised of a *Leishmania major* strain lacking the *Centrin* gene. As *Leishmania* vaccines are being developed, LST could also be used as a surrogate of vaccine immunogenicity. Characterization of leishmanin antigens using high-dimensional flow cytometry analysis of the cell populations isolated from the DTH sites in murine models showed an enrichment of CD69+ and CD4+ skin resident memory T cells. Additionally, the presence of activated macrophages and Langerhans cells at the DTH site was detected, consistent with previous studies. These studies provide evidence for the large-scale production of a well-characterized cGMP-LST antigen that can be used in future vaccine clinical trials as a marker for immunity and in

active surveillance studies of endemic and emerging areas of *Leishmania* infection. *"My contributions are an informal communication and represent my own best judgement. These comments do not bind or obligate FDA."*

6006

VISCERAL LEISHMANIASIS DIAGNOSIS WITH DIGITAL MICROSCOPY AND EDGE-AI MODELS

Lin Lin[1], Ramón Vallés-López[2], Daniel Cuadrado[2], David Bermejo-Peláez[2], Alexander Vladimirov[2], Maria Postigo[2], Fabiana Alves[3], Eleni Ayele[4], Arega Yeshanew[5], Miguel Luengo-Oroz[2], Israel Cruz[6], Elena Dacal[2]

[1]Spotlab, Madrid & Spain Biomedical Image Technologies, ETSI Telecomunicación, Universidad Politécnica de Madrid, Madrid, Spain & CIBER de Bioingeniería, Biomateriales y Nanomedicina, Madrid, Spain, [2]Spotlab, Madrid, Spain, [3]Drugs for Neglected Diseases initiative, Geneva, Switzerland, [4]University of Gondar Department of Internal Medicine, Gondar, Ethiopia, [5]University of Gondar Department of Internal Medicine. Leishmaniasis Research Treatment Center, Gondar, Ethiopia, [6]National School of Public Health, CIBERINFEC, Instituto de Salud Carlos III, Madrid, Spain

Visceral leishmaniasis (VL) remains a significant public health concern, with an estimated 30,000 new cases/year and the highest burden in rural settings in eastern Africa and Brazil. Tissue aspirate (mainly spleen and bone marrow) remains a cornerstone in the VL diagnostic algorithm and is the reference method to assess parasitological cure. Therefore, continuous quality assurance for both sample preparation and microscopist expertise is essential to ensure an accurate diagnosis.We developed an innovative digital microscopy solution enhanced with edge-based artificial intelligence (AI), integrated within a mobile app for real-time detection and quantification of Leishmania amastigotes. This consists of two modules: a smartphone-based app able to transform conventional microscopes into digital ones, and TeleSpot, a web-based platform for remote collaborative image analysis. This system was deployed in the Leishmaniasis Research Treatment Center, Gondar, Ethiopia following an implementation research approach and conducting a technology usability and acceptability evaluation with the participation of 5 laboratory staff after training. The solution allows evaluation of the quality of the smear and its staining, as well as the score (from 0 to 6+) that determines the parasite load. Six independent samples were digitised with 13 different smartphones. For AI model development, experts annotated 85 images, resulting in 1060 labels. Using a Yolo v8 algorithm, a precision of 85.9% and a recall of 79.3% were retrieved.This project represents a significant advancement in the standardization and automation of VL diagnosis, facilitating remote quality control in resource-limited settings and allowing for a potential improvement in the accuracy of microscopic diagnosis.

6007

DYNAMICS OF DENGUE VIRUS-REACTIVE B CELLS IN PEDIATRIC CASES FROM A HOSPITAL STUDY IN NICARAGUA

Tulika Singh[1], Amir Balakhmet[1], Rohan Shinkre[1], Nharae Lee[1], Aaron Graber[1], Raul Zapata[2], Walter Brenes[2], Angel Balmaseda[3], Eva Harris[1]

[1]Division of Infectious Diseases and Vaccinology, School of Public Health, University of California, Berkeley, Berkeley, CA, United States, [2]Sustainable Sciences Institute, Managua, Nicaragua, [3]Laboratorio Nacional de Virología, Centro Nacional de Diagnóstico y Referencia, Ministerio de Salud, Managua, Nicaragua

Dengue virus serotypes 1-4 (DENV 1-4) cause an estimated 100 million febrile infections annually in the tropics. DENV-reactive memory B cells are important for protective immunity in the subsequent infection as they can differentiate to secrete antibodies (Abs). Yet, their dynamics and persistence are not well understood. To enumerate DENV-reactive B cells, we developed a novel and sensitive approach that does not depend on B cell stimulation. We fluorescently labelled whole mature virions of DENV1, DENV2, and DENV3 with Alexa Fluor (AF)-488 and AF647, yielding 6 single-fluor antigens that retain key neutralizing epitopes only present on the quaternary structure of the virus. A cocktail of antigens was applied to peripheral mononuclear cells (PBMCs) from Nicaraguan pediatric hospitalized cases of dengue. DENV-reactive B cells were defined as positive for both fluorescent antigens: $CD3^-/CD14^-/CD16^-/CD19^+$/DENV-$AF488^+$/DENV-$AF647^+$. We detected counts of DENV-reactive live B cells from early convalescent PBMCs across primary (1°) and secondary (2°) infections of DENV1-3 at frequencies 2-11-fold higher than a naïve sample. To analyze the dynamics of DENV-specific B cell populations over time, we selected a set of 6 longitudinal PBMCs collected at acute phase, early convalescence (days 14-28), and 3-, 6-, 12-, and 18-months post-infection in 1° DENV1 and 2° DENV1 cases. In preliminary results, we found DENV-reactive B cells at 18 months, with peak levels between 6-12 months. DENV-reactive activated memory B cells (MBCs; $IgD^-/CD20^+/CD21^-/CD27^+$) were highest in acute 2° DENV1. Interestingly, DENV-reactive atypical MBCs ($IgD^-/CD20^+/CD21^-/CD27^-$) were higher 6-18 months post-2° than the same time post-1° DENV1. Our ongoing analyses support that: 1) levels of DENV-reactive B cells may increase with each exposure; 2) activated MBCs participate in the acute 2° immune response; 3) levels of atypical DENV-reactive B cells increase post-2° infection; and 4) peak DENV-reactive B cells are found at 6-12 months post-infection and persist up to 18 months. Our approach will enable a better understanding of protective B cell immunity against DENV.

6008

ORDER MATTERS: DENV2-ZIKV AND ZIKV-DENV2 SEQUENTIAL INFECTIONS DIFFERENTIALLY MODULATE THE MAGNITUDE AND BREADTH OF HOMOTYPIC AND DENV CROSS-REACTIVE ANTIBODY RESPONSES

Sandra Bos[1], Elias Duarte[1], Nharae Lee[1], Jose Victor Zambrana[2], Aaron Graber[1], Angel Balmaseda[3], Eva Harris[1]

[1]Division of Infectious Diseases and Vaccinology, School of Public Health, University of California, Berkeley, Berkeley, CA, United States, [2]Department of Epidemiology, School of Public Health, University of Michigan, Ann Arbor, MI, United States, [3]Sustainable Sciences Institute, Managua, Nicaragua

Dengue (DENV) and Zika (ZIKV) viruses, closely related flaviviruses, present significant public health and vaccine development challenges due to their complex immune interactions. Based on our prospective pediatric cohort study in Nicaragua, we have shown an increased risk of symptomatic DENV infection following a DENV-ZIKV but not a ZIKV-DENV infection sequence. Here, we further investigate how the order of DENV/ZIKV infections influences antibody (Ab) responses. Using samples from participants with DENV2-ZIKV (n=28) and ZIKV-DENV2 (n=12) infection sequences collected post-first (1°) and post-second infection, we analyzed the neutralizing Ab (nAb) potency and Ab binding profiles to DENV1-4 and ZIKV antigens. We found that the order of ZIKV/DENV2 infections impacts homotypic (DENV2, ZIKV) responses in a non-reciprocal way. Higher ZIKV nAb titers were generated after DENV2-ZIKV compared to after 1° ZIKV infection, suggesting that prior DENV2 exposure increases the magnitude of the ZIKV nAb response; in contrast, comparable nAb titers to DENV2 were measured after 1° DENV2 and ZIKV-DENV2 infection, showing no impact of prior ZIKV infection. Moreover, the sequence of infection significantly affected homotypic and heterotypic DENV Ab responses both in terms of magnitude of binding and neutralization. The DENV2-ZIKV sequence showed consistently lower Ab responses to DENV1, 2, 3, 4 and ZIKV compared to the ZIKV-DENV2 sequence, suggesting that a second ZIKV infection may limit the development of DENV-induced Ab responses. Further, the order of infection affected the breadth of the Ab repertoire; NS1 Abs from the ZIKV-DENV2 group were highly cross-reactive to DENV1, 2, 3, and ZIKV, unlike those from the DENV2-ZIKV group, which predominantly cross-reacted to only DENV1 and 2. Overall, these results demonstrate that the order of DENV2/ZIKV infections strongly modulates the magnitude and breadth of Ab responses, with a second ZIKV infection dampening the DENV Ab response. This has significant implications for vaccine development strategies, underscoring the importance of considering prior flavivirus exposure history.

6009

MECHANISTIC MODELING OF HOST-VIRAL INTERACTIONS TO ELUCIDATE IMMUNE MECHANISMS UNDERPINNING DISPARATE RESPONSES TO DENGUE VIRUS INFECTION BY PRIOR EXPOSURE HISTORY

Rosemary A. Aogo[1], Kelsey E. Lowman[1], Chloe M. Hasund[1], Charlie Voirin[1], Gitanjali Bhushan[1], Patrick I. Mpingabo[1], Saba Firdous[1], Silvia Blanco-Rivera[1], Melissa Law[1], Daniela Weiskopf[2], Viviane Callier[3], Sally Hunsberger[4], Robbie Kattappuram[5], Jeffrey R. Strich[6], Heather L. Teague[6], Lauren Knabe[6], Jeffery I. Cohen[7], Anna Durbin[8], Stephen S. Whitehead[9], Camila D. Odio[1], Leah C. Katzelnick[1]

[1]Viral Epidemiology and Immunity Unit, Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States, [2]Department of Medicine, Division of Infectious Diseases and Global Public Health, University of California San Diego (UCSD), La Jolla, CA, United States, [3]Clinical Monitoring Research Program Directorate, Frederick National Laboratory for Cancer Research, Bethesda, MD, United States, [4]Division of Clinical Research, Biostatistics Research Branch, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States, [5]Department of Pharmacy, NIH Clinical Center, National Institutes of Health, Bethesda, MD, United States, [6]Critical Care Medicine Department, National Institutes of Health Clinical Center, Bethesda, MD, United States, [7]Medical Virology Section, Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States, [8]Johns Hopkins Bloomberg School of Public Health, Department of International Health, Baltimore, MD, United States, [9]Arbovirus Vaccine Research Section, Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States

The immunological mechanisms underpinning disease pathogenesis and protection against dengue, especially the dysregulated host response in secondary dengue virus (DENV) infection, remain only partially understood. We have developed a mechanistic mathematical model that explicitly considers interactions between DENV target cells, including monocytes, dendritic cells, and macrophages, as well as virus, NK cells, neutrophils, CD4 T cells, CD8 T cells, B cells, antibodies, and a milieu of cytokines to model innate responses and how they modulate adaptive responses. First, we have modeled both intrinsic and extrinsic antibody-dependent enhancement using a sub-model of the interaction between the virus, target and infected cells, and IFN-beta and IL-10 assuming the rate of viral production is proportional to IL-10 and inhibited by IFN-beta. Under this assumption, our model simulations showed rapid viral clearance and target cells returning to homeostasis, indicating effective viral resolution. However, lack of IFN production led to increased viremia and infection of all the target cells. Assuming increased viral infectivity and production, we observed delayed viral clearance, infected cells persistence, and high IFN and IL-10 concentrations beyond day 30, suggesting an inflammatory disease phenotype. Our ongoing work involves fitting the sub-model and full model to data from the literature as well as our unique Phase 1 trial (NCT05691530) to safely measure how distinct immune histories impact host response to a live-attenuated DENV3 monovalent vaccine. The clinical trial has so far enrolled 20 of 45 healthy adult participants with no (naive, n=15), one (non-DENV3 heterotypic, n=15), or more than one (polytypic, n=15) previous natural DENV infection(s). We are measuring at serial timepoints; immune cell populations, cytokine profiles, DENV-specific plasmablasts, viral load, and antibody titers. Our full model will be calibrated to these data to elucidate how recall responses perturb *de novo* responses in secondary infection, identifying immune mechanisms driving severe dengue pathology, and potential therapeutic targets.

6010

IMMUNOLOGICAL FEATURES ASSOCIATED WITH SEVERE DENGUE IN CHILDREN AND YOUNG ADULTS WITH OBESITY AND NORMAL WEIGHT

Michaela Gregorova[1], Marianna Santopaolo[1], Divya Diamond[1], Vi Tran Thuy[2], Nguyet Nguyen Minh[2], Vuong Nguyen Lam[2], Hoa Vo Thi My[2], Chanh Ho Quang[2], Chau Nguyen Thi Xuan[2], Tam Dong Thi Hoai[2], Duyen Huynh Thi Le[2], Tam Cao Thi[3], Sophie Yacoub[2], **Laura Rivino**[1]

[1]University of Bristol, Bristol, United Kingdom, [2]OUCRU, HCMC, Vietnam, [3]Hospital of Tropical Diseases, HCMC, Vietnam

Dengue virus infections are spreading globally. Host immune responses are believed to drive dengue pathogenesis, through mechanisms which remain poorly understood. Obesity is emerging as a risk factor for severe dengue (SD). The reasons for this remain unknown but may involve chronic low-grade inflammation leading to ineffective anti-viral immunity. In this study we test the hypothesis that SD associates with dysfunctional T/NK-cell responses, and that these defects are exacerbated in overweight/obese (OB) individuals, thus underpinning their increased susceptibility to SD. We analysed the phenotypical/functional features of $CD4^+$, $CD8^+$-T and NK-cells using multiparametric flow cytometry in peripheral blood mononuclear cells (PBMCs) of Vietnamese OB dengue patients aged 10-30 years, with each OB patient matched by age, sex and illness phase to one of normal weight. PBMCs from a total of 124 patients (94 non-SD, 30 SD) were analysed at admission (days of illness: 2-5) and 3 days later. Our data shows altered $CD4^+$/$CD8^+$-T and NK-cell responses in SD compared to non-SD. Dengue-specific and total $CD4^+$-T and $CD8^+$-T-cells of SD patients displayed features of T-cell exhaustion with high expression of PD-1 and other inhibitory receptors (e.g, TIGIT, LAG-3, TIM-3), and decreased granzyme B levels. These features were exacerbated in OB patients compared to their normal weight counterparts. *Ex vivo*-blockade of PD(L)-1 with anti-PD(L)-1 antibodies enhanced the cytotoxic potential of DENV-specific $CD8^+$-T-cells in some patients, suggesting their function can be restored. SD patients also displayed NK-cell dysfunction, whereby NK-cells expressed inhibitory receptors (LILRB1, NKG2A, PD-1, PDL-1, TIGIT and LAG-3) and decreased cytotoxicity. Importantly, T and NK-cell inhibitory receptor expression associated strongly with markers of dengue severity and endothelial dysfunction (e.g., plasma leak, ferritin, Angiopoietin-2, syndecan-1, VCAM-1). Our data suggests that $CD8^+$-T and NK-cell dysfunction leading to poor clearance of dengue-infected cells may underlie SD, and potentially contribute to the increased risk of OB individuals to SD.

6011

NEW INSIGHTS INTO AN OLD VACCINE: HETEROLOGOUS FLAVIVIRUS INFECTION ENHANCES THE POTENCY AND BREADTH OF 17D-ELICITED NEUTRALIZING ANTIBODIES AGAINST A PANEL OF WILD-TYPE YELLOW FEVER VIRUSES

Felicity J. Coulter, Abram E. Estrda, Samantha R. Osman, Shuhua Luo, Courtney A. Micheletti, Peter D. Sullivan, Brian L. Booty, William B. Messer

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

Mosquito-borne yellow fever virus (YFV) remains a pathogen of public health concern, with recent outbreaks of yellow fever in South America and Africa. While the live-attenuated YFV vaccine, 17D, elicits neutralizing antibodies (NAbs) in >99% of vaccinees by 28 days post vaccination, and immunity from a single dose of 17D is touted as “lifelong,” the durability of 17D-elicited NAbs differs between populations, and the need for booster doses remains controversial. A major limitation of most NAb studies of 17D-elicited immunity is the almost exclusive reliance on 17D as the test virus in serologic studies, and the breadth of human serum NAbs against antigenically diverse wild-type (WT) YFVs implicated in human disease remains largely uncharacterized. Here we address two knowledge gaps: (1) the potency and breadth of 17D-mediated NAbs against WT viruses from all 7 genotypes, and (2) the effect of heterologous flavivirus

immunity on potency and breadth of NAbs in humans. Using non-endemic 17D-vaccinee sera ≤10 years post vaccination, from participants with diverse heterologous flavivirus immunity (n=55), and a unique panel of 14 YFV strains isolated from 10 countries between 1927 and 2018, we performed focus reduction neutralization tests (FRNT). Here we show (1) a 5- to 35-fold reduction in potency of NAbs against WT viruses compared to 17D, with a strong correlation between FRNT50 and amino acid similarity to 17D; (2) poor neutralization of South America genotype I (SA-I) strains, where up to 56% of vaccinees (28/50) were seronegative (FRNT50 <1:10); and (3) significantly increased potency of NAbs against SA-I strains amongst vaccinees with heterologous flavivirus immunity exhibiting 94% seropositivity (15/16), compared to just 25% seropositivity in vaccinees without heterologous immunity (6/24). We hypothesize that a glycosylation site, N67, shared exclusively between SA-I strains and all four serotypes of dengue viruses, confers the greater breadth of neutralization observed in heterologously infected 17D vaccinees. These findings have the potential to significantly impact future YFV vaccine design and deployment strategies.

6012

PROTECTIVE VACCINATION OF NONHUMAN PRIMATES AGAINST AEROSOL EXPOSURE TO MARBURG VIRUS USING A VESICULAR STOMATITIS VIRUS-VECTORED VACCINE: IMPLICATIONS FOR MUCOSAL VACCINE STRATEGIES AND UNPREDICTABLE FILOVIRUS TRANSMISSION

Christopher L. Cooper[1], Gavin Morrow[1], Thomas Postler[1], Yesle Choi[1], Aaron Wilson[1], Zhou Liu[1], Karina Peregrina[1], Fuxiang Hou[1], Shui Li[1], Suzane Ramos da Silva[1], Denise Wagner[1], Alexei Carpov[1], Michal Gazi[2], Yenny Goez-Gazi[2], Kendra J. Alfson[2], Ricardo Carrion[2], Anne Ercolini[1], Nina Malkevich[3], Mark B. Feinberg[3], Swati B. Gupta[3], Christopher L. Parks[1]

[1]Vaccine Design and Development Laboratory, The International AIDS Vaccine Initiative, Inc. (IAVI), Brooklyn, NY, United States, [2]Applied Science & Innovation, Texas Biomedical Research Institute, San Antonio, TX, United States, [3]The International AIDS Vaccine Initiative, Inc. (IAVI), New York, NY, United States

Recent increases in outbreaks caused by filoviruses, including Marburg virus (MARV) pose a serious global health threat due to a lack of countermeasures shown to be effective in people. Zoonotic spillover of MARV likely from Rousettus bat virus reservoirs followed by human-to-human transmission through contact with infected body fluids, has been associated with outbreaks. However, lethal infection by aerosol exposure, an unnatural route, has been demonstrated in preclinical models, which further establishes MARV as a substantial bioweapon threat. We have previously shown that a single intramuscular (IM) injection with a clinical-ready replication-competent recombinant vesicular stomatitis virus vaccine vector encoding the MARV glycoprotein GP (rVSVΔG-MARV-GP) was highly efficacious in protecting cynomolgus macaques exposed to MARV-Angola by systemic infection. Here, we demonstrate that either IM or mucosal intranasal (IN) rVSVΔG-MARV-GP vaccination regimens can elicit immunity that protects cynomolgus macaques following MARV-Angola aerosol exposure. We found that all rVSVΔG-MARV-GP vaccinated macaques developed potent systemic immunity as measured by anti-MARV GP binding titers and viral neutralization responses, regardless of the route of vaccine delivery and that the humoral responses appeared to be predictive of MARV aerosol protection. Moreover, macaques vaccinated by the IN route displayed superior protection against MARV aerosol exposure as indicated by improved control of MARV viremia, decreased clinical pathologies and increased survival. Together, these results support that rVSV-based vaccines have broad utility as effective countermeasures against natural and unpredictable pathogen exposures. Moreover, this work highlights that rVSV-based vaccines can be safely deployed within the mucosal environments and can provide significant benefits for protection against respiratory pathogen exposure.

6013

FIRST, DO NO HARM: FIELD EVALUATION OF AN INDEPENDENT RIFT VALLEY FEVER VACCINATION CAMPAIGN AND THE IMPACT ON PREGNANT LIVESTOCK IN A SEMI-PASTORAL AREA IN KENYA

Keli N. Gerken[1], Abraham Rereu[2], Fredrick Sururu[3], Alice Kiyong'a[4], Cynthia M. McMillen[5], Amy L. Hartman[6], Bernard Bett[4], Andrew P. Stringer[7], Matthew Baylis[7], Eric M. Fèvre[1]

[1]International Livestock Research Institute, Nairobi, Kenya and Institute of Infection, Veterinary and Ecological Sciences, University of Liverpool, Liverpool, United Kingdom, Liverpool, United Kingdom, [2]Loitokitok Sub-County Department of Veterinary Services, Kajiado County, Kenya, Loitokitok, Kenya, [3]Loitokitok Sub-County Department of Veterinary Services, Kajiado County, Kenya, Liverpool, United Kingdom, [4]International Livestock Research Institute, Nairobi, Kenya, [5]4Department of Microbiology and Molecular Genetics, Center for Vaccine Research, University of Pittsburgh, Pittsburgh, PA, United States, [6]Department of Microbiology and Molecular Genetics, Center for Vaccine Research, University of 4Department of Microbiology and Molecular Genetics, Center for Vaccine Research, University of Pittsburgh, Pittsburgh, PA, United States, [7]Institute of Infection, Veterinary and Ecological Sciences, University of Liverpool, Liverpool, United Kingdom, Liverpool, United Kingdom

Rift Valley fever (RVF) is a devastating zoonotic and livestock disease causing abortion and death of young animals. There are currently no human vaccine options and, therefore, livestock vaccination is key tool to protect public health and livelihoods. The effectiveness of RVF livestock vaccination is complicated by the population covered and reaction time following early warnings. This study was initiated following an independent vaccine event in Loitokitok sub-county, southern Kenya. We sampled vaccinated animals and had a unique opportunity to understand the impact of the live-attenuated Smithburn vaccine on pregnancy in a field setting. Vaccinated pregnant dams were prioritized for sampling. We sampled 150 animals including 123 females (76 sheep, 47 goats) and 23 males (6 sheep, 17 goats) across two large herds in Imbirikani and Risa villages, with 41 and 47 days between vaccination and sampling. Over this time, farmers reported 23 sheep abortions and nine goat abortions and at sampling, we counted 289 sheep and 79 goats in total. Despite these abortions, anti-RVFV IgG antibodies were detected only in 43.3% (65/150) of adult animals, and we found no evidence of a statistical association with the animals' village, sex, species, or age. We sampled 108 dams and matched them to 74 offspring. Seroprevalence was higher in the aborted dams present for sampling (75%, 6/8) compared to all adults ($p=0.08$). Abortions occurred between 18-32 (median: 24.5) days post-vaccination, and dams vaccinated earlier in gestation were more likely to abort ($p=0.001$). Offspring had low seroprevalence (21.6%, 16/74) and seropositivity was associated with having a seropositive mother ($p=0.05$). Abortions following the vaccination event is concerning given the low seroprevalence. This study design does not support causation, yet the Smithburn vaccine's association with abortion suggests an improved strategy including safe vaccines for pregnant animals is needed as abortions contribute to farmers' loss of livelihood. In addition, models to assess efficacy before programme delivery can mitigate vaccine hesitancy in vulnerable livestock owning populations.

6014

USING A ONE HEALTH APPROACH IN INVESTIGATING A CRIMEAN-CONGO HEMORRHAGIC FEVER OUTBREAK IN LYANTONDE DISTRICT, UGANDA 2024.

Luke Nyakarahuka[1], Sophia Mulei[1], Joanita Mutesi[1], Jimmy Baluku[1], Alex Tumusiime[1], Jackson Kyondo[1], Calvin R. Torach[1], Dianah Namanya[1], Stephen K. Balinandi[1], Trevor Shoemaker[2], John Klena[2], Joel Montgomery[2], Julius Lutwama[1]

[1]Uganda Virus Research Institute, Kampala, Uganda, [2]United States Centers for Disease Control and Prevention, Atlanta, GA, United States

In January 2024, a cluster of deaths in Buyanja village, Lyantonde district, Uganda, initially suspected to be anthrax, prompted investigation and identification of Crimean-Congo Hemorrhagic Fever (CCHF) cases. This

study describes the outbreak investigation, including case findings, public health actions, and control measures. Initially, there was an outbreak of Anthrax in the district neighboring Lyantonde. The first two cases were suspected to be anthrax and were sent to the Uganda Virus Research Institute (UVRI) for Anthrax testing. The samples tested negative for anthrax, prompting a request for them to be tested for other Viral Hemorrhagic Fevers. Both samples were tested by RT-PCR and were positive for Crimean-Congo Hemorrhagic Fever (CCHF) and were negative for Ebola, Marburg, and Rift Valley Fever viruses. Following confirmation of these two cases, the Rapid Response Team conducted an investigation involving both veterinary and human medicine following a one health approach to ascertain the risk factors for these cases and sampled more humans and livestock, which were tested by both PCR and ELISA. We sensitized community leaders on CCHF causes, advised on control measures, and collected GPS coordinates for mapping purposes. Following the confirmation of two initial CCHF cases in Lyantonde, three additional cases were confirmed, totaling five cases. The outbreak was linked to close contact with infected body fluids, primarily from slaughtering animals. Investigations revealed that all cases were males involved in animal-related occupations, such as butchering and livestock care. Public health actions included community sensitization, supply of health education materials, and extensive sampling of animals and humans for testing. This outbreak highlights the importance of early detection, prompt public health response, and community engagement in controlling zoonotic diseases like CCHF. Efforts to enhance surveillance, improve laboratory capacity, and educate at-risk populations are crucial for preventing future outbreaks.

6015

AN EPIZOOTIC OF DEER TICK VIRUS ON MARTHA'S VINEYARD DUE TO AMPLIFICATION OF A SINGLE VIRAL GENOTYPE

Heidi Goethert[1], Alanna O'Callahan[1], Richard Johnson[2], Sam Telford[1]

[1]Tufts Cummings School of Veterinary Medicine, Grafton, MA, United States, [2]Martha's Vineyard Tick Initiative, Edgartown, MA, United States

Deer tick virus (DTV), or lineage II Powassan virus, is the cause of a rare encephalitis with a high case fatality rate. During longitudinal studies of the ecology and epidemiology of tick-transmitted pathogens on Martha's Vineyard, Massachusetts, we detected a >5-fold increase in DTV prevalence in 2021,4.5% of 2194 host-seeking nymphs tested, up from 0.8% in 2020 and 0.5% in 2019. We used molecular epidemiologic methods to generate hypotheses for the biological basis for this increase. Transmission on the island was highly focal; most of the 72 sites sampled did not yield infected ticks. From the 14 sites with infected ticks, 99 were determined to be positive by RT-qPCR screening and confirmed by sequencing. Prevalence estimates at these sites ranged from 1% to 65.3%, with 75% of positives derived from only 4 sites. Whole virome sequencing determined that there were 3 distinct genotypes on the island. Genotype 2 was found primarily on Chappaquiddick, which is connected to the main island by a narrow beach. Types 1 and 3 were identified across the main island with some sites yielding both. Type 1 was responsible for 78% of the infected ticks, most of which were from the few hot foci. We tested the hypothesis that foci were due to horizontal transmission from a single reservoir species with individuals serving as super-spreaders. We identified the host upon which the infected ticks had fed as larvae using our previously described bloodmeal analysis assay. None of these sites yielded a single bloodmeal source from their infected ticks, indicating that a super-spreader host was not likely to be responsible for the great prevalence of infections. Almost half of the infected ticks (47%) had fed on shrews, 7% on mice, 5% on birds, 5% on deer and 35% did not yield bloodmeal results. It is possible that ticks acquired infection by inheritance (transovarial transmission); sequencing of whole tick mitochondrial genomes to determine whether the infected ticks could derive from the same egg batch is ongoing. It may be that the 2021 DTV epizootic on Martha's Vineyard was caused by the amplification of a single viral genotype maintained by transovarial transmission.

6016

GENOMIC SURVEILLANCE OF TICK AND MOSQUITO POOLS FROM GEORGIA (SOUTH CAUCASUS), SCREENED FOR VIRUSES ASSOCIATED WITH ACUTE FEBRILE ILLNESSES

Chanel A. Mosby-Tourtellot[1], Quinn K. Thomas[2], Adrian C. Paskey[2], J. Alex Chitty[2], Andrea E. Luquette[2], Maren C. Fitzpatrick[2], Jennetta Green[3], Maggie Bartlett[4], Danielle Ali[4], Malik Kadir[4], Drew D. Reinbold-Wasson[5], Tamar Chunashvili[5], Giorgi Kirkitadze[5], Anano Shubashishvili[5], Gregory K. Rice[2], Regina Z. Cer[3], Francisco Malagon[2], Darci R. Smith[3], Kimberly A. Bishop-Lilly[3]

[1]Defense Threat Reduction Agency, Fort Belvoir, VA, United States, [2]Leidos, Reston, VA, United States, [3]Naval Medical Research Command, Fort Detrick, MD, United States, [4]Parsons Corporation, Centreville, VA, United States, [5]Walter Reed Army Institute of Research Europe – Middle East, Tbilisi, Georgia

Genomic surveillance of arthropod-borne viral diseases is an important aspect of infectious disease surveillance that allows for monitoring of viral activity in vectors. Understanding the amount of viral activity in a region assists in predicting the likelihood or risk of human infection in an area, which in turn informs disease prevention strategies for force health protection. Here, we present findings from sequencing of 100 pools of ticks and mosquitoes screened for viruses associated with acute febrile illnesses (AFI) which were collected in the country of Georgia from May to October 2022 after an outbreak of human cases of Crimean-Congo hemorrhagic fever virus. RNA was extracted from tick and mosquito pools prior to shotgun sequencing. A total of 7,570 million reads were processed through in-house pipelines MetaDetector and VirusSeeker 2.0, an enhanced version of the public VirusSeeker pipeline. Viral sequences present in the results of both pipelines were investigated further. AFI-associated viruses were found in approximately 18% of the sample pools. The emerging pathogens Jingmen tick virus (JMTV) and Haseki tick virus (HTV) were the most frequently identified AFI-associated viruses. Both these viruses were previously identified in patients suffering from febrile illnesses following tick bites. JMTV is a segmented virus belonging to the *Flaviviridae* family and was identified in our study in pools of *Rhipicephalus bursa* ticks. Complete JMTV genomes were assembled from two tick samples allowing for phylogenetic analysis that revealed the nearest sequenced neighbor for both strains is a JMTV from Turkey. Similarly, HTV belongs to the *Flaviviridae* family and was seen in pools of both *Dermacentor reticulatus* and *Haemaphysalis punctata* ticks. We were able to extract partially complete coverage of the HTV polyprotein from pooled samples resulting in a phylogenetic analysis that showed clustering with HTV strains from Russia. This study expands our knowledge of AFI-associated viral pathogens in the region and provides further understanding of the range of these emerging pathogens and the vectors from which humans may be exposed to them.

6017

THE BAT BUSHMEAT TRADE AS AN INTERFACE FOR FILOVIRUS AND HENIPAVIRUS SPILLOVER IN THE REPUBLIC OF CONGO

McKenna Roe[1], Eeva Kuisma[2], Evrard Missamou[2], Alain Ondzie[2], Chastel Mapanguy[3], Providence Sita[3], Robert J Fischer[4], Claude Kwe Yinda[4], Morgane Cournarie[2], Diane Detoeuf[2], Francine Ntoumi[3], Eric Laing[1], Vincent J. Munster[5], Sarah H. Olson[2]

[1]Department of Microbiology and Immunology, Uniformed Services University, Bethesda, MD, United States, [2]Wildlife Conservation Society, New York, NY, United States, [3]Fondation Congolaise pour la Recherche Médicale (FCRM), Brazzaville, Congo, Republic of the, [4]Laboratory of Virology, Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Hamilton, MT, United States, [5]Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Hamilton, MT, United States

Outbreaks of Ebola virus and the potential for emergence of henipaviruses in sub-Saharan Africa underscores the need to identify when and where

these viruses spillover into human populations. Molecular evidence of Ghana virus (GhV) and antibodies to Ebola virus (EBOV) have been detected in African straw-colored fruit bats (*Eidolon helvum*), the most commonly hunted bat in sub-Saharan Africa. Therefore, hunters of this bat species may be at risk for henipavirus and ebolavirus spillover. A cross-sectional surveillance study of 164 bat hunters and 420 *E. helvum* bats in the Republic of Congo was conducted. Serum from hunters and bats was collected and tested by an envelope glycoprotein-based multiplex microsphere immunoassay for IgG against ten filoviruses and seven paramyxoviruses. We detected henipavirus seropositivity in 18.3% (7.1 - 29.5%) of bats sampled, including 11.3% (5 – 17.6%) GhV seropositivity, 5.2% (1.9 – 8.6%) Nipah virus (NiV) seropositivity, and 6.3 (1.2 – 11.4%) Mòjiāng virus (MojV) seropositivity. There was also evidence of filovirus seropositivity in 25.4% (4.3 - 46.4%) of bats sampled, including 15.1% (0.7 – 29.5%) Ebola virus (EBOV) seropositivity and 21.3% (3.6 – 39.1%) Bundibugyo (BDBV) seropositivity. Additionally, we detected henipavirus seropositivity in 17% (4.3 – 29.9%) of bushmeat hunters sampled, including 15.6% (4.3 – 29.8%) MojV seropositivity. We also detected 23% (17.7 – 29.8%) filovirus seropositivity, including 12.8% (6.7 – 18.9) EBOV seropositivity and 11.6% (3.7 – 19.5%) BDBV seropositivity. Here, we report the first estimates of filovirus and henipavirus serological prevalence in African straw-colored fruit bats and bushmeat hunters in the Republic of Congo. Overall, henipavirus and EBOV seroprevalence in this species of bat has been reported in other countries in sub-Saharan Africa but estimates of seroprevalence of GhV and other antigenically distinct henipaviruses in bats is limited. Additionally, our detection of MojV-like henipavirus infections humans suggests exposure to novel henipaviruses that are distantly related to the prototypic species.

6018

NEXT-GENERATION SEQUENCING SURVEY OF ACUTE FEBRILE ILLNESS IN SENEGAL (2020-2022)

Gregory S. Orf[1], Ambroise D. Ahouidi[2], Maximillian Mata[1], Cyrille Diedhiou[2], Aminata Mboup[2], Abdou Padane[2], Noel Magloire Manga[3], Agbogbenkou Tevi Dela-del Lawson[4], Francisco Averhoff[1], Michael G. Berg[1], Gavin A. Cloherty[1], Souleymane Mboup[2]

[1]Abbott Laboratories, Abbott Park, IL, United States, [2]Institut de Recherche en Santé, de Surveillance Epidémiologique et de Formation, Dakar, Senegal, [3]Hôpital de la Paix, Ziguinchor, Senegal, [4]Hôpital Mame Abdou Aziz Sy Dabakh, Tivaouane, Senegal

Acute febrile illnesses (AFI) in developing tropical and sub-tropical nations are challenging to diagnose due to the numerous causes and non-specific symptoms. The proliferation of rapid diagnostic testing and successful control campaigns against malaria have revealed that non-Plasmodial pathogens still contribute significantly to AFI burden. Thus, a more complete understanding of local trends and potential causes is important for selecting the correct treatment course, which in turn will reduce morbidity and mortality. Next-generation sequencing (NGS) in a laboratory setting can be used to identify known and novel pathogens in individuals with AFI. In this study, plasma was collected from 228 febrile patients tested negative for malaria at clinics across Senegal from 2020-2022. Total nucleic acids were extracted and converted to metagenomic NGS libraries. To identify viral pathogens, especially those present at low concentration, an aliquot of each library was processed with a viral enrichment panel and sequenced. Corresponding metagenomic libraries were also sequenced to identify non-viral pathogens. Sequencing reads for pathogens with a possible link to febrile illness were identified in 51/228 specimens, including (but not limited to): *Borrelia crocidurae* (N=7), West Nile virus (N=3), *Rickettsia felis* (N=2), *Bartonella quintana* (N=1), human herpesvirus 8 (N=1), and Saffold virus (N=1). Reads corresponding to *Plasmodium falciparum* were detected in 19 specimens, though their presence in the cohort was likely due to user error of rapid diagnostic testing or incorrect specimen segregation at the clinics. Mosquito-borne pathogens were typically detected just after the conclusion of the rainy season, while tick-borne pathogens were mostly detected before the rainy season. The three West Nile virus strains were phylogenetically characterized and shown to be related to both European and North American clades. Surveys such as this will increase the understanding of the potential causes of non-malarial AFI, which may help inform diagnostic and treatment options for clinicians who provide care to patients in Senegal.

6019

INVESTIGATION OF YELLOW FEVER VIRUS, VECTOR AND HOST NETWORK IN THE METROPOLITAN REGION OF MINAS GERAIS, BRAZIL IN 2023, INDICATES THE CONTINUED CIRCULATION OF YELLOW FEVER VIRUS

Matheus Soares Arruda[1], Daniel C. Jacob[1], Mikaelly F. Testa[1], Marcelle Alves de Oliveira[1], Ana Maria Paschoal[1], Gabriel Dias Moreira[1], Anna Catarina D.S. Guimarães[1], Natália Lima Pessoa[1], Gabriela Fernanda Garcia Oliveira[1], Thais Alkifeles Costa[1], Samantha S F M Viegas[1], Daniel A. Rocha Vilela[2], Marina do Vale Beirão[1], Kathy A. Hankey[3], Nikos Vasilakis[4], **Betânia Paiva Drumond**[1]

[1]Federal University of Minas Gerais, Belo Horizonte, Brazil, [2]IBAMA, Belo Horizonte, Brazil, [3]New Mexico State University, Las Cruces, NM, United States, [4]University of Texas - Medical Branch, Galveston, TX, United States

In 2016-18, Southeast Brazil experienced a huge yellow fever outbreak, notably in Minas Gerais state, the epicenter of the outbreak. Since then, yellow fever virus (YFV) has been detected in non-human primates (NHP) in the state, including the Metropolitan region of Belo Horizonte (BH). To investigate the virus-host-vector network that could sustain YFV maintenance and transmission, we have been performing NHP and mosquito surveillance in six urban parks of BH, since mid 2023. So far, a total of 2,548 mosquitoes have been collected in six urbans parks of BH. 45.64% of individuals have already been identified to genus level as *Aedes, Haemagogus, Sabethes, Culex, Limatus, Uranotaenia, and Psorophora.* Sera from 63 free-living black-tufted marmoset (*Callithrix penicillata)* captured or rescued in 2023, tested negative for YFV, ZIKV, CHIKV by RTqPCR, indicating that none of the animals were actively infected by these arboviruses. Additionally, 36 sera have been screened via plaque reduction neutralization test (PRNT) against YFV showing 20 positives (PRNT 50% at 1:20 dilution). These samples were from *C. penicillata (*3 infant (0-5 months of age), 6 young (6-17 months of age) and 11 adult (more than 18 months of age), sampled in the Metropolitan region of BH (n=19), and southwest Minas Gerais (n=1). Four out of 20 PRNT positive sera also tested positive for IgM anti-YFV (3 adults, 1 young). These four *Callithrix i*ndividuals were collected in urban areas from the Metropolitan region of BH, in 2023. Although it is known that IgM can persist after orthoflavirus infection, these NHP were sampled in 2023, more than 4 years since the end of yellow fever outbreaks in 2018, Minas Gerais. The detection of neutralizing and IgM against YFV in NHP (including young and adults sampled in 2023) reinforces the circulation of YFV in urban areas of Minas Gerais. Urbans parks in BH present the host-vector network: NHPs and sylvatic mosquitoes (*Haemagogus and Sabethes),* that could contribute to the maintenance and transmission of YFV. Continued surveillance and studies are vital to public health in Southern Brazil due to ongoing YFV circulation.

6020

DYNAMICS AND REGULATION OF SEXUAL COMMITMENT IN *PLASMODIUM FALCIPARUM*

Surendra K. Prajapati[1], Jeffrey X. Dong[1], Kim C. Williamson[2]

[1]Uniformed Services University of the Health Sciences and Henry M Jackson Foundation for the Advancement of Military Medicine, Inc., Bethesda, MD, United States, [2]Uniformed Services University of the Health Sciences, Bethesda, MD, United States

Human-to-human malaria transmission via mosquitoes requires the production of sexual stage parasites, known as gametocytes. In vitro, during each asexual cycle this important developmental transition takes place during schizogony in a sub population of blood stage parasites. We generated a series of transgenic reporter lines to assess the dynamics of sexual commitment from RBC invasion through schizogony to the next generation of parasites. The results reveal that translation of *gametocyte development 1* gene (*gdv1*) tagged with nanoluciferase is first detected

26-27 hpi and TdTomato-tagged GDV1 is visible before the first nuclear division (30-32 hpi) just prior to the initial detection of AP2 transcription factor, *ap2-g,* RNA (31-32 hpi). The initial increase in *ap2-g* transcription plateaus until 37-38 hpi before continuing to increase through schizogony. The marked increase in *ap2-g* transcript after 37-38 hpi fits well with the first detection of mScarlet-tagged AP2-G at 39-40 hpi and is consistent with AP2-G transcriptional autoregulation. Two hours later (41-42 hpi) the cells already positive for mScarlet AP2-G also begin to express mNeonGreen-tagged MSRP1. This dual AP2-G/MSRP1 positive schizont population continues to increase through schizogony indicating that it is only the mature schizont population that can be used to determine the sexual conversion rate. The regulatory roles of GDV1 and AP2-G were then investigated using GDV1 inducible lines containing nanoluciferase-tagged active or inactive *ap2-g* and demonstrated that the initial increase in *ap2-g* transcription requires GDV1 and is AP2-G independent, whereas the further increase after 38 hpi requires active AP2-G. Together the findings define the time course of sexual commitment through schizogony and the distinct roles of GDV1 and AP2-G. The transgenic reporter lines developed are also powerful tools to study the initial signaling events underlying sexual commitment and screen molecules with transmission-blocking potential. Disclaimer: The opinions expressed are those of the authors, not the affiliated Institutions.

6021

SPECIALIZED SPOROZOITE-TYPE RIBOSOMES IN *PLASMODIUM YOELII* DRIVE INITIAL RAPID ASEXUAL BLOOD STAGE GROWTH AND SEXUAL DEVELOPMENT

James P. McGee[1], Sylvie Briquet[2], Olivier Silvie[2], **Scott E. Lindner**[1]

[1]Pennsylvania State University, University Park, PA, United States, [2]Centre d'Immunologie et des Maladies Infectieuses, INSERM, CNRS, CIMI-Paris, Sorbonne Universite, Paris, France

Eukaryotes use ribosome specialization to promote specific translational outcomes in distinct cell types and stages of development. Specialization is often derived from rRNA sequence heterogeneity within the expansion segments (ESs) that emanate from the conserved core, as well as differential ribosome protein (RP) paralog expression and incorporation. However, it has been challenging to study individual ribosome types genetically because most eukaryotes encode hundreds of tandem copies of rRNA genes. Therefore, we have overcome this challenge by studying the rodent-infectious malaria parasite *Plasmodium yoelii*, a eukaryote with only four 18S/5.8S/28S rRNA genes on Chromosomes 5, 6, 7, and 12. These four rRNAs have different temporal expression patterns that enable their characterization as "Asexual" A-type rRNAs (Chr 7 and 12, with nearly identical sequences) and "Sporozoite" S-type (Chr 5 and 6) rRNAs that vary in sequence from the A-type and each other primarily at ESs. Previous genetic studies have only identified that S-type rRNAs impact oocyst growth and sporozoite development in mosquitoes. However, our phenotypic characterization of parasites lacking either or both S-type rRNA genes surprisingly revealed two biologically and statistically significant defects during the blood stage, when S-type rRNAs are only weakly expressed. Deletion of the Chr 5 rDNA locus led to the disruption of the initial rapid wave of asexual blood stage growth. In contrast, deletion of the Chr 6 rDNA locus decreased the production of mature male gametocytes at this same point. Moreover, the Chr 6 deletion phenotype dominated when both loci were deleted, thus restoring the initial wave of asexual growth. Based on these data and follow-on studies focused on identifying the contributing components of these rRNAs, we propose a model in which S-type ribosomes provide specialized functions, with one S-type ribosome (Chr 5) driving asexual blood stage growth and acting to dampen the promotion of gametocytogenesis by the other (Chr 6).

6022

IDENTIFICATION OF NOVEL ANTI-GAMETOCYTE TRANSMISSION BLOCKING VACCINE TARGETS

Fiona Angrisano[1], Hayley Bullen[1], Amelia Ford[2], Katarzyna Sala[3], Andrew Blagborough[2]

[1]Burnet Institute, Melbourne, Australia, [2]Cambridge University, Cambridge, United Kingdom, [3]Imperial College, london, United Kingdom

Inhibiting transmission of *Plasmodium* is a central strategy in malaria eradication, and the biological process of gamete fusion during fertilisation is a proven target for this approach. The lack of knowledge of the mechanisms underlying fertilisation have been a hindrance in the development of transmission blocking interventions. Here we describe a protein disulphide isomerase essential for malarial transmission (*PDI Trans* PBANKA 0820300) to the mosquito. We show that *PDI Trans* activity is male specific, surface expressed, essential for fertilisation and transmission, and exhibits disulphide isomerase function which is up regulated post gamete activation. We demonstrate that *PDI Trans* is a viable anti malarial drug and vaccine target blocking malarial transmission with the use of repurposed the PDI inhibitors and anti *PDI Trans* peptide antibodies. These results reveal that protein disulphide isomerase function is essential for malarial transmission and emphasise the potential of anti PDI agents to act as an anti malarial, facilitating the development of novel transmission blocking interventions.

6023

LOSS OF FUNCTION OF THE *PLASMODIUM FALCIPARUM* PROLINE TRANSPORTER *PF*APIAT2 MEDIATES HALOFUGINONE RESISTANCE BUT RESULTS IN OOCYST DEVELOPMENTAL DYSFUNCTION

Malhar Khushu[1], Lola Fagbami[2], Alexandra Probst[1], Tasneem Rinvee[1], Robert Summers[1], Amanda K. Lukens[3], Flaminia Catteruccia[4], Dyann Wirth[3], Selina Bopp[1]

[1]Harvard T. H. Chan School of Public Health, Boston, MA, United States, [2]Harvard T. H. Chan School of Public Health/Metabolomics Platform, Broad Institute, Boston/Cambridge, MA, United States, [3]Harvard T. H. Chan School of Public Health/Infectious Disease and Microbiome Program, Broad Institute, Boston, MA, United States, [4]Harvard T. H. Chan School of Public Health/Howard Hughes Medical Institute, Boston, MA, United States

Resistance to halofuginone (HFG), a potent antimalarial targeting the cytoplasmic proline-tRNA synthase of the human malaria parasite *Plasmodium falciparum*, can be mediated through loss-of-function (LOF) mutations in the Apicomplexan Amino acid Transporter 2 gene (*pfapiat2)*. During the asexual stage, *Pf*ApiAT2-LOF results in accumulation of cytoplasmic proline which competes with HFG for target site binding. Previous work in the *Plasmodium berghei/Anopheles stephensi* mouse malaria system pointed to an essential role of *Pb*ApiAT2 in oocysts, but there is nothing known of its role in the transmission stages of *P. falciparum*. To understand the role of *Pf*ApiAT2 during mosquito stages, we generated HFG-resistant parasites in the transmissible NF54 line. These *Pf*ApiAT2-HFGR parasites were 10-fold more resistant than the parental line to HFG. Sequencing of the *pfapiat2* locus revealed two independent non-synonymous mutations (G449R and R345I) in these lines. Both lines were fed to female *Anopheles gambiae* mosquitoes, and we observed no defect in early stages of parasite development in the mosquito midgut but saw strongly reduced growth during the oocyst stage. Preliminary analysis showed that *Pf*ApiAT2-HFGR oocysts were five-fold smaller than wild-type oocysts on day 7 and could not complete proper development to sporozoites by day 14 and day 21. To determine localization of *Pf*ApiAT2 in the mosquito stages, we generated an NF54 *Pf*ApiAT2-HA line. Pilot immunofluorescence assay microscopy suggests that *Pf*ApiAT2 localizes at peripheral membranes of developing oocysts, colocalizing with *Pf*s25, a known plasma membrane marker. We are further validating the essentiality of *Pf*ApiAT2 in oocyst development through genetic knockouts. Our data

suggests a crucial role of *Pf*ApiAT2 during oocyst development, and we are characterizing proline transport activity of *Pf*ApiAT2 through heterologous expression in a *Xenopus laevis* oocyte system.

6024

PARTIAL CLEARANCE OF PRE-ESTABLISHED *PLASMODIUM FALCIPARUM* INFECTION IN MOSQUITOES BY MIMICKING A BLOODMEAL ON TREATED PATIENTS WITH ARTEMETHER+LUMEFANTRINE + ATOVAQUONE-PROGUANIL

Priscilla Adjei-Kusi[1], Serge Rakiswendé Yerbanga[2], Thierry Lefèvre[3], Esi Bart-Plange[1], Esther Naadu Placca[1], Melvin Agbogbatey[1], John Asiedu Larbi[4], John Amuasi[1], Jerome Clain[5], Oumou Maiga Ascofaré[6], Anna Cohuet[3], ASAAP Consortium --[7]

[1]Kumasi Centre for Collaborative Research in Tropical Medicine (KCCR), Kumasi, Ghana, [2]Institut des Sciences et Techniques (INSTech - BOBO), Bobo-Dioulasso, Burkina Faso, [3]MIVEGEC, Université de Montpellier, IRD, CNRS, Montpellier, France, [4]Department of Theoretical and Applied Biology, College of Science, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana, [5]Université de Paris Cité, UMR 261 MERIT, Paris, France, [6]Department of Infectious Disease Epidemiology, Bernhard Nocht Institute for Tropical Medicine (BNITM), Hamburg, Germany, [7]--, --, VA, United States

In addition to curative treatment against the pathogenic asexual stage of the malaria parasite, targeting the transmissible sexual stage is essential to impede the spread of drug resistance. The present study relies on a clinical trial testing the benefits of combining Atovaquone Proguanil (AP) with Artemether-lumefantrine (AL) in the treatment of uncomplicated malaria. AP has been shown to prevent transmission post-treatment by affecting parasite development in mosquito vectors in mice models. Atovaquone exhibits further properties by persisting in the blood of treated patients for days, possibly hindering parasite development during ookinete to oocyst and oocyst to sporozoite transition. We aim to assess the parasite clearance properties of plasma from AL *versus* AL+AP treated patients in mosquitoes with pre-established *Plasmodium falciparum* infection. Eight time points (Day 0 to 28) plasma from 17 patients treated with either AL+placebo or AL+AP was collected for mosquito feeding. Infectious blood meal was provided to laboratory reared female *Anopheles* mosquitoes and infected mosquitoes were exposed to a second blood meal containing the plasma from treated patients. A total of 9,259 mosquitoes were dissected, 4,850 at 7 and 4,409 at 14 days post-infection (dpi). Infection rate and intensity in the control groups range from 20% to 89.19% and 8.75 to 71.96 respectively at 7 dpi. Our preliminary analysis demonstrates that in *P. falciparum* infected mosquitoes, a blood meal with plasma from AL+AP treated patients significantly inhibits parasite development with a reduction in the prevalence of infected mosquitoes and infection intensity and slower sporogonic development, whereas plasma from patients treated with AL alone had no effect. The AL+AP effect however decreases after 5 days post-treatment. Further analysis including correlation with drug dosage, mosquito survival and quantification of sporozoites in the salivary glands is ongoing. These results highlight a benefit of triple ACT containing AP as when treated patients are bitten by infected mosquitoes, they may be partially “cured” of the infection and therefore reduce transmission.

6025

UNRAVELING THE JOURNEY OF *PLASMODIUM FALCIPARUM* PARASITES INSIDE THEIR MOSQUITO VECTOR AT THE SINGLE CELL RESOLUTION

Yan Yan[1], Elaine Cheung[1], **Lisa H. Verzier**[1], Duo Peng[2], Federico Appetecchia[1], Tasneem A. Rinvee[1], Esrah Du[1], Alexandra S. Probst[1], Naresh Singh[1], W. Robert Shaw[3], Flaminia Catteruccia[3]

[1]Harvard TH Chan School of Public Health, Boston, MA, United States, [2]The Chan Zuckerberg Biohub, San Francisco, CA, United States, [3]Harvard TH Chan School of Public Health & Howard Hughes Medical Institute, Boston, MA, United States

Plasmodium parasites, often incorrectly referred to as obligate intracellular parasites, replicate exclusively within host cells in humans. Their cycle within the *Anopheles* mosquito vector, however, takes a markedly different path. Following the mosquito’s ingestion of an infected blood meal, newly formed zygotes evolve into ookinetes that traverse the midgut epithelium and transform into oocysts underneath the basal lamina. This extracellular replicative stage develops over more than a week, maturing into thousands of sporozoites—the form able to infect humans. The processes mediating ookinete traversal and oocyst growth remain largely unknown. We employed single cell RNA sequencing of both parasite and mosquito cells across four critical timepoints for parasite survival and growth to generate a new parasite single cell atlas spanning from invading ookinetes to segmenting oocysts. We found an unexpected preferential invasion route followed by ookinetes during epithelial cell traversal, which is currently being validated by genetic and microscopy means. Moreover, we detected and validated processes that are essential for oocyst growth and sporozoite segmentation, identifying previously unknown players and mechanisms. Our study enhances our understanding of the host-pathogen dynamics during the mosquito development phase and opens avenues for the identification of novel targets for mosquito-specific, transmission-blocking strategies.

6026

DEFINING TRANSCRIPTIONAL SIGNATURES OF *PLASMODIUM FALCIPARUM* HEMATOPOIETIC INFECTION AT THE SINGLE CELL LEVEL

Barbara Stokes[1], Fiona Achcar[1], Charles Ndovi[2], James Nyirenda[2], Priscilla Ngotho[1], Edward Agboraw[1], Thomas Otto[1], Christopher Moxon[2], Matthias Marti[1]

[1]University of Glasgow, Glasgow, United Kingdom, [2]Malawi Liverpool Wellcome Clinical Research Programme, Blantyre, Malawi

During blood stage malaria infection, most parasites undergo asexual reproduction, exponentially increasing the parasite load and leading to clinical manifestations of the disease. However, a small subset of asexually replicating blood-stage parasites differentiate to become transmission-competent gametocytes in a process known as sexual stage conversion. Recent work has established that the hematopoietic niche of the bone marrow is an essential site for gametocyte development, in addition to being an under-appreciated reservoir of asexual parasites. Whether the bone marrow microenvironment specifically promotes commitment to the sexual development trajectory in addition to serving as the major site for gametocyte maturation is not known. Several factors have been implicated as potential mediators of sexual commitment within the bone marrow, including a local depletion of the host phospholipid lysophosphatidylcholine (LysoPC), levels of which have been shown to regulate stage conversion in *P. falciparum in vitro*, as well as an enrichment of immature red blood cells (reticulocytes), which have been shown to promote gametocytogenesis in *P. berghei*. Nonetheless, the mechanisms whereby these and other features of the bone marrow microenvironment might contribute to parasite development and differentiation remain poorly understood. Here, we apply single-cell RNA sequencing to study *P. falciparum* development within the hematopoietic niche using complementary *in vitro* and *ex vivo* approaches. For our *in vitro* studies, we compare parasites cultured in primary human bone marrow cells to parasites cultured in peripheral blood cells, focusing on pathways relating to sexual commitment. For our *in vitro* studies, we

compare orthopaedic samples from naturally infected donors in Malawi to donor-matched peripheral blood samples. Results suggest that a combination of host cell intrinsic and extrinsic factors contribute to parasite development and differentiation within the hematopoietic niche and provide insight into mechanisms whereby parasites can regulate the balance within-host growth and between-host transmission.

6027

ENHANCING MALARIA DATA QUALITY IN BENIN: IMPACT OF MONTHLY DATA VALIDATION AND DEATH DATA AUDIT

Julien Aissan[1], Cyriaque Affoukou[1], Achille Batonon[1], Raoul Oloukoi[2], Virgile Gnanguenon[2], Pascal Zinzindohoue[2], Achille Couao-Zotti[1], Christian Godjo[1], Koffi Emmanuel Yovo[3], Michael Humes[4], Ashley Garley[4], John Bernon[4]

[1]Benin National Malaria Control Program, Cotonou, Benin, [2]U.S. President's Malaria Initiative, USAID, Cotonou, Benin, [3]Clinton Health Access Initiative (CHAI), Cotonou, Benin, [4]U.S. President's Malaria Initiative, USAID, Washington, DC, United States

In Benin, malaria incidence and mortality rates stagnated from 2020 to 2022 despite increased prevention efforts. Concerns about the accuracy of rapid diagnostic test (RDT) results reporting, overall data quality, and a need for strengthened governance and leadership at regional and district levels led to guidance and procedural revisions. In 2023, the Benin malaria control program introduced new guidelines for malaria data validation meetings and death audits. Data review meetings went from quarterly at regional levels to monthly at district levels and emphasized data accuracy, investigating discrepancies, and peer-to-peer cross-checking of patient registers including RDT cassette verification. Benin found a decline in malaria incidence from 221 per 1,000 population in 2022 to 168 in 2023, with regional reductions ranging from 1% to 38%. In 2023, as compared with 2022, suspected malaria cases remained at 4.0M and patients tested by RDT increased by 3% from 3.4M to 3.5M while confirmed cases decreased from 2.7M to 2.1M (-21%), and ACT prescriptions from 2.2M to 1.6M (-24%), and RDT test positivity decreased by 25% from 66% to 51%. Reductions in confirmed cases appear to be related to improved oversight in RDT result reporting. Death audits led to reductions in seven of the 10 hospitals reporting 66% of malaria-related deaths, driving reductions in malaria mortality from 25.4 to 24.2 per 100,000 inhabitants. Like many malaria-endemic countries, malaria in Benin is often seen as a syndrome that can lead to reporting non-malaria fevers as malaria. Increased focus on quality assured RDT result reporting and malaria death definition and verification led to more accurate estimates of malaria cases and deaths and ACT consumption. This allows for improved impact and trend monitoring, and targeting of limited resources

6028

INTEGRATED COMMUNITY CASE MANAGEMENT (ICCM) COMMUNITY HEALTH WORKERS AND THEIR IMPACT ON SEVERE MALARIA AND MALARIA MORTALITY IN LUAPULA PROVINCE, ZAMBIA'S HIGHEST MALARIA BURDEN PROVINCE, 2016-2023

Melody N. Simataa[1], Webby Phiri[1], Ellen Ferris[2], Sarah Shankwaya[1], Mulakwa Kamuliwo[3], Chabu Kangale[1], Bupe M. Kabamba[1], Marie-Reine I. Rutagwera[1], Caroline Phiri-Chibawe[1], Jennifer Somtore[4], Busiku Hamainza[5]

[1]PATH PAMO Plus, Lusaka, Zambia, [2]PATH, Seattle, WA, United States, [3]Jhpiego, Lusaka, Zambia, [4]U.S. President's Malaria Initiative, Lusaka, Zambia, [5]Zambia Ministry of Health National Malaria Elimination Centre, Lusaka, Zambia

Luapula Province has Zambia's highest malaria burden at 63.3% parasite prevalence in children under age 5. Since deployment of integrated community case management (iCCM) in 2018, 2,115 community health workers (CHWs) have been trained and deployed in 9 of the 12 districts using a ratio of 1 CHW per 500 population. iCCM provides accessible testing and treatment of uncomplicated malaria and pre-referral treatment of severe malaria for children ages 2 months to 6 years. Over an 8-year period from 2016 to 2023, an analysis was conducted on health management information systems and malaria rapid reporting data. Negative binomial regression models were developed for 4 outcomes to assess the impact of iCCM in the 9 identified districts. ITNs, IRS, rainfall, temperature, and vegetation covariates were included in the models to reduce potential confounding over the years. From 2016 to 2023, there were 99,449 severe malaria admissions and 2,553 malaria deaths for all ages. On average, in 2023, CHWs detected 40.3% of total confirmed malaria cases in Luapula, compared to 0% in 2016. CHW activity declined in 2020-2021 coinciding with the COVID-19 pandemic, which disrupted health systems and affected commodity supply, and recovered in 2022-2023 with improved commodity availability. The analysis showed that each 10% increase in the proportion of cases detected by CHWs was associated with a 7% decrease in severe malaria in all ages, an 8% decrease in severe malaria for all children under age 5, a 9% decrease in deaths in all ages, and a 9% decrease in deaths in all children under age 5. More cases detected in the community means quicker access to care and fewer cases progressing to severe disease. The National Malaria Elimination Program can use these data to advocate for continued scale-up of iCCM in high malaria burden areas.

6029

ANTENATAL CARE SURVEILLANCE FOR MONITORING PREVALENCE AND COVERAGE OF INSECTICIDE-TREATED NETS - A MULTI-COUNTRY ANALYSIS

Anna Munsey[1], Patrick G. T. Walker[2], Peder Digre[3], Joseph Wagman[4], Molly Robertson[5], Joseph Hicks[2], Manzidatou Alao[6], Aurore Hounto[7], Adama Gansane[8], Siaka Debe[8], Baltazar Candrinho[9], Perpetua Uhomoibhi[10], Okefu Oyale Okoko[10], Ruth Lemwayi[11], Sijenunu Aaron[12], Chabu Kangale[13], Bupe Kabamba[13], Julie R. Gutman[1], ANC Surveillance Working Group[1]

[1]CDC, Atlanta, GA, United States, [2]MRC Centre for Global Infectious Disease Analysis, School of Public Health, Imperial College, London, United Kingdom, [3]PATH, Seattle, WA, United States, [4]PATH, Washington, DC, United States, [5]The Global Fund to Fight AIDS, Tuberculosis, and Malaria, Geneva, Switzerland, [6]U.S. Presidents' Malaria Initiative Impact Malaria Project, Medical Care Development Global Health, Cotonou, Benin, [7]Unité de Parasitologie/Faculté des Sciences de la Santé, Université d'Abomey, Calavi, Cotonou, Benin, [8]Centre National de Recherche et Formation sur le Paludisme, Ouagadougou, Burkina Faso, [9]National Malaria Control Program, Ministry of Health, Maputo, Mozambique, [10]National Malaria Elimination Programme, Abuja, Nigeria, [11]Jhpiego, Dar es Salaam, Tanzania, United Republic of, [12]National Malaria Control Program, Dodoma, Tanzania, United Republic of, [13]PATH, Lusaka, Zambia

Estimates of malaria prevalence and intervention coverage in Africa are primarily based on nationally representative household (HH) surveys. However, their expense and infrequency limit their utility for operational action by malaria programs. We assessed whether data collected during the first antenatal care (ANC1) visit, consisting of data on malaria prevalence using rapid diagnostic tests and ownership of insecticide-treated nets (ITNs), could provide relevant data to guide decision-makers. Malaria prevalence among ANC1 attendees in select areas of six countries (Benin, Burkina Faso, Mozambique, Nigeria, Tanzania, and Zambia) were compared to prevalence data among children under five (u5) from cross-sectional HH surveys in the same areas. To examine the relationship between prevalence among ANC1 attendees and u5 prevalence we fitted a linear trend to the log-odds ratio of the risk of testing positive. The predictive performance of the model was assessed by leave-one-out cross-validation (LOOCV). District-level ANC1 prevalence and u5 prevalence have a linear relationship (Pearson correlation coefficient, r = 0.80, 95% confidence interval, CI = 0.66-0.88, $p < 0.001$) and ANC1 prevalence is predictive of prevalence among u5s (LOOCV mean absolute error = 6.2%). To understand whether data on ITN ownership collected at ANC1 are representative of that collected in HH surveys, we assessed the district-level proportion of household ownership in five countries (Benin, Burkina Faso, Mozambique, Nigeria, and Zambia). ITN ownership at the level of one ITN per HH as assessed by ANC1 questionnaires was strongly correlated with ITN ownership at the level of one ITN per HH in HH questionnaires (r = 0.80, 95% CI = 0.63-0.90, $p < 0.001$). There was moderate correlation in ITN

ownership at the level of one ITN per two persons (r = 0.64, 95% CI = 0.38-0.81, p < 0.001). Estimates of malaria prevalence and ITN coverage derived from ANC1 attendees correlate with estimates derived from HH surveys and may be useful in monitoring malaria prevalence and prevention efforts.

6030

MALARIA SURVEILLANCE TO PREVENT THE RE-ESTABLISHMENT OF MALARIA IN MOBILITY DYNAMIC SETTING OF RAMREE TOWNSHIP IN MYANMAR

Khin Mon Mon[1], Wah Wah Thaw[1], Hnin Su Su Khin[1], Sway Min Htet[1], Nu Nu Khin[2], Gunawardena Dissanayake[3], Ersin Topcuoglu[4], Kyaw Myint Tun[1], Danielle Awabdeh[4]

[1]PMI Eliminate Malaria, University Research Co., LLC, Yangon, Myanmar, [2]U.S. President's Malaria Initiative, USAID, Yangon, Myanmar, [3]U.S. President's Malaria Initiative, USAID, Bangkok, Thailand, [4]PMI Eliminate Malaria, University Research Co., LLC, Chevy Chase, MD, United States

The President's Malaria Initiative Eliminate Malaria (PMI-EM) Activity in Myanmar provides technical and operational assistance to implement case-based and community-based surveillance in malaria-elimination townships. The receptivity and vulnerability assessments were conducted from May to July 2023 using qualitative methods, applying participatory mapping, focus group discussions, and reviewing the program data to prevent malaria re-establishment in Ramree Township. Regarding receptivity of 228 villages, 11 at the forest fringes were high, 193 along paddy fields/coastal areas were moderate, and the remaining 24 in urban plains were low. Regarding vulnerability, 11 villages were high, and 122 were moderate based on the influx of migrants and visits to forested worksites related to logging, tobacco plantation, fishing, and oil exploration. The indigenous transmission was interrupted in 2021 after five malaria cases were detected in one village in 2020. Five malaria cases were detected but maintained zero active focus in 2022. In 2023, 17 cases were detected and classified as 11 imported (65%), two relapses (12%), and three indigenous/one introduced (23%). Integrated Community Malaria Volunteers (ICMVs) were deployed in 162 villages (71%), and the remaining 66 villages (29%) were covered by facility-based surveillance of basic health staff. In 2023, 11,212 RDTs were performed for the township population 96,957, and the township's annual blood examination rate (ABER) was 11.6%. Community surveillance through ICMVs contributed to 67% of RDTs performed, 71% of cases detected, and 100% case-based surveillance in 2023. Only two cases were detected in an area with high receptivity, and no cases were detected in areas with high vulnerability in 2023. After the coup in February 2021, there was a resurgence of malaria nationally, and there was an increase of 240% in malaria cases from 5 in 2020 to 17 in 2023 in Ramree. It was unpredictable where the cases would be caught. To prevent the re-establishment of malaria in dynamic and conflict-affected mobility areas like Ramree, community surveillance through ICMVs should be sustained.

6031

DATA INTEGRATION FOR DECISION-MAKING: A MALARIA DATA DASHBOARD THAT MERGES ROUTINE SURVEILLANCE AND GENOMIC RESEARCH DATA WITH MODELED OUTPUTS FOR PROGRAMMATIC ACTION IN SENEGAL

Katherine E. Battle[1], Médoune NDiop[2], David Kong[1], Joshua L. Proctor[1], Emily K. Driano[1], Caitlin A. Bever[1], Punam Amratia[3], Mouhamad Sy[4], Bassirou Ngom[4], Ibrahima Diallo[2], Doudou Sene[2], Dyann F. Wirth[5], Sarah K. Volkman[5], Daouda Ndiaye[4]

[1]Bill & Melinda Gates Foundation, Seattle, WA, United States, [2]Programme National de Lutte contre le Paludisme, Dakar, Senegal, [3]Malaria Atlas Project, Dar es Salaam, Tanzania, United Republic of, [4]Centre International de recherche, de formation en Genomique Appliquee et de Surveillance Sanitaire, Dakar, Senegal, [5]Harvard T. H. Chan School of Public Health, Boston, MA, United States

The National Malaria Control Program (NMCP) in Senegal has been working in collaboration with research groups in Senegal and around the world to leverage genomic surveillance and modeled data for operational decision-making. To bring together the NMCP epidemiological data with novel molecular and modeled data, a data dashboard was conceived from a research consortium led by Le Centre International de recherche et de formation en Génomique Appliquée et de Surveillance Sanitaire (CIGASS; Dakar, SN) with direction from the NMCP. Software development and design was led by the Institute for Disease Modeling (IDM; Bill & Melinda Gates Foundation, Seattle, USA). NMCP routine surveillance data were used by the Malaria Atlas Project (MAP; Perth, AU and Dar es Salaam, TZ) to generate a Senegal-specific geospatial model of malaria incidence that accounts for data challenges such reporting completeness and access to care. Molecular surveillance data, including molecular markers for drug resistance and parasite population relatedness statistics, were generated by CIGASS in partnership with the Harvard T.H. Chan School of Public Health and The Broad Institute (Boston, USA). With the permission and partnership of the NMCP, all data were added to the digital dashboard platform. The resulting dashboard allows the NMCP routine data to be compared side by side with modeled outputs from MAP. These could each be overlaid with genomic summary statistics. Maps and time series plots allow data comparisons to be made across both space and time. The ability to visualize changes in parasite populations and drug resistance markers over space and time, referenced to programmatic data, is an important step towards making novel molecular surveillance data operationally relevant. The Senegal data dashboard highlights the potential breadth of data that NMCPs can add to their toolkits, and also the quality of outputs made possible through research and programmatic collaboration.

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FORECASTING GLOBAL NEED AND DEMAND FOR CRITICAL MALARIA COMMODITIES TO ANTICIPATE POTENTIAL MARKET DISRUPTIONS

Jessica Floyd[1], Monica Golumbeanu[2], Tasmin Symons[3], Anna Trett[1], Punam Amratia[3], Graziella Scudu[1], Ioana Ursu[4], Salome Muchiri[1], Oliver J. Watson[5], Abigail Ward[1], Tara Seethaler[1], Peter W. Gething[3], Emilie Pothin[2], Aaron Woolsey[1]

[1]Clinton Health Access Initiative, Boston, MA, United States, [2]Swiss Tropical and Public Health Institute, Allschwil, Switzerland, [3]Telethon Kids Institute, Perth, Australia, [4]Innovative Vector Control Consortium, Liverpool, United Kingdom, [5]Imperial College, London, United Kingdom

Challenges for malaria elimination such as increasing populations, emerging treatment and insecticide resistance, the spread of HRP2 gene deletions and global budgetary constraints are driving the need to be more selective with how malaria commodities are chosen and distributed. The Malaria Commodities Forecasting Project produces comprehensive forecasts to generate consensus views on market trends, highlight gaps in demand for essential commodities and identify supply risks. We use statistical modeling to predict future trends in commodity needs from historical data, dynamic modeling to explore the impact of future interventions, and scenario analysis of the impact of factors such as policy, pricing, and global budgets. In 2023 the project published its second long-term forecast of global need and demand for insecticide-treated nets (ITNs), insecticides for indoor residual spraying (IRS), rapid diagnostic tests (RDTs), and antimalarial treatments. Under the baseline scenario, global ITN demand is expected to grow from 227 million in 2023 to 309 million ITNs in 2032, with an estimated 46% of this demand being for newer (dual active ingredient) ITNs. The global public sector demand for ACTs is also projected to increase, from 365 million in 2026 to 394 million treatments in 2032. We identify high levels of incorrect treatment in sub-Saharan Africa, with a predicted 438 million antimalarials used to treat non-malarial fevers in 2032. The need for non-HRP2 RDTs could rise to 56% of the African market by 2032 according to modelled HRP2 deletion spread. Demand for SP+AQ, used for seasonal malaria chemoprevention, is expected to increase from 225 million in 2026 to 249 million treatments in 2032, far below the projected need. These forecasts play a crucial role in identifying areas with insufficient funding and coverage, predicting commodity demand under various scenarios and uptake of new tools to fight malaria. Publicly accessible and updated annually, they provide

policymakers, donors, manufacturers and the broader global malaria community with a platform to anticipate market risks and disruptions in the ongoing efforts to fight malaria.

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TEMPORAL TRANSCRIPTOMICS UNRAVEL MOLECULAR SIGNATURES OF SEVERE COVID-19

Clinton Onyango[1], Ivy Hurwitz[2], Qiuying Cheng[2], Kristan Schneider[2], Douglas J. Perkins[3]

[1]Maseno University, Maseno, Kenya, [2]University of New Mexico Health Sciences Center, Albuquerque, NM, United States, [3]University of New Mexico Health Sciences Center, Alubquerque, NM, United States

Hospitalized COVID-19 patients often experience severe symptoms, with some progressing to respiratory failure necessitating prolonged ICU care. To better understand the underlying molecular characteristics of severe COVID-19 pathogenesis, transcriptomics by next-generation sequencing was performed on a matched subset (n=36) of temporally (Days 0,1, 2, 3, and 6 following enrollment) collected RNA from peripheral blood of COVID-19 patients with severe disease (n=21), defined as admission to the ICU and/or death, or those who did not require ICU support (non-severe, n=15). Differential gene expression analysis between severe and non-severe patients revealed 3690, 2605, 4289, 3879, and 3454 differentially expressed genes (DEGs, $P<0.05$) at D0, D1, D2, D3, and D6 respectively. Enrichment analysis using Metacore™ revealed significant enhancement of the COVID-19: Immune Dysregulation (FDR=4.54e-7), COVID-19 Associated Coagulopathy (FDR=9.20e-2), and COVID-19: SARS-CoV-2 Entry into Target Cells (FDR=8.22e-2) pathways. MHC class I, MHC class II, and CD4 were downregulated, while GLUT1, PKM2, and GATA were upregulated across all time points in the Immune Dysregulation pathway, indicating impaired antigen presentation, reduction in functional CD4 T cells and altered metabolic state. In addition, the upregulation of P2Y1, CD147, P-selectin, von Willebrand factor, and GP-IB alpha genes were identified in the COVID-19 Associated Coagulopathy pathway, suggesting platelet activation, adhesion, and inflammation dysregulation, which could contribute to COVID-19 severity. Syndecan-4 and CD147 were also upregulated across time in the COVID-19: SARS-CoV-2 Entry into Target Cells pathway. CD147 upregulation may enhance viral entry into host cells, while Syndecan-4 may contribute to the dysregulation of endothelial cells. Collectively, these findings underscore the complex interplay between immune dysregulation, coagulopathy, and viral pathogenesis in severe COVID-19 cases in a diverse population of patients, offering insights for potential therapeutic interventions.

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EVALUATING THE BURDEN OF RESPIRATORY TRACT INFECTIONS IN DECEASED IN KARACHI, PAKISTAN: A POST-PANDEMIC MORTALITY SURVEILLANCE ANALYSIS

Furqan Kabir[1], Raheel Allana[1], Sameer Belgaumi[2], Chrisitina Arif[1], Saima Jamal[1], Sehrish Amir Ali[1], Saad B Omer[2], Abdul Momin Kazi[1]

[1]Aga Khan University, Hospital, Karachi, Pakistan, [2]UT Southwestern Medical Center, Dallas, TX, United States

Respiratory tract infections (RTIs) are of critical concern in the wake of the COVID-19 pandemic, necessitating extensive examination of their impact on mortality rates. We assessed the prevalence of COVID-19 and other RTIs among deceased individuals within peri-urban areas in Karachi, Pakistan in the post-pandemic period. We also aimed to assess the efficacy of nasal swabs stored in dry tubes (dry nasal swabs) compared to standard nasal swabs stored in transfer medium (wet nasal swabs) for RT-PCR COVID-19 detection. Samples from the recently deceased were collected between September 2022 and October 2023 using both wet and dry swabs. 350 samples underwent PCR testing for common respiratory pathogens and genomic sequencing for variant identification. 6% (21/350) of cases tested positive for COVID-19 on either wet or dry swabs. 57% of positive cases occurred in those aged 60 or higher. A majority of COVID-19 positive deaths (47.6%) occurred at home. Seasonality revealed spikes in positive cases in October (5/20) and June (5/33). 22F omicron was the most prevalent strain. 19 COVID-19 positive cases had co-infection with another organism with *K. pneumonia* found in 62% of these cases. Among all cases, *K. pneumonia* and *S. aureus* were the two most common organisms detected. *K. pneumonia* had a slightly higher prevalence in home deaths (43%) and *S.* aureus (42%) in hospital deaths. *K. pneumonia complex* was most common in children under five years with *S. aureus* most common in all other age groups. Dry nasal swabs detected more positive COVID-19 cases (19/21) than standard wet swabs (16/21) and had a negative predictive value of 99.3% compared to 98.5% in wet swabs. In the post-pandemic period, there remains a higher-than-expected prevalence of COVID-19 in our target population. Dry nasal swabs storage showed slightly better efficacy than standard transfer media storage for collecting/preserving COVID-19 genetic material for RT-PCR testing. Furthermore, variations in respiratory pathogen predominance showcases age-specific mortality trends and the pivotal role of healthcare setting, urging targeted interventions and improved end-of-life care.

6035

BIOMARKERS FOR PROGNOSTIC PREDICTION OF CHILDHOOD CLINICAL PNEUMONIA IN SUB-SAHARAN AFRICA

Isabelle Silber[1], Yasir Shitu Isa[2], Yekini Ajauoi Olatunji[2], Rasheed Salaudeen[2], Sarwar Golam[2], Galega Lobga[2], Megan Carelus[1], Patricia Hibberd[1], Edward F. Knol[3], Grant Mackenzie[4], Clarissa Valim[1]

[1]Boston University School of Public Health, Boston, MA, United States, [2]MRC Unit, The Gambia at the London School of Hygiene & Tropical Medicine, Fajara, Gambia, [3]Center of Translational Immunology and Department of Dermatology/Allergology, University Medical Center Utrecht, Utrecht, Netherlands, [4]Department of Disease Control, Faculty of Infectious and Tropical Diseases, London School of Hygiene & Tropical Medicine, London, United Kingdom

Pneumonia is a leading cause of pediatric hospitalization and mortality. Prompt diagnosis and prognosis in patients with clinical pneumonia would optimize prioritization of patient care, particularly in resource limited settings. Current studies have highlighted procalcitonin and C-Reactive Protein as markers to prognosticate disease in patients with pneumonia or sepsis, but these markers have only moderate accuracy (80% sensitivity, 50% specificity). An improved signature of markers is needed to increase accurate classification of clinical pneumonia prognosis. We studied 140 patients in 2 hospitals in rural Gambia, aged 2-59 months who sought care for symptoms of clinical pneumonia and followed-up for five days during admission, at discharge, and 30 days after admission with a phone call. Poor-moderate prognosis (n=82) was defined as either readmission, over 3 days of hospitalization, diminished feeding ability, or death. Good prognosis (n=58) was defined as discharged well, had a hospital duration under 3 days, and did not re-seek care. 45 inflammatory proteins were quantified in plasma at admission through Luminex immunoassay. We sought a combination of these proteins (biomarker signatures) that allowed for distinction between children with poor-moderate vs. good prognosis through classification trees. 12 proteins showed statistically significantly ($P < 0.05$) differences between children with poor-moderate prognoses and good prognoses: IL18, SCF, IL1R2, TNFR2, YKL40, Resistin, TIMP1, IL6, IL8, SCF, sIL2R, and PAI. A biomarker signature of TIMP1, TNFa, IL1R2, sIL6R, and PARC had high (91%) sensitivity and moderate (60%) specificity to discriminate between those with good and moderate/prognosis. Markers in this signature could be incorporated in a future a point-of-care test to identify children with clinical pneumonia and poor-moderate prognosis to support decisions about care.

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THE EFFECT OF AZITHROMYCIN ON *STREPTOCOCCUS PNEUMONIAE* CARRIAGE AMONG KENYAN CHILDREN DISCHARGED FROM THE HOSPITAL

Tanya E. Libby[1], Angela Karani[2], J. Anthony G. Scott[2], Donald Akech[2], Benson Singa[3], Kirkby Tickell[1], Doreen Rwigi[3], Kevin Kariuki[3], Nancy Onamu[3], Derrick Ounga[3], James A. Berkley[2], Judd L. Walson[4], Patricia B. Pavlinac[1]

[1]University of Washington, Seattle, WA, United States, [2]Kenya Medical Research Institute-Wellcome Trust Research Programme, Kilifi, Kenya, [3]Kenya Medical Research Institute, Nairobi, Kenya, [4]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States

More than 2.8 million deaths occur each year among children under five years in sub-Saharan Africa, primarily due to preventable infectious conditions, most commonly pneumonia. Azithromycin reduces child mortality in some settings and may work through reductions in the nasopharyngeal carriage of *Streptococcus pneumoniae*. Data are lacking on the impact of such interventions on *S. pneumoniae* carriage over time. We analyzed data from a double-blind, randomized placebo-controlled trial which followed 1,400 hospitalized Kenyan children to evaluate the impact of a 5-day course of azithromycin prescribed at discharge on mortality and re-hospitalization (published elsewhere) and carriage of *S. pneumoniae* at 3- and 6-months post-discharge. Randomization to azithromycin or placebo arm (1:1) was stratified by enrollment site (Kisii or Homa Bay counties in western Kenya). We calculated prevalence ratios (PRs) for *S. pneumoniae* carriage in the two arms at 3- and 6-months post-discharge using generalized estimating equations (GEE) with a Poisson link and exchangeable correlation structure, adjusting for enrollment site. We assessed effect modification by *S. pneumoniae* status at discharge using the likelihood ratio test. Overall prevalence of *S. pneumoniae* was 24% at hospital discharge and increased to 67% at 3- and 6-months post-discharge. Prevalence was similar between azithromycin and placebo arms at month 3 (65.8% versus 67.2%, PR 0.98, 95% CI 0.70-1.27) and month 6 (66.7% versus 66.5%, PR 1.00, 95% CI 0.68-1.32). Results were similar after adjustment for factors that were slightly imbalanced following randomization: baseline *S. pneumoniae,* breastfeeding, and household crowding. There was no evidence for statistical interaction between the intervention and baseline carriage of *S. pneumoniae*. Any effect of azithromycin treatment on the prevalence of *S. pneumoniae* carriage was not seen 3- or 6-months post-discharge. Future work will examine the intervention's impact on resistance to azithromycin and other antimicrobial agents among *S. pneumoniae* isolates.

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COMPARISON OF ANTIBIOTIC RESISTANCE PATTERNS OF *STREPTOCOCCUS PNEUMONIAE* IN CASES OF INVASIVE PNEUMOCOCCAL DISEASE AND PAIRED NASOPHARYNGEAL COLONIZATION ISOLATES

Morr cham, Grant Mackenzie, Isaac Osei, Rasheed Salaudeen, Hendry Badji, Baleng-Mahama Wutor, Molfa Minteh, Ousman Barjo, Yusuf Abdulsalam Olawale, Golam Sarwar, Abdoullah Nyassi

Medical Research Council The Gambian at London School of Hygiene and Tropical Medicine(MRC@LSHTM), Basse santa su, Gambia

The successful treatment of pneumococcal disease significantly depends on the right choice and use of antibiotics. This study assessed whether nasopharyngeal isolates of *Streptococcus. pneumoniae* from hospitalized children in rural Gambia could be used to predict the prevalence of antimicrobial resistance (AMR) of strains that cause invasive pneumococcal diseases (IPD). Population-based surveillance for pneumonia, septicaemia, and meningitis was conducted among children under 5 years in a rural area of The Gambia under demographic surveillance from September 2019 to December 2023. Nasopharyngeal swabs (NPS), blood cultures, cerebrospinal fluid (CSF) and lung aspirates were collected from eligible children. Conventional microbiological culture was used to identify and isolate *S. pneumoniae*. Antibiotic susceptibility testing was performed using the Kirby-Bauer disc diffusion method. We used descriptive statistics to determine and compare the proportions of AMR in patients with IPD in whom the homologous *S. pneumoniae* was detected in NPS. Of the 49 IPD cases detected with homologous NPS collected, *S. pneumoniae* was isolated in 45 (91.8%) NPS cultures. Of the paired IPD and NPS homologous *S. pneumoniae* samples, 19 (42.2%) of oxacillin, 0 (0%) of chloramphenicol, 23 (51.1%) of tetracycline, 42 (93.3%) of trimethoprim-sulfamethoxazole, 40 (88.9%) of vancomycin and 0 (0%) of ceftriaxone showed resistance in NPS samples. 23 (51.1%) of oxacillin, 2 (4.4%) of chloramphenicol, 26 (57.8%) of tetracycline, 45 (100%) of trimethoprim-sulfamethoxazole, 5 (11.1%) of vancomycin and 0 (0%) of ceftriaxone demonstrated resistance in invasive samples. The proportions of AMR of *S. pneumoniae* isolates from invasive samples and NPS were comparable. These findings demonstrate that nasopharyngeal isolates of *S. pneumoniae* from children with suspected pneumonia may be considered as a tool to monitor for AMR in a defined setting. Using NPS, a relatively non-invasive procedure as a tool for AMR surveillance programs in developing countries will enable a rational and effective use of antibiotics in the clinical management of IPD including pneumonia.

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HIGH RESIDUAL NASOPHARYNGEAL CARRIAGE OF VACCINE SEROTYPE PNEUMOCOCCI AFTER 12 YEARS OF INTRODUCTION OF PNEUMOCOCCAL CONJUGATE VACCINE IN THE GAMBIA

Isaac Osei[1], Emmanuel Mendy[1], Effua Usuf[2], Christian Bottomley[3], Rasheed Salaudeen[1], Henry Badji[1], Ikumapayi U. Nurudeen[1], Phillip Hill[4], Brian Greenwood[5], Grant Mackenzie[6]

[1]Medical Research Council The Gambia at the London School of Hygiene and Tropical Medicine, Banjul, Gambia, [2]Medical Research Council The Gambia at the London School of Hygiene and Tropical Medicine, Fajara, Gambia, [3]Department of Infectious Disease Epidemiology, London School of Hygiene & Tropical Medicine, London, UK, London, United Kingdom, [4]Centre for International Health, University of Otago, Otago, New Zealand, [5]Faculty of Infectious and Tropical Diseases, London School of Hygiene & Tropical Medicine, London, United Kingdom, [6]Medical Research Council The Gambia at the London School of Hygiene and Tropical Medicine, Basse, Gambia

The introduction of Pneumococcal Conjugate Vaccines (PCVs) into routine immunization programs has led to a substantial decrease in the incidence of Invasive Pneumococcal Diseases attributable to vaccine type (VT) serotypes globally. The impact of PCVs is measured through a reduction in disease or nasopharyngeal (NP) carriage of VT serotypes. Despite the long-term use of PCVs in most African countries, VT carriage persists, in contrast to similar post-PCV introduction time points in high-income countries. The residual VT carriage in Sub-Saharan Africa is of concern as it has the potential to sustain pneumococcal transmission and a persisting disease burden. We conducted population-based cross-sectional surveys of NP carriage before the introduction of PCV13 in 2009, and after in 2015, 2017, and 2022. Nasopharyngeal swabs were taken from selected household members of all ages in the Basse and Fuladu West HDSS, transported, stored, and cultured. *Streptococcus pneumoniae* isolation and serotyping were performed using standardized methods according to WHO guidelines. The prevalence of PCV13 VT pneumococcal carriage among all ages was 19%, 12%, 13%, and 8.5% in 2009 (n=2,988), 2015 (n=3,162), 2017 (n=2,709), and 2022 (n=3,822) respectively. The prevalence of VT carriage decreased from 46% in those aged 2-59 months in 2009 to 15%, 17%, and 10% in 2015, 2017 and 2022 respectively. In those aged <60 days, VT carriage prevalence fell from 27% in 2009 to 10%, 13%, and 11% respectively in 2015, 2017, and 2022. The prevalence of VT serotypes in 2009, 2015, 2017, and 2022 was 17%, 14%, 15%, and 9% among those 5-17 years, and 5%, 6%, 6%, and 2% among adults ≥18 years. In 2022, serotypes 3, 19F, 14, 23F, and 6A were the most abundant VT serotypes. Dry season, younger age group, and having a runny nose in the preceding two weeks were associated with VT carriage. Twelve years after the introduction of PCV13 in the Gambia, substantial direct effects on VT carriage have been

observed in children with limited indirect effects in adults. While additional VT reductions were observed in 2022 in those 5-17 years old, the effect in those aged <60 days appears to have plateaued in 2015.

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EVALUATION OF THE USABILITY, ACCEPTABILITY, AND FEASIBILITY OF TWO DEVICES FOR THE DELIVERY OF INTRANASAL VACCINES IN LOW-AND-MIDDLE INCOME COUNTRIES

Jennifer Foster[1], Erin Rowand[1], Wilkister Musau[2], Edith Jepleting[2], Sandeep Kumar[3], Priyanka Bajaj[3]

[1]PATH, Seattle, WA, United States, [2]PATH, Nairobi, Kenya, [3]PATH, New Delhi, India

The COVID-19 pandemic highlighted the need for rapid and equitable global access to vaccines, including methods to enhance vaccine acceptability, uptake, and supply chain. An intranasal vaccine with an optimized delivery system has potential to expand global access and transform the vaccine delivery landscape for COVID-19 and other respiratory diseases. Intranasal vaccines could offer advantages for health workers and clients over intramuscular injections by eliminating the need for sharps disposal, decreasing risk of contamination and injuries, and reducing discomfort associated with needles. Intranasal vaccines can potentially induce local immunity in the nasal passages and upper airways, which could prevent infection at the virus entry site, directly reduce transmission, and enable a stronger immune response. PATH evaluated the usability and feasibility of two intranasal delivery devices from a provider and program perspective through simulated use by target users in Kenya, Nepal, and the U.S., and through interviews with country and global stakeholders. The devices tested were the Mucosal Atomization Device and the ZEOx1 Orion Delivery System. Simulated use participants described the devices as easy to use and successfully delivered a mock vaccination with minimal difficulties, although use errors and deviations were observed. Participants described several advantages of intranasal delivery. Having a needle-free option was the most important advantage since it is painless, eliminates sharps waste, and may be more acceptable for some clients, although a few concerns were raised around suitability for young children. Factors influencing acceptability of the individual devices included the number of steps for use, perceived comfort of the nasal tip, training required, and waste management, with community sensitization being an important component. In conclusion, this study highlights the potential of intranasal vaccine delivery devices for use in global vaccination efforts against infectious diseases and the importance of intranasal product profile characteristics in supporting usability and acceptability.

6040

THE LIVED EXPERIENCES OF UGANDAN COMMUNITY HEALTH WORKERS ENGAGED IN PREVENTION OF VERTICAL TRANSMISSION OF HIV AND A CAPACITY-BUILDING INTERVENTION

Victor Mocanu[1], Hannah M. Brooks[1], Sophie Namasopo[2], Robert O. Opoka[3], **Michael T. Hawkes**[4]

[1]University of Alberta, Edmonton, AB, Canada, [2]Kabale District Hospital, Kabale, Uganda, [3]Medical College East Africa, Aga Khan University, Nairobi, Kenya, [4]University of British Columbia, Vancouver, BC, Canada

An estimated 220,000 new HIV infections are averted annually with coordinated efforts toward the elimination of vertical transmission (EVT). This study explored the lived experiences of community health workers (CHW) engaged in EVT of HIV and to assess the impact of a capacity-building intervention. The study consisted of: (1) a qualitative assessment of lived experiences of CHWs; (2) a capacity-building intervention tailored to identified needs; and (3) assessment of the intervention using pre- and post-intervention questionnaires. Focus group discussions (FGD) and semi-structured key informant interviews (KII) were conducted. Interactive CHW training sessions for HIV/EVT were held in one rural and one semi-urban setting in Uganda, based on training materials developed by the WHO and USAID. We used standardized pre- and post-intervention questionnaires to assess comprehensive knowledge and accepting attitudes toward HIV. Qualitative exploration of the lived experience of 152 CHWs in ten FGDs and four KIIs revealed several themes: (1) CHWs as bridges between health system and community; (2) CHW assets (tacit knowledge and shared social networks); (3) CHW challenges (stigma, secrecy, and ethical quandaries); (4) favorable community reception; and (5) need for continuing education and reinforcement of skills. In response to identified needs, a capacity-building intervention was designed and implemented with 143 CHWs participating in 10 sessions. The proportion of participants with comprehensive knowledge of HIV increased from 45% to 61% (p=0.006) and the proportion endorsing accepting attitudes increased from 63% to 76% (p=0.013). In summary, CHWs are valuable players in global EVT efforts. Ongoing training is needed to support community-level initiatives.

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FACTORS CONTRIBUTING TO LOW LINKAGE TO HIV TREATMENT IN GHANA, 2023

Jennifer Nai-Dowetin[1], Vincent Ganu[2], Grace Ocansey[1], Anthony Ashinyo[3], Stephen Ayisi-Addo[3], Ernest Kenu[4]

[1]Ghana Field Epidemiology and Laboratory Training Programme, Accra, Ghana, [2]Infectious Disease Unit, Department of Medicine and Therapeutics, Korle-Bu Teaching Hospital, Accra, Ghana, [3]National HIV/AIDS/STI Control Programme, Ghana Health Service, Accra, Ghana, [4]Department of Epidemiology and Disease Control, School of Public Health, University of Ghana. Ghana Field Epidemiology and Laboratory Training Programme, Accra, Ghana

Successful linkage of persons living with HIV ensures prevention of new infections, prolong survival, and improved quality of life. Despite the importance of early linkage of HIV cases to care, Ghana recorded suboptimal linkage rate of 53% and 59% for 2020 and 2021 respectively and attained 78-81-68 of UNAIDS target in 2022. We analyzed issues resulting in low linkage to care in Ghana. A cross-sectional design with a mixed method approach employed. Sixteen health facilities were selected with multi-stage sampling across three geographical zones. Data on HIV testing, evidence of profiling of clients (i.e., probing of patients whether they are re-testing for HIV) assessing HIV testing services, confirmation of tests, linkage to care, and ART initiation and retention in care were reviewed from July 2022 to June 2023 in each facility. We observed the linkage process while key informant interview and FGDs were conducted among service providers. Ten clients per facility diagnosed with HIV within six months were interviewed. We used proportions to estimate patients profiled, confirmatory tests, linkage to care and retention. Of the 47,493 persons tested over the period, HIV positivity was 5.2% (2472). Of those testing positive, only 70% (1732/2472) were actual newly positive with the rest being those re-testing. About 44% (1082/2472) of those testing positive for HIV were not profiled. Confirmatory tests for HIV were limited in 63% (10/16) facilities while 80% (13/16) facilities did not have linkage registers. Of the 1732 new cases, 80% (1379/1732) were successfully referred to ART care and 62.4% (1081/1732) were linked and initiated on ART and 37.6% (651/1732) were not linked and initiated on ART because of sub-optimal referral system, non-use of triplicate referral forms and non-use of linkage registers. Inadequate profiling of patients, reporting of re-testing patients as new HIV positives and, sub-optimal referral system and non-use of the linkage registers accounted for low linkage to care. Intensive orientation and monitoring of healthcare workers to adherence to linkage to ART care implementation activities is key for improving linkage rates.

6042

ASSESSING THE RISK OF ADVERSE PREGNANCY OUTCOME AMONG HIV-POSITIVE AND HIV-NEGATIVE PREGNANT WOMEN: ANALYSIS FROM A COHORT OF WOMEN PARTICIPATING IN TWO INDIVIDUALLY RANDOMIZED CONTROLLED TRIALS IN WESTERN KENYA

EVERLINE DELYLAH ONDIEKI, Eric Donald, George O. Olilo, Hellen C. Barsosio

Kenya Medical Research Institute, Kisumu, Kenya

The intersection of maternal human immune deficiency virus (HIV) infection and pregnancy outcomes has been a subject of critical importance in the realm of maternal and child health. Numerous studies have shown that maternal HIV infection is associated with adverse pregnancy outcomes. However, this has been contradicted with the introduction of antiretroviral therapy (ART) for the prevention of mother-to-child HIV transmission (PMTCT). The Improving Pregnancy Outcome (IMPROVE) trials have provided a comprehensive platform to investigate the intricate relationship between maternal HIV status and the risk of adverse pregnancy outcomes among pregnant women. HIV-negative data was extracted from IMPROVE 1, a randomized, double-blind, three-arm trial conducted in regions with elevated sulfadoxine-pyrimethamine resistance in Kenya. Data for HIV-positive women were extracted from IMPROVE 2, a randomized, double-blind, two-arm, placebo-controlled trial focused on monthly IPTp with dihydroartemisinin-piperaquine for malaria in HIV-infected participants on daily cotrimoxazole eligible for (or on) daily tenofovir-lamivudine-dolutegravir (TLD) and with an undetectable viral load. Women with viable singleton pregnancies between 16-28 weeks gestation were enrolled. A total of 1224 HIV-negative and 701 HIV-positive pregnant women were recruited. Multivariable logistic regression was employed to identify associations between maternal HIV status and adverse pregnancy outcomes. The median age at enrolment was 25 years, with an interquartile range (IQR) of 21 to 30 years. HIV- positive women had increased odds of experiencing adverse pregnancy outcomes (AOR = 1.39, 95% CI: 1.04, 1.85), miscarriage (AOR=1.83, 95% CI: 1.06, 2.77) and stillbirth (AOR =1.80, 95% CI: 1.16–3.09) whereas Cohabiting women exhibited nearly a two-fold increase in the odds of adverse pregnancy outcomes (AOR = 1.75, 95% CI: 1.08, 2.82). Despite the study recruiting HIV-positive women on effective ART and with undetectable viral loads, adverse pregnancy outcomes remained higher among this group as compared with HIV negative counterparts.

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AFRICAN BRAIN POWERED GAMES APPS AVAILABLE ON COMPUTER TABLETS CAN BE USED TO DYNAMICALLY ASSESS BRAIN/BEHAVIOR INTEGRITY AND NEUROCOGNITIVE PERFORMANCE IN UGANDAN AND MALAWIAN SCHOOL-AGE CHILDREN AFFECTED BY HIV

Michael J. Boivin[1], Itziar M. Familiar-Lopez[1], Lillian Wambuzi Ogwang[2], Sufia Dadabhai[3], Brian Winn[1], Alla Sikorskii[1], Bruno Giordani[4]

[1]Michigan State University, East Lansing, MI, United States, [2]Makerere University - Johns Hopkins University, Kampala, Uganda, [3]Malawi College of Medicine - Johns Hopkins University, Blantyre, Malawi, [4]University of Michigan, Ann Arbor, MI, United States

Brain Powered Games (BPG) is an app with 10 games designed to improve attention, working memory and learning, visual-spatial analysis, and problem solving. In Phase II we developed Village Builder (VB) to engage school-age children in a prosocial game using reasoning and planning skills to garner the resources needed to build a simulated village community. The goal was to evaluate the use of both BPG and VB as dynamic measures of brain/behavior integrity and neurocognitive function in school-age Ugandan and Malawian children (HIV, HEU, HUU cohorts). 60 HIV, 120 HEU, and 120 HUU boys and girls (MU-JHU Kampala Uganda; MCM-JH Blantyre Malawi) were randomized to either 12 one-hour training sessions of BPG over 2 months (Phase I), or "wait-listed" to 12 sessions of VB in Phase II. Performance on BPG or VB before and following the training period was then compared to performance on our "gold standard" neuropsychological battery of the Kaufman Battery for Children (KABC-II), Tests of Variables of Attention (TOVA), CogState computerized cognitive ability test, and the Achenbach Child Behavior Checklist (CBCL; completed by caregiver). Ugandan and Malawian children randomized to 12 BPG sessions in Phase I had significantly higher post-training performance on all principal outcomes of our gold-standard neuropsychological tests, when compared to "wait-listed" children. These included the KABC-II Mental Processing Index (MPI), Nonverbal Index (NVI), TOVA attention errors, TOVA impulsivity errors, CogState attention and maze learning, and CBCL internalizing symptoms (emotional problems). Similar Findings were obtained for children receiving VB in Phase II of the study. Although neuropsychological performance of HIV and HEU children was below that of the reference group (HUU) on both game achievement and gold standard assessment outcomes, all three group significantly benefitted from the game app training. Improvements in performance on the game apps were significantly related to improvements on neuropsychological tests for all three cohorts of study children. We also report on adaptation of these apps to the cloud for community-based scalability.

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ONE AND TWO DOSE TYPHOID CONJUGATE VACCINE SAFETY AND IMMUNOGENICITY IN HIV-EXPOSED UNINFECTED AND HIV-UNEXPOSED UNINFECTED MALAWIAN CHILDREN

Nginache Nampota-Nkomba[1], Osward M. Nyirenda[2], Divya Hosangadi[1], Victoria Mapemba[2], Priyanka D. Patel[3], Happy C. Banda[3], Felistas Mwakiseghile[3], Theresa Misiri[3], Richard Wachepa[3], John Ndaferankhande[3], Bright Lipenga[3], Robert S. Heyderman[4], Marcela Pasetti[1], Leslie P. Jamka[1], Shrimati Datta[1], Melita A. Gordon[3], Kathleen M. Neuzil[1], Matthew B. Laurens[1]

[1]University of Maryland School of Medicine, Baltimore, MD, United States, [2]Blantyre Malaria Project, Blantyre, Malawi, [3]Malawi Liverpool Wellcome Programme, Blantyre, Malawi, [4]University College London, London, United Kingdom

Vaccine safety and immunogenicity data in HIV-exposed uninfected (HEU) children are important for decision-making in HIV and typhoid co-endemic countries. In an open-label study, we recruited Malawian HEU and HIV unexposed uninfected (HUU) infants aged 9-11 months. For HEU participants, HIV exposure was determined by documented maternal history of HIV. For HUU participants, a negative maternal HIV rapid test was obtained. HIV status for all participants was confirmed by non-detectable infant HIV viral load at enrollment. HEU participants were randomized to receive: Vi-tetanus toxoid conjugate vaccine (Vi-TT) at 9 months (HEU9), 15 months (HEU15), or 9 and 15 months (HEU9+15). HUU participants received Vi-TT at 9 and 15 months. Safety outcomes included solicited and unsolicited adverse events (AE) within 7 and 28 days of vaccination, respectively. Serum was collected before and 28 days after vaccination to measure anti-Vi immunoglobulin G (IgG) antibodies by enzyme-linked immunosorbent assay (ELISA). Seroconversion was defined as ≥4-fold rise in antibody titers from day 0. Enrollment occurred from 2 December 2019 and was paused on 25 March 2020 due to the COVID-19 pandemic and resumed from 1 March - 27 August 2021. A total of 166 participants were vaccinated; 50 HEU9, 43 HEU15, 48 HEU9+15, and 25 HUU. Solicited AEs were mostly mild, and occurrence did not differ significantly in HEU and HUU participants, or one- and two-dose groups. At day 28 post-9 months vaccination, HEU (HEU9 and HEU9+15), and HUU participants had similar significant geometric mean titers (GMT) increases from day 0, reaching 3111.8 ELISA Units (EU)/mL (95% CI 2301.1- 4208.1) and 3493.7 EU/mL (95% CI 2729.4-4471.9), respectively. At 28 days post-15 months vaccination, GMT ranged from 2572.0 EU/mL (95% CI 1844.6-3586.2) to 4117.6 EU/mL (95% CI 2362.8-7175.8) and were similar in the first dose HEU15 group and second dose HEU9+15 and HUU groups. All participants seroconverted by the final study visit. Our findings of comparable safety and immunogenicity of Vi-TT in HUU and HEU children support country introductions with single-dose Vi-TT in HIV-endemic countries.

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THE CLINICO-EPIDEMIOLOGICAL EXPERIENCE OF AN MPOX OUTBREAK AT A LARGE HEALTHCARE SYSTEM IN LOUISIANA, USA.

Mary Ellen Owings[1], Latha Rajan[1], Obinna Nnedu[2]

[1]Tulane University School of Public Health and Tropical Medicine, New Orleans, LA, United States, [2]Ochsner Medical Center, Infectious Diseases Department, New Orleans, LA, United States

Mpox, a viral zoonosis of the Orthopoxvirus genus, garnered attention in May 2022 when cases emerged in non-endemic countries, including the United States. The outbreak quickly spread, with Louisiana reporting its first case in July 2022. This study analyzed Mpox cases seen across the Ochsner health system, which comprises 46 hospitals and several clinics in Louisiana and southern Mississippi. The goal of this study was to use the results as lessons learned to improve outbreak response and optimize care. Patient data from January 1, 2022, to February 28, 2023, across all Ochsner facilities was analyzed. One hundred and forty-two confirmed and suspected cases of Mpox were identified in the electronic medical record. Of these 142 cases, 77 tested positive for Mpox (confirmed) while 65 had inconclusive test results (suspected). 90.9% of confirmed cases were male, and 68.8% were black. Most patients were aged between 18 and 49 (93.6%) and Medicaid was the predominant insurance type (42.9%). At the time of testing for Mpox, 69% of patients were tested for sexually transmitted infections (STI), including syphilis, chlamydia, and gonorrhea. Of those tested for STIs, 14.1% were infected with one or more STIs. Of the 77 confirmed cases, 45.5% were living with HIV. Of those living with HIV with available CD4 measurements, 25% had a CD4 count below 200. It took an average of 5.8 days for inconclusive results and 4.8 days for positive results to return, leading to delays in diagnosis and decision-making. Patients with severe immune compromise (including HIV patients with a CD4 count <200 and HIV patients not on treatment) met the criteria for tecovirimat. Of those living with HIV who met the criteria, 41.7% received tecovirimat. The results of this study serve as a valuable foundation for enhancing clinical management strategies in future outbreak responses, potentially leading to enhanced patient outcomes.

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PERFORMANCE EVALUATION OF FIVE POINT-OF-CARE TESTS FOR MPOX DETECTION

Devy M. Emperador[1], Elie Ishara[2], Jacob Parkes[3], Juvenal Nkeramahame[4], Mikaela Watson[5], Anushri Somasundaran[3], Yusra Hussain[3], Nadia Kontogianni[3], Daniel Mukadi[2], Marithé Mukoka[2], Hugues Mirimo[2], Emile Milonde[2], Susan Logoose[4], Audrey Albertini[6], Berra Erkosar[5], Emmanuel Agogo[1], Jake Dunning[7], Ana Cubas Atienzar[3], Hugo Kavunga-Membo[2]

[1]Pandemic Threats Program, FIND, Geneva, Switzerland, [2]Laboratoire Rodolphe Mérieux - Institut National de Recherche Biomédicale, Goma, Congo, Democratic Republic of the, [3]Center for Drugs and Diagnostics, Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [4]Clinical Trial Unit, FIND, Geneva, Switzerland, [5]Data Science Unit, FIND, Geneva, Switzerland, [6]Program Management Unit, FIND, Geneva, Switzerland, [7]Pandemic Sciences Institute, University of Oxford, Oxford, United Kingdom

The global outbreak of mpox in 2022 spurred the rapid availability of commercial diagnostics for this disease. However, given the limited data on test performance, particularly for tests suitable for use in decentralized settings and those that detect multiple monkeypox virus (MPXV) clades, we conducted a diagnostic accuracy study in 2 countries to determine the clinical performance of 5 point-of-care tests (3 antigen-based rapid tests [AgRDTs] and 2 point-of-care [POC] molecular tests) compared to PCR for the detection of MPXV. Individuals >=2 years of age suspected to have mpox were enrolled prospectively in the Democratic Republic of the Congo (DRC) and retrospectively in the United Kingdom (UK) in 2023. Paired lesion and oropharyngeal (OP) samples were collected at time of enrolment. All samples were tested with both PCR reference test and all 5 index tests. A total of 105 individuals (DRC: n=68, UK: n=37) were enrolled, providing 79 lesion swabs (DRC: n=68, UK: n=11) and 82 OP swabs (DRC: n=68, UK: n=14). Overall MPX positivity in the combined cohort was 37% (N=29; DRC: 28% [19/68], UK: 91% [10/11]). Clinical sensitivity on lesion samples was highest among the two POC molecular tests (SN: 76-79%) compared to the AgRDTs (SN: 7-10%), while clinical specificity was high among all tests (SP: 85-100%). Clinical performance trends were similar when analyzed by country, although POC molecular tests had higher point sensitivity in the UK (SN: 100%) compared to DRC (SN: 63-68%). Clinical performance of POC tests on OP samples was comparable to lesion samples. Based on this, POC molecular tests on lesion samples can be used for screening, while the utility of AgRDTs for screening is unclear. In addition, OP swabs may be an alternative sample type for diagnosis.

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BURDEN OF CHAGAS DISEASE RELATED TO CARDIOMYOPATHY IN THE UNITED STATES

Steffany Vucetich, Kelly DeToy, Yeonsoo Baik, Robert H. Gilman, Bryan Patenaude

Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States

Chagas disease (CD) presents a growing concern in the United States (US). It stems largely from chronic Chagas cardiomyopathy, which causes progressive heart failure, arrhythmias, and sudden death. This condition not only has health impacts (morbidity and premature mortality) but also carries significant economic burden. Furthermore, the under-diagnosis of CD in the US limits effective monitoring and care. Our study assesses the health and economic burden of Chagas cardiomyopathy caused by adult inpatient population in the US from a health system perspective. We used the 2019 Healthcare Cost & Utilization Project National Inpatient Sample (HCUP-NIS) database to assess the economic and health impacts of Chagas cardiomyopathy, using six concurrent conditions. We evaluated treatment costs and health effects in the form of Disability Adjusted Life Years (DALYs) based on survival rates and life expectancy data. Prevalence rates were estimated using age-specific data and population data from publicly available sources. We calculated the number of individuals developing cardiomyopathy within age groups based on infection prevalence. Costs were computed by age group for those with and without CD. We included survival rates from various sources and disability weights from the Global Burden of Disease Study (GBD). We modified our results to account for multimorbidity and performed a one-way sensitivity analysis to account for uncertainty. The economic burden of Chagas cardiomyopathy in the US in 2019 was estimated to be $7.14 billion as a total, yielding 1.65 million total DALYs, or 376.42 DALYs per 100,000 population. After considering multimorbidity, the burden was $5.34 billion, yielding 1.25 million DALYs or 291.6 DALYs per 100,000 population. Our study highlights the significant health and economic burden of Chagas cardiomyopathy in the US. Comparison with the 2019 GBD reveals that the burden of Chagas cardiomyopathy ranks above Tuberculosis and HIV/AIDS in total DALYs in the US. These findings stress the importance of implementing effective early diagnosis strategies to prevent unnecessary adverse health and economic consequences.

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WHAT IS THE EFFECT OF DEFORESTATION OF THE ATLANTIC FOREST ON THE OCCURRENCE OF *PANSTRONGYLUS TIBIAMACULATUS* IN URBAN AREAS?

Gilmar Ribeiro-Jr[1], Fernanda C. Lanza[1], Diego L P Miranda[1], Renato B. Reis[2], Cristiane W. Cardoso[3], Fabiano Simões[3], Eliaci C L Costa[3], Márcia Castro[4], Rodrigo Gurgel-Gonçalves[5], Mitermayer G. Reis[1]

[1]Oswaldo Cruz Foundation, Salvador, Brazil, [2]Facudade Salvador, Salvador, Brazil, [3]Secretaria de Saúde de Salvador, Salvador, Brazil, [4]Harvard School of Public Health, Boston, MA, United States, [5]Universidade de Brasília, Brasília, Brazil

In Salvador, Bahia, Brazil, we have observed a systematic reduction of the Atlantic Forest, as well as an increase in the records of triatomines. Our hypothesis is that the landscape influences the occurrence of *Panstrongylus*

tibiamaculatus in houses in Salvador. Our objective was to investigate the effect of disturbances in the natural landscape of the Atlantic Forest on the occurrence and spatial distribution of *P. tibiamaculatus* in neighborhoods of Salvador. Triatomines were recorded between 2007 and 2019 in Salvador by the Center for Zoonosis Control. We evaluated the influence of forest cover, deforestation, urban infrastructure, and population on the occurrence and spatial distribution of *P. tibiamaculatus*, the main species recorded. We obtained data from the deforestation module of MapBiomas. We analyzed the association between variables using bivariate and multivariate zero-inflated generalized linear models with negative binomial distribution (glmmTMB). Multivariate models were evaluated by the Akaike Information Criterion (AIC). We obtained 1511 records of *P. tibiamaculatus* between 2007 and 2019. We observed a clustered spatial distribution of triatomines, mainly in neighborhoods with higher deforestation rates, such as the Patamares neighborhood (78.97%, n=1199). The models indicated that deforestation is the most important factor in explaining the number of triatomine records per neighborhood in Salvador. Bivariate models indicated that deforested area, forest cover, and urban structure showed a positive and significant association ($p < 0.05$) with the occurrence of triatomines. Multivariate models indicated that the model with the best performance (lower AIC) was composed of deforested area and forest cover. We observed that deforestation of Atlantic Forest areas is the main effect influencing the spatial distribution of *P. tibiamaculatus* in neighborhoods of Salvador. We recommend strengthening triatomine surveillance in the most affected neighborhoods, and environmental management of palm trees and other natural refuges of triatomines in Atlantic Forest remnants

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SYSTEMIC CLINICAL PARAMETERS AND INFECTIVITY IN CANINE LEISHMANIOSIS

Max Waugh[1], Karen Cyndari[2], Tom Lynch[3], Soomin Koh[2], Ferney Henao-Ceballos[2], Jacob J. Oleson[2], Paul Kaye[2], Christine A. Petersen[2]

[1]The University of Iowa, Coralville, IA, United States, [2]The University of Iowa, Iowa City, IA, United States, [3]The Ohio State University, Columbus, OH, United States

Visceral leishmaniasis (VL), caused by the zoonotic intracellular protozoal parasite *Leishmania infantum*, is a significant public health concern for humans and domestic dogs, the primary reservoir. In areas where sand fly transmission occurs, public health interventions often focus on dogs, especially sick dogs, as a source of human infections, with a belief that the sicker the dog the more likely they are to transmit parasites. However, rates of canine leishmaniosis and human leishmaniasis have not significantly improved when public health interventions remove sick dogs from the population. Additionally, xenodiagnoses studies investigating infectivity and clinical disease, based on the LeishVet scoring guidelines, found that dogs with mild to moderate disease scores were the most infective to sand flies. This study aimed to evaluate, using RNAscope, dermal and systemic factors that predict infectiousness of *L. infantum*. The number and distribution of dermal parasitized phagocytes (amastin+/CD14+) in dogs at different LeishVet stages (a clinical scoring system from 1-4 commonly used in the field) was evaluated against systemic clinicopathology of the two predominant clinical values that assess renal failure and anemia (creatinine and hematocrit). In a subset of dogs used for xenodiagnoses, dermal parasitized phagocytes were also evaluated against infectivity to sand flies. Individuals with the lowest score, had significantly fewer parasitized phagocytes than individuals with scores 2-4, which did not significantly differ from each other. Additionally, parasitized phagocyte counts significantly increased as hematocrit decreased. There was an inverse correlation between hematocrit and infectivity. In contrast, no significant changes were associated with increasing creatinine despite these being characterized as the sickest animals with the highest LeishVet scores. Future work will articulate how and why infectivity correlates with low hematocrit. These initial findings suggest that disease presentation, not severity, influences infectivity and should be considered in VL-focused public health interventions.

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EPIDEMIOLOGY OF VISCERAL LEISHMANIASIS AND OTHER PARASITIC INFECTIONS IN REFUGEE CAMPS OF ETHIOPIA

Bortola A. Ayana[1], Adugna Abera[1], Aman Yesuf[2], Habtamu Belay[1], Mahlet Belachew[1], Tesfahun Bishaw[3], Fransisco Averhoff[4], Alan Landay[5], Paulina A. Rebolledo[6], Geremew Tasew[1]

[1]Ethiopian Public Health Institute, Addis Ababa, Ethiopia, [2]St. Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia, [3]Ministry of Health of Ethiopia, Addis Ababa, Ethiopia, [4]Abbott Diagnostics, Abbott Park, IL, IL, United States, [5]University of Texas, Austin, TX, United States, [6]Emory University, Atalanta GA, GA, United States

While national guidelines exist for controlling Visceral Leishmaniasis (VL) and other parasitic infections in Ethiopia, data on their prevalence among refugees remains scarce. The aim of this study is to describe the prevalence of VL and co-occurrence of other parasitic infections among refugees in Gambella and Benishangul-Gumuz Regions. A community-based cross-sectional study was conducted at four refugee camps located in Gambella and Benishangul Gumuz Regions of Ethiopia during May to August 2023. Sociodemographic, clinical, behavioral, housing, and environmental characteristics were collected. Blood and Stool specimens were obtained and underwent testing. Data were analyzed using SPSS 25. Finally, a binary logistic regression analysis was fit to identify risk factors to VL infection at p-value <0.05. Of 2702 participants, 2670 were willing to participate in the study. Based on Direct Agglutination test (DAT) and/or rK39 test, the overall prevalence of VL was 6.3%, with higher rates observed in refugees from the Sudan (7.4%) refugees residing in Benishangul Gumuz region (9.3%) and Tsore camp (11.3%). Among refugees with VL, the prevalence of VL co-infection with Malaria, Schistosoma mansoni, Ascaris lumbricoides, Trichuris Trichuria and Hookworm were 7.8%, 10.2%, 6.6%, 3.4% and 1.2%, respectively. In multivariable analysis, Age (<15 years) AOR 0.496, 95% CI: 0.264 -0.932; residing in the Gambella refugee camp AOR 3.860, 95% CI: 2.173 - 6.644; household size (&ge5) AOR 1.48, 95% CI: 1.022 - 2.149; no previous contact history of VL AOR 0.022, 95% CI: 0.007- 0.065; close proximity of termite hills to the home AOR 2.729, 95% CI: 1.770-4.210 and presence of stagnant water near to house AOR 2.11, 95% CI:1.405-3.183 were factors associated with VL infection. This study found high VL prevalence among refugee populations in Ethiopia. These findings suggest that screening for multiple parasites should be considered for refugee population. Finally, alongside early diagnosis and treatment, public health efforts should prioritize environmental interventions like drainage and termite control to combat VL in displaced populations.

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SPECIFIC PATHOGEN TESTING FOR OPPORTUNISTIC INFECTIONS IN PERSONS WITH HIV IN PERU AND BOLIVIA

Hannah E. Steinberg[1], Andrea Diestra[2], Beth J. Condori[2], Edith Malaga[2], Christian Guerra[3], Margot Ramirez Jaldin[4], Lynn Pinchi[5], Ricardo Medrano Colmenares[2], Maria J. Pessoa[4], Sergio Burgoa[4], Cusi Ferradas[6], Daniela E. Kirwan[7], Monica M. Diaz[8], Michael Sciaudone[9], Maritza Calderón[2], Manuela Verastegui[2], Lilia Cabrera[5], Viviana Pinedo Cancino[3], Frine Samalvides[2], Freddy Tinajeros[4], Cesar Ramal Asayag[10], Robert H. Gilman[11], Natalie M. Bowman[8]

[1]University of Washington, Seattle, WA, United States, [2]Universidad Peruana Cayetano Heredia, Lima, Peru, [3]Universidad Nacional de la Amazonía Peruana, Iquitos, Peru, [4]AB Prisma, Santa Cruz, Bolivia, Plurinational State of, [5]AB Prisma, Lima, Peru, [6]University of California Davis, Davis, CA, United States, [7]St George's, University of London, London, United Kingdom, [8]University of North Carolina, Chapel Hill, NC, United States, [9]Tulane University, New Orleans, LA, United States, [10]Hospital Regional de Loreto, Iquitos, Peru, [11]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States

Neurological opportunistic infections are a significant cause of morbidity and mortality in people living with HIV (PLWH). Over the last five years, we enrolled 162 PLWH with new-onset neurological symptoms and 422 PLWH controls (52% hospitalized non-neurological, 48% ambulatory) in

Santa Cruz, Bolivia, Iquitos, and Lima, Peru. Specific pathogen testing was completed for *Toxoplasma gondii, Trypanosoma cruzi,* and *Cryptococcus spp*. Site-specific testing was completed for *Mycobacterium tuberculosis* and *Histoplasma* spp. *T. gondii* seroprevalence was 76% across all sites, with Iquitos having the highest at 97% and Lima having the lowest at 60%. Seroprevalence of *T. gondii* did not vary substantially between cases and controls. This study identified 19 cases of neurological toxoplasmosis across all three sites using qPCR in cerebral spinal fluid (CSF). All toxoplasmosis cases, except for 1, were in the neurological case group. Seroprevalence of *T. cruzi* was measured in Iquitos (1%) and Santa Cruz (21%). 26 cases of Chagas disease were identified using qPCR in blood or CSF, 25 from Santa Cruz and 1 in Iquitos; 24 specimens were positive in the blood specimen alone, while 2 were positive in both blood and CSF, concerning for CNS Chagas disease. Nine of the Chagas cases were in the neurological group, while 16 were in the control group. We identified 55 cases of Cryptococcus using Immy's CrAg lateral flow assay. 38 cases were in the neurological group and 17 were in the control group. Tuberculosis testing via microscopic-observation drug-susceptibility (MODS) occurred in Lima where 2 cases were identified and Santa Cruz where 15 cases were identified; all positive results were sputum, and no cultured CSF was found to be positive. There are 4 positive TB cases in the neurological group and 13 in the negative group. Histoplasmosis testing was only completed in Lima, where four cases were identified in urine; 1 in the neurological group and 3 controls. This limited pathogen testing allowed for the identification of a potential disease etiology for 44% of the neurological patients, 19% of hospitalized controls, and 4% of ambulatory controls.

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COHORT ESTIMATION ANALYSIS OF CUTANEOUS AND MUCOCUTANEOUS LEISHMANIASIS, 1990-2021

Cathleen Keller, Michael Celone, Ewerton Cousin, Taren Gorman, Quince Hara, Olivia Nesbit, Lydia Plante, Joanna Whisnant, Steph Zimsen, Jon Mosser

Institute for Health Metrics and Evaluation, Seattle, WA, United States

Leishmaniasis is a vector-borne parasitic disease caused by species of the genus Leishmania and transmitted via female phlebotomine sandflies. Several factors are important to consider in estimating the disease including geographic location, species and dissemination of treatment. We aim to describe method updates to the cohort estimation process for calculating prevalence of cutaneous and mucocutaneous leishmaniasis (CL) for the Global Burden of Disease Study (GBD), from 1990 to 2021. We used estimates of CL from GBD 2021 and preliminary results from GBD 2023 to describe the impact of the results. We first updated incidence data to include reported cases of zero. To estimate incidence, we ran a Spatiotemporal Gaussian Process Regression (ST-GPR). The regression stage of ST-GPR was updated to a negative binomial, as opposed to linear, to improve the fit of the model. Assumptions around duration, percent experiencing chronic sequelae, and healthcare access were combined with incidence to estimate prevalence. Previously, prevalence estimates were calculated by running a cohort model from 1990 using GBD age bins. The updated model began in 1890 and ran with single-year age bins to accumulate chronic cases across ages from year to year. To estimate burden, we calculated disability-adjusted life years (DALYs), calculated as the sum of years lived with disability. Percent change and counts were estimated at 1-year intervals spanning 1990 to 2021. Globally, model updates resulted in prevalence and DALY increases of 42.1% (95% UI 38.0-47.7) and 50.2% (95% UI 34.4-71.2), respectively, in 2021. Total DALYs increased from 393,000 to 591,000. Afghanistan, Brazil and Syrian Arab Republic were estimated to have the highest prevalence estimates in 2021 across both analyses. The updated model showed decreases in prevalence and DALYs in 55 of 93 endemic countries. The significant differences between models describes a need for including lower data points and continuing to update methods for estimating CL. These estimates can be utilized as a resource for policymakers to develop control and treatment programs targeted to reduce the impact of CL burden.

6053

LOW RISK FOR LOCALLY ACQUIRED CHAGAS DISEASE IN CALIFORNIA: A REVIEW OF HUMAN CASES AND TRIATOMINE SUBMISSIONS, 2013-2023

Andrea Lund[1], Marco E. Metzger[2], Vicki L. Kramer[1], Anne M. Kjemtrup[1]

[1]California Department of Public Health, Sacramento, CA, United States, [2]California Department of Public Health, Ontario, CA, United States

Chagas disease in humans is caused by infection with *Trypanosoma cruzi* parasites that are transmitted by triatomine bugs via infected feces. Most infections are acquired in rural parts of Mexico and Central and South America, though triatomines and *T. cruzi* are also endemic to the southern United States. Chagas disease is considered non-endemic in the United States. Most identified US patients with Chagas disease report travel history to Latin America, yet the presence of both the vector and causative agent in California has prompted questions about the risk of locally acquired infections. We summarize 226 triatomine bug submissions and 50 human case reports to the California Department of Public Health between 2013 and 2023. Of the 226 triatomines tested, 63 (28%) were positive for *T. cruzi* via multi-target PCR. We use a draft surveillance case definition and five criteria to evaluate evidence of *T. cruzi* infection and local transmission, respectively. Forty-three (86%) patients had evidence of infection and were classified as cases. We found limited overlap in the spatial and temporal distribution of triatomine collections and human cases. Country of birth, travel history outside the United States, and absence of triatomine detections in the county of residence ruled out local transmission for 26 (60%) cases. Local transmission could not be ruled out for the remaining 17 (40%) cases, though missing demographic information prevented full assessment of local transmission criteria for these cases. This is consistent with the documented behavior of triatomine bugs in California, which, in contrast to anthropophilic species in Latin America, typically defecate away from the bite site, reducing the risk of transmission. Results suggest low risk for locally acquired Chagas disease in California, though more complete collection of demographic data in human case reporting would improve our understanding.

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EFGH CATCHMENT AREA, DHAKA, SECONDARY *SHIGELLA* TRANSMISSION AND PREDISPOSING FACTORS FOR DEVELOPING SHIGELLOSIS AMONG HOUSEHOLD CONTACTS IN THE BANGLADESH

Md. Taufiqul Islam, Farhana Khanam, Ismail Hossen, Nazmul Hasan Rajib, Mahzabeen Ireen, Syed Qudrat-E- Khuda, Md Golam Firoj, Prasanta Kumar Biswas, Amirul Islam Bhuiyan, Faisal Ahmmed, Firdausi Qadri

International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh

Shigellosis, caused by *Shigella* spp., is a major global health concern, leading to millions of deaths annually, and under 5 children are more vulnerable. Previous studies showed the susceptibility of infection among household (HH) contacts of index cases. The purpose of the study was to illustrate the secondary transmission among HH contacts of *Shigella*-positive patients. Information on index cases was captured from EFGH study carried out in Bangladesh. The *Shigella*-positive patient was defined as an index case and the HH contacts who permanently resided in the same HH or were present for at least 7 days of the last 14 days, were enrolled in this study. Stool specimens and other information were collected from the HH contacts within 7 days of index cases enrollment. Microbiological culture and quantitative PCR were carried out to detect *Shigella* within HH contact. Descriptive statistics, bivariate, and multiple logistic regression models were used to evaluate the association of the secondary transmission of *Shigella* infection with different factors. A total of 235 HH contacts of the index cases (n=78) were enrolled. We found 23 culture-confirmed *Shigella* among HH contacts, which represented ~10% secondary transmission. The quantitative PCR revealed 79% (n=73/92)

Shigella infection. No significant association was found with secondary *Shigella* transmission and other predicted risk factors. However, in multiple regression analysis, individuals with secondary-level education had a 4-fold higher risk of secondary *Shigella* transmission compared to those with higher levels of education. Additionally, individuals who did not use soap after defecation had a 7-fold higher risk of secondary *Shigella* transmission compared to soap users.Evidence generated from this study on secondary *Shigella* transmission and household-level risk factors will help to plan strategies to reduce risk of transmission in the communities.

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GENETIC FACTORS CONTRIBUTING TO DISEASE IN *SHIGELLA*

Charlotte E. Chong[1], Xiaoliang Ba[1], Rebecca Ji Bengtsson[2], P. Malaka De Silva[1], Mark A. Holmes[1], Karen Kotloff[3], Sharon M. Tennant[3], Kate S. Baker[1]

[1]University of Cambridge, Cambridge, United Kingdom, [2]University of Liverpool, Liverpool, United Kingdom, [3]University of Maryland, Baltimore, MD, United States

Shigella spp. are the leading bacterial cause of moderate-to-severe childhood diarrhoea in lower- and middle- income (LMICs) and the causative agent of shigellosis. Increasing antimicrobial resistance (AMR) and a lack of widely available licenced vaccine means *Shigella* is now classed by WHO as an AMR priority pathogen. Understanding pathogen-specific factors responsible for the manifestation of clinical disease is critical to inform future treatment and management strategies. To identify key genetic determinants associated with clinical disease we performed bacterial Genome Wide Association Studies (GWAS) on > 1000 *S. flexneri* and *sonnei* isolates from South Asia and sub-Saharan Africa collected during the Global Enteric Multicentre Study (GEMS), using case-control and severity score data. Our findings indicate that specific genotypes of *S. sonnei* and *S. flexneri* are more significantly associated with disease and clinical severity than others. Through bGWAS we identified multiple SNPs (*n*= 206) and accessory genes (*n*= 171) significantly associated with disease. Some accessory genome elements showed an epidemiological interaction with genotype, which subsequent laboratory investigation revealed was associated with an unstable virulence plasmid in *S. flexneri* Phylogroup 1 and variation in a transporter protein was shown to be associated with increased clinical severity in *S. sonnei.* These genetic factors might act as predictors of severe disease or be developed as novel therapeutic targets, and demonstrate the potential of bGWAS and functional microbiology to provide insight into genetic contributors of disease to support diagnostics, vaccine and drug development.

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ANTIBODY-MEDIATED PROTECTION AGAINST SHIGELLOSIS

Biana Bernshtein[1], Julia Zhiteneva[1], Galit Alter[2], Edward T. Ryan[3]

[1]Ragon Institute of MGH, MIT and Harvard, Cambridge, MA, United States, [2]Ragon Institute of MGH, MIT and Harvard, CAMBRIDGE, MA, United States, [3]MGH, Boston, MA, United States

Shigella is the second leading cause of diarrheal disease-related death in young children in low and middle income countries. Efforts to develop an effective *Shigella* vaccine have been hindered by the limited understanding of immunological correlates of protection against shigellosis. To address this gap, we developed and applied a systems approach to analyze antibody-mediated responses to *Shigella* across individuals living in Shigella endemic and non-endemic regions. Using this approach we interrogate antibody specificities, isotypes, Fc-receptor binding and antibody-mediated phagocytic-cell activation to construct high-resolution antibody profiles. First, we analyzed serum samples collected from individuals experimentally challenged with *Shigella* and found that functional IgG against *Shigella* virulence factor IpaB bind to Fc-receptors and activate neutrophils and monocytes to elicit protective phagocytosis. More recently, we analyzed *Shigella*-specific antibody responses over time in the context of endemic resistance or breakthrough infections in a *Shigella* high burden location. Here, we unraveled a novel functional role for oligo-polysaccharide-specific FcaR binding IgA, found in resistant individuals, that activates bactericidal neutrophil functions including phagocytosis, degranulation and reactive oxygen species production. Finally, we analyzed serum samples of adults and kids hospitalized with shigellosis, to elucidate age dependent differences in antibody mediated protection against severe shigellosis. Overall, our findings suggest that *Shigella*-specific antibodies protect individuals by binding to Fc receptors on the surface of phagocytic cells and leveraging bactericidal activities of phagocytes. These findings will assist in the development and evaluation of vaccines against *Shigella* and other enteric pathogens.

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DEVELOPMENT OF A SEROEPIDEMIOLOGY TOOL FOR *SHIGELLA*

Claire E. Munroe[1], Ashraful I. Khan[2], Fahima Chowdhury[2], Paul Kovac[3], Peng Xu[3], Polina Kamenskaya[1], Meagan Kelly[1], Jeshina Janardhana[1], Chanchal Wagh[1], Jessica C. Seidman[4], Kristen Aiemjoy[5], Firdausi Qadri[2], Edward T. Ryan[1], Richelle C. Charles[1]

[1]Division of Infectious Diseases, Massachusetts General Hospital, Boston, MA, United States, [2]Infectious Diseases Division, International Centre for Diarrhoeal Disease Research, Dhaka, Bangladesh, [3]National Institutes of Health, Bethesda, MD, United States, [4]Sabin Vaccine Institute, Washington, DC, United States, [5]Department of Public Health Sciences, University of California Davis School of Medicine, Davis, CA, United States

Shigella is the leading cause of diarrheal mortality worldwide, with children under the age of five in low-and-middle-income countries (LMICs) bearing the highest burden of disease. A robust pipeline of *Shigella* vaccines are in clinical development, however, our understanding of the Shigella burden in LMICs remains limited due to the lack of accurate rapid diagnostic tests. Such data are essential to inform vaccine introduction and evaluation of impact. A cost-effective, seroepidemiology tool for Shigella can potentially overcome these challenges. We have previously developed a seroepidemiology tool for enteric fever based on leveraging antibody decay kinetics of anti-HlyE post-infection to estimate seroincidence in cross-sectional serosurveys. To apply this model to Shigella, we used a multiplex bead-based assay to measure semi-quantitative IgG and IgA levels to IpaB and the O-specific polysaccharide of the four most prevalent Shigella serotypes (*Shigella sonnei* and *Shigella flexneri* (Sf) 2a, Sf 3a, and Sf 6) in blood samples. We modeled the longitudinal antibody kinetics in Bangladeshi cases with culture or PCR-confirmed Shigella infections and evaluated the antibody distributions across endemic communities in Asia and Africa. We found that all antigens could discriminate convalescent Shigella cases and healthy controls with Areas Under the Curve of 0.98-1.00. IgA responses to all antigens peaked by day 7, while IgG peaked by day 7 to 30, varying according to age. Responses were highest to the homologous OSP of the infecting serotype, with the highest cross-reactivity seen between Sf 2a and Sf 3a, which are structurally similar. Children under the age of 5 years exhibited lower peak antibody responses and faster antibody decay compared to older children and adults. Additionally, we observed varied reactivity to these antigens across endemic communities with differences by age. These data highlight the potential utility of this assay to estimate Shigella seroincidence in communities where culture- or PCR-based serosurveillance is limited or unavailable.

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PROTECTION CONFERRED BY A SINGLE DOSE OF TYPHOID CONJUGATE VACCINE AMONG BANGLADESHI CHILDREN AFTER FIVE YEARS OF VACCINATION: ANALYSIS OF A CLUSTER RANDOMIZED CONTROLLED TRIAL

Farhana Khanam[1], Firdausi Qadri[1], Yiyuan Zhang[2], Prasanta Kumar Biswas[1], Merryn Voysey[3], Yama F Mujadidi[2], Sarah Kelly[2], Amirul Islam Bhuiyan[1], Nazmul Hasan Rajib[1], Ismail Hossen[1], Nazia Rahman[1], Sadia Islam[1], Virginia E. Pitzer[4], John D. Clemens[5], Andrew J. Pollard[2], Xinxue Liu[2]

[1]International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh, [2]Oxford Vaccine Group, Department of Paediatrics, University of Oxford, Oxford, UK, UK, United Kingdom, [3]UKNIHR Oxford Biomedical Research Centre and Oxford University Hospitals NHS Foundation Trust, Oxford, UK, UK, United Kingdom, [4]Department of Epidemiology of Microbial Diseases and Public Health Modelling Unit, Yale School of Public Health, New Haven, Connecticut, United States of America, Connecticut, CT, United States, [5]International Vaccine Institute, Seoul, Republic of Korea, Korea, Korea, Republic of

Typhoid fever in low- and middle-income countries continues to be a serious public health concern. Large randomised controlled trials of typhoid conjugate vaccine (TCV) demonstrated high efficacy among children aged 9 months to 15 years after two years of receiving single dose of TCV. A cluster randomised controlled trial was conducted in Dhaka, Bangladesh, between 2018 and 2021. To generate data on medium-term protection, follow-up of the trial population was extended until August 2023 to evaluate the immunogenicity and protection of the vaccine 4-5 years after immunization. Japanese encephalitis (JE) vaccine was the control vaccine. Children who received JE vaccine were invited to receive TCV in 2021. Primary endpoint of the follow-up was to compare incidence of typhoid fever between children who received TCV in 2018/2019 and those in 2021 to evaluate the decline of vaccine efficacy (VE). An immunogenicity study was conducted on 1500 children. Previous TCV recipients (vaccinated in 2018/19) demonstrated higher risk of typhoid than recent recipients (vaccinated in 2021); the adjusted incidence rate ratio of 3.10 (95%CI: 1.39-6.95) indicated a decline in protection with a single-dose TCV after 4-5 years. The estimated VE after 4-5 years of follow-up was 50% (95%CI: -13-78). Using test-negative design (TND) analysis, the estimate of decline of VE was confirmed. Compared to non-vaccinees, VE was 84% (95%CI: 74-90) and 57% (95%CI: 39-70) in recent and previous TCV vaccinees, respectively. Over the study period, anti-Vi-IgG responses declined. Children who received vaccinations before the age of two showed the highest rate of decline. Negative correlation between age and decay of antibodies was also seen in subgroup analysis of VE, where the youngest age group (<7 years at fever visits) exhibited the fastest waning, with VE dropping to 31% (95%CI: -19-60) at 4-5 years post-vaccination. Children who received vaccine at a younger age showed the highest decline in immune responses and protection. To maintain protection against typhoid fever, a booster dose of TCV for children vaccinated under the age of two years could be recommended.

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SAFETY AND IMMUNOGENICITY OF A BIVALENT VACCINE AGAINST SALMONELLA TYPHI AND SALMONELLA PARATYPHI A: INTERIM DATA FROM A PHASE 1 RANDOMIZED CONTROLLED OBSERVER-BLIND, TRIAL AMONG HEALTHY ADULTS IN EUROPE

Usman N. Nakakana[1], Ilse de Coster[2], Marie-Annick Götze[2], Pierre Van Damme[2], Eleanna Sarakinou[1], Chiara Fineschi[1], Elisa Marchetti[1], **Mohammad A. AbdelGhany**[1], Kanchanamala Withanage[2], Francesco Berlandascorza[1], Giulia Luna Cilio[3], Alimamy Serry-Bangura[3], Iris Sarah De Ryck[3], Martina Carducci[1], Luisa Massai[1], Simona Rondini[1], Valentino Conti[1], Omar Rossi[1], Ashwani Arora[1]

[1]GSK Vaccines Institute for Global Health, Siena, Italy, [2]Centre for the Evaluation of Vaccination, University of Antwerp, Antwerp, Belgium, [3]GSK Vaccines Srl, Siena, Italy

Enteric fever remains a major cause of disability and death. In 2019, there were 13 million cases of enteric fever globally, 28% of these being caused by Salmonella Paratyphi A. There is a trend of increased incidence of S. Paratyphi A in parts of Asia, estimated as ~35% of cases in India and Nepal and >60% of enteric fever in China, with a similar trend of antimicrobial resistance. GVGH is developing a novel Typhoid and Paratyphoid A conjugate vaccine (bivalent Vi-CRM197+O:2-CRM197), for the prevention of both typhoid and paratyphoid A enteric fever in infants and older age groups. We present here the interim results from a first-time-in-human study aimed to evaluate the safety and immunogenicity of this candidate vaccine. Overall, 96 healthy adult participants were randomised 2:1 to receive 2 injections at 0 and 6 months, with the investigational product or comparator vaccines. There was a dose-escalation approach with 2 different doses (Low and Full, containing 5 µg Vi/ 5 µg O:2 and 25 µg Vi/ 25µg O:2, respectively), formulated with or without Alum. Interim analysis was performed 28 days after the first dose administration. Results showed no safety signals or concerns from the analysis of data collected up to 28 days after the first dose administration. Majority of Adverse Events (AEs) reported were of mild to moderate severity. No Serious AEs were reported. Both dose levels (with or without Alum) induced a robust immune response: 100% of the participants in the Low or Full Dose without Alum groups, 88.9% and 95.7% of participants in the Low and Full dose with Alum groups respectively, achieved Anti-Vi Ag IgG ≥4.3 µg/ml. 100% of the participants in the Low or Full Dose without Alum groups, 81.8% and 83.3% of the participants in the Low or Full Dose with Alum groups respectively, achieved at least 4-fold increase in Anti-O:2 IgG levels, with functionality against S. Paratyphi A confirmed using Serum Bactericidal Activity (SBA) assay. Based on these interim results, the clinical development with the Full dose without Alum formulation is progressing to phase 2 study in target population. Post dose-2 data are expected in Q3 2024.

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EFFECT OF BIANNUAL AZITHROMYCIN MASS DRUG ADMINISTRATION ON ENTERIC FEVER TRANSMISSION INTENSITY IN NIGER

Kristen Aiemjoy[1], Jessica C. Seidman[2], Ahmed M. Arzika[3], Kristina Wen-Jeh Lai[1], Ramatou Maliki[3], Amza Abdou[4], Denise Garrett[2], Claire Munroe[5], Abel Gonzalez[5], Leah Sukri[6], Elodie Lebas[7], Catherine Cook[7], Benjamin F. Arnold[7], Thomas M. Lietman[7], Kathleen M. Neuzil[6], Jason R. Andrews[8], Jeremy D. Keenan[9], Richelle C. Charles[5]

[1]University of California Davis, Davis, CA, United States, [2]Sabin Vaccine Institute, Washington, DC, United States, [3]Centre de Recherche et Interventions en Santé Publique, Niamey, Niger, [4]Nationale de Santé Oculaire, Niamey, Niger, [5]Massachusetts General Hospital, Boston, MA, United States, [6]Center for Vaccine Development and Global Health, Baltimore, MD, United States, [7]UCSF Francis I. Proctor Foundation, San

Francisco, CA, United States, [8]Stanford University School of Medicine, Palo Alto, CA, United States, [9]UCSF-Francis I. Proctor Foundation, San Francisco, CA, United States

Enteric fever, a systemic bacterial infection caused by *Salmonella enterica serovars* Typhi and Paratyphi, remains a significant health concern in many low- and middle-income countries, where it is primarily managed with antimicrobials. Historically, assessing the enteric fever burden has been challenging in regions lacking blood culture surveillance. New methods coupling specific serologic markers with modeled antibody decay trajectories from blood-culture-confirmed enteric fever patients now allow accurate estimation of the force of infection from cross-sectional serosurveys. This secondary analysis aimed to assess enteric fever incidence within a population devoid of blood culture surveillance and evaluate the impact of biannual azithromycin mass drug administration on exposure. The MORDOR study, a cluster-randomized, placebo-controlled trial, allocated 30 communities in rural Dosso, Niger, to receive biannual azithromycin or placebo. All children 1-59 months weighing >3.8kg were eligible for treatment. Annually, a random sample of 40 children from each community provided capillary blood samples collected on filter paper. Samples from the 2015 and 2020 surveys were tested for IgA and IgG antibodies against Hemolysin E using ELISAs. Seroincidence rates were estimated by maximizing the likelihood of the cross-sectional antibody response data based on age-specific antibody kinetics using the Serocalculator package in R. Samples from 1455 children were available for analysis, 449 from baseline and 1006 from year 5 after 10 rounds of treatment. The median age was 2.9 years (IQR 1.7-3.9). The force of infection increased from 1.35 (95% CI 1.2 - 1.5) per person-years in 2015 to 2.8 (95% CI 2.5 - 2.1) and 3.0 (95%CI 2.7-3.3) in the intervention and control groups, respectively, by 2020. These results demonstrate a substantial and increasing burden of enteric fever among young children in rural Niger, with biannual azithromycin showing no discernible impact on transmission intensity. Interventions including introduction of typhoid conjugate vaccine are urgently needed to reduce the burden of enteric fever in this population.

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MACHINE LEARNING CAN REVEAL GENOMIC SIGNALS ASSOCIATED WITH ANTIGENIC DISTANCE IN DENGUE VIRUSES

Ozan Kiratli[1], Lin Wang[2], Angkana T. Huang[2], Derek A. Cummings[3], Henrik Salje[2], Matthew A. Conte[1]

[1]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [2]University of Cambridge, Cambridge, United Kingdom, [3]University of Florida, Gainesville, FL, United States

Dengue virus (DENV), a mosquito-borne flavivirus with four distinct serotypes (DENV1-4), affect between 100-400 million people each year. Given that dengue virus spreads through infected mosquitoes (Aedes aegypti or Ae. albopictus) prevalent in tropical and sub-tropical areas, nearly half of the world population faces the threat of contracting DENV. A previously published antigenic cartography map encompassing 348 Thailand dengue viruses over two decades discovered oscillating patterns in DENV antigenicity. One notable oscillating pattern corresponding with the magnitude of DENV epidemics in Thailand. However, the genetic processes underlying antigenic shifts remain undiscovered. Expanding on this previous work, we used random forest machine learning models to explore mutational space of DENV and the distances from the antigenic cartography to determine the genes and specific mutations associated with to the variance in antigenic distance First, we compared the prediction power of different genetic distance models and found that position-wise nucleotide pairs performed best. Next, we found that genetic signals in NS5, NS3, and E proteins were best able to predict antigenic distance. Finally, we used these findings to model epidemic outbreak prediction. To increase prediction power, we also tested our models using deep learning architecture. Our research identifies the signals in the DENV genome corresponding to antigenic changes, which cannot be identified through traditional statistical methods, such as linear regression. While this research focuses on DENV, our methodology exhibits versatility for application to other viruses for which the antigenic cartography maps are present. The utilization and ongoing development of this model alongside with other machine learning techniques are poised to empower researchers in anticipating the shifts in antigenicity through analyzing viral genomes. This proactive stance will facilitate preparedness for potential outbreaks impacting global populations and foster the strategic development of vaccines to combat various diseases.

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GENOMIC EPIDEMIOLOGY OF ARBOVIRUSES REVEALS NEW VIRUS INTRODUCTIONS AND SIMULTANEOUS VIRUS CIRCULATION DURING DENGUE AND CHIKUNGUNYA OUTBREAKS IN BRAZIL

Lívia Sacchetto[1], Beatriz Marques[1], Victoria Bernardi[1], Victor Hernandes[1], Igor Teixiera[1], Andreia Negri[2], Nikos Vasilakis[3], Mauricio Nogueira[1]

[1]Faculdade de Medicina de Sao Jose do Rio Preto, Sao Jose do Rio Preto, Brazil, [2]Departamento de Vigilancia Epidemiologica, Sao Jose do Rio Preto, Brazil, [3]The University of Texas Medical Branch, Galveston, TX, United States

Viral genomic surveillance is crucial for epidemic response and public health preparedness, as well as for providing insights into virus transmission patterns and dynamics. Between 2019 and 2024, we conducted an active arbovirus investigation on 8,000 samples from patients with acute febrile illness from a dengue hyperendemic municipality in Sao Paulo, Brazil, identifying 1,650 positive samples. Full-genome deep sequencing and phylogenetic analyses of approximately 1,000 samples confirmed the prevalence of DENV-2 genotype III from 2019 to 2020. In 2021, we observed a serotype replacement, with DENV2-III (Asian-American) replaced by DENV-1 genotype V, which remains the prevalent serotype to date. However, at the beginning of 2024, we detected circulation of DENV-2 genotype II (Cosmopolitan) and demonstrated an increase in the number of cases caused by this serotype. Furthermore, we characterized autochthonous cases of DENV-3 genotype III in 2024, emphasizing the resurgence of DENV-3 after 15 years of absence. The molecular surveillance of CHIKV revealed the region's most significant CHIKV outbreak, with increased virus detection since December 2023. Phylogenetic analysis classified all the genomes as ECSA lineage, closely related to strains from the Northeast and Southeast of Brazil. We have also analyzed the circulating viruses during these years correlating it with epidemiological and clinical data. We demonstrated that differential diagnosis for DENV serotypes and other arboviruses is essential to understand the epidemiological landscapes in areas with simultaneous circulation of viruses with similar clinical profiles. Our results stress the importance of sustainable active arbovirus surveillance to detect new introductions and ensure rapid deployment of control strategies to mitigate outbreaks in regions with high epidemic potential.

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SEROLOGICAL AND GENETIC CHARACTERIZATION OF THE DENGUE VIRUS SEROTYPE 3 (DENV-3) INFECTING CHILDREN'S POPULATIONS DURING A DENGUE OUTBREAK IN MERIDA, MEXICO

Henry Nelson Puerta Guardo[1], Hannah C. Dakanay[2], K. Jacqueline Ciau[1], Manuel Alejandro Parra Cardeña[1], James Earnest[3], Gloria Barrera Fuentes[1], Oscar D. Kirstein[4], Azael D. Che Mendoza[1], J. Kevin Yam[1], Mathew Collins[5], Daniel Espinoza[5], Pablo Manrique Saide[1], Norma Pavía Ruz[1], Guadalupe Ayora Talavera[1], Anne L. Piantadosi[6], Gonzalo Vazquez Prokopec[3]

[1]Universidad Autonoma de Yucatan, Merida, Mexico, [2]Department of Pathology and Laboratory Medicine, Emory University, Atlanta, GA, United States, [3]Department of Environmental Sciences, Emory University, Atlanta, GA, United States, [4]Dept. of Environmental Sciences, Emory University, Atlanta, GA, United States, [5]Division of Infectious Diseases, Department

of Medicine, Emory University, Atlanta, GA, United States, [6]Department of Pathology and Laboratory Medicine, Emory University School of Medicine, Atlanta, GA, United States

The dengue virus (DENV, *Flaviviridae* family), one of the most important mosquito-borne viruses that causes significant morbidity and mortality every year around the word. While DENV exists as four antigenically distinct serotypes (1 to 4), these serotypes are genetically clearly distinct "genotypes" which demonstrate varying pathogenicity and infectivity. DENV strains currently circulating in Mexico are not well studied at either serological or molecular level. Here, we performed a serological and molecular characterization of DENV strains from a dengue outbreak that affected children living in the city of Merida, Yucatan in 2023. Briefly, during an active surveillance phase (July and December 2023), 163 out of 536 (30.4%) clinically suspected febrile cases for arbovirus infection were identified as DENV-RNA positive by RT-qPCR. Of these, 30% (49/163) underwent full DENV genome sequencing using a combination of metagenomic library preparation and reference-based assembly. Preliminary molecular analyses identified all sequences as DENV 3 genotype III and were closely related to samples from the U.S, Cuba, and Brazil collected in 2023. Phylogenetic analysis revealed three distinct clades that differed by up to 3% at the nucleotide level and clustered independently on a maximum likelihood tree, suggesting that the outbreak was not due to a single new introduction. Additionally, screening of neutralizing antibody responses (NT_{50}) using a set of these DENV (PCR+) samples identified a higher prevalence of neutralizing antibodies mainly against DENV-1 and -2 and ZIKV but not DENV-3 (NT_{50} titer<1:20-100) suggesting that flavivirus cross-reactive immunity might not be equally protective against all four DENV serotypes. As DENV disease outcome is determined by complex interactions between immunopathologic, viral, and human genetic factors, understanding the genetic and serological variations elicit during DENV infections in endemic areas have important implications for the emerging of future outbreaks as well as the evaluation of dengue vaccines, and vector control trials.

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GENOMIC SURVEILLANCE OF DENGUE VIRUS FROM AN ACUTE FEBRILE ILLNESS STUDY IN EL SALVADOR, 2022-2023

Alexander R. Kneubehl[1], Avery A. Kaye[1], Elías Aguilar[2], Kenni Campos[1], Delmy Lisseth Recinos[2], Sarah M. Gunter[1], Gilberto A. Santiago[3], Jorge L. Muñoz-Jordán[3], Rafael Chacon[4], Beatriz Lopez[4], Emily Zielinski-Gutierrez[4], Rhina Dominguez[2], Xochitl Sandoval[2], Kristy O. Murray[1], Shannon E. Ronca[1]

[1]Baylor College of Medicine, Houston, TX, United States, [2]National Institute of Health of El Salvador, San Salvador, El Salvador, [3]Division of Vector-Borne Diseases, Centers for Disease Control and Prevention, San Juan, PR, United States, [4]Centers for Disease Control and Prevention. Central America Region, Guatemala City, Guatemala

Recent dengue virus (DENV) epidemics in Central and South America highlight the need for improved surveillance, particularly regarding viral genomic surveillance. El Salvador is a Central American country where data regarding which DENV serotypes are circulating are sparse, and circulating DENV genotypes are largely unknown. To address the current gap in knowledge and to supplement El Salvador Ministry of Health's surveillance, DENV surveillance was conducted as part of an acute febrile illness study. Samples from acutely febrile patients were collected from two study sites located in the Santa Ana department of El Salvador from 2022-2023. Whole blood samples were screened for DENV using a quadraplex real-time PCR assay to define the serotype. A total of 134 samples tested positive for DENV in our study and included all four serotypes, with the majority positive for DENV-4 (70.9%), followed by DENV-1 (22.4%), DENV-3 (5.2%), and DENV-2 (1.5%). These samples were further investigated through viral genomic sequencing using a tiled amplicon PCR assay coupled with Oxford Nanopore Technologies' MinION platform. These data were analyzed using the viralrecon Nextflow pipeline to generate the genome sequences, which were genotyped using Genome Detective's Dengue Virus Typing Tool. Initial analysis revealed that DENV-4 samples were genotype IIB, consistent with reports from neighboring countries. Phylogenetic analysis of the DENV-4 samples in the context of all other DENV-4 sequences on GenBank from 2022-2024 indicated a monophyletic clade with distinct subclades. These subclades suggested circulation within El Salvador however two subclades were also related to autochthonous DENV-4 isolates from Florida, USA (2022 and 2023) and two Nicaragua isolates (2022). Work is ongoing to incorporate geospatial analysis with phylogenetic information to investigate how the viruses are circulating in Santa Ana department. This work is key to understanding the molecular epidemiology of DENV in El Salvador, which can be used to define introduction events and inform countermeasure development and deployment.

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ECOLOGICAL AND GENETIC DETERMINANTS OF WEST NILE VIRUS PERSISTENCE IN FORT COLLINS, COLORADO

Robert Tobias Koch, Gregory Ebel

Colorado State University, Fort Collins, CO, United States

Despite being a semi-arid environment, Fort Collins, CO has emerged as a prominent hot spot for West Nile Virus (WNV) in the United States, with the 2023 season being one of the worst on record. To explore factors that contribute to successful introductions and persistence of West Nile virus in Fort Collins, we constructed a maximum likelihood time-resolved phylogenetic tree from 671 sequences derived from WNV-positive mosquito pools collected and sequenced from Fort Collins' surveillance program combined with 2,595 sequences from the rest of the United States. We found 91 discrete introductions of West Nile virus into Fort Collins with an average local persistence of 2.72 years. To investigate determinants of WNV persistence we developed a linear regression model. This preliminary analysis found a negative association between the persistence of West Nile virus and the year of introduction (estimate -0.41, CI 95%; -0.78, -0.04) indicating conditions become less favorable for WNV persistence with each passing year. These results are intriguing, given the rise in the incidence of West Nile cases despite a decrease in the persistence of new introductions, indicating that existing introductions have some advantages over novel introductions. A two-pronged investigation is ongoing to investigate these findings further. Firstly, computational modeling will scrutinize weather patterns, land types, avian immunity, and geographical locations during the introduction of these strains. Secondly, experimental analysis will explore strain phenotypes, focusing on replication under varying conditions. This interdisciplinary approach seeks to uncover critical insights into the factors influencing the introduction and persistence of WNV strains within a defined geographical focus. By combining advanced phylogeographic analyses with experimental investigations, we aim to inform targeted control strategies and contribute to a broader understanding of WNV transmission dynamics.

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GENOMIC EPIDEMIOLOGY OF RIFT VALLEY FEVER VIRUS INVOLVED IN THE 2018 & 2022 OUTBREAKS IN LIVESTOCK IN RWANDA

Isidore Nsengimana[1], John Juma[2], Method N. Gasana[3], Emmanuel Hakizimana[4], Claude M. Muvunyi[4], Jean N. Hakizimana[5], Gillian Eastwood[6], Augustino Chengula[1], Bernard Bett[2], Samuel O. Oyola[2], Christopher J. Kasanga[1]

[1]Sokoine University of Agriculture, Morogoro, Tanzania, United Republic of, [2]International Livestock Research Institute, Nairobi, Kenya, [3]Rwanda Agriculture and Animal Resources Development Board, Huye, Rwanda, [4]Rwanda Biomedical Center, Kigali, Rwanda, [5]SACIDS Foundation for One Health, Morogoro, Tanzania, United Republic of, [6]Virginia Polytechnic Institute and State University, Blacksburg, VA, United States

Rift Valley Fever (RVF) caused by RVF virus (RVFV) is a mosquito-borne transboundary zoonosis which is endemic in many African countries and the Arabian Peninsula. This phlebovirus was first molecularly confirmed in Rwanda's livestock in 2012 and since then sporadic cases have been reported almost every year until 2018 when the first largest outbreak occurred followed by the second in 2022. The objective of this study was to determine the genetic characteristics of the circulating lineages and their

ancestral origin. Following the emergency of the 2022 RVF outbreak and the need to protect the public health, Rwanda established six temporary RVF testing centers to carry out the pre-slaughter screening by RT-qPCR of all animals arriving at slaughterhouses for meat production. A total of 157 initially RT-qPCR-confirmed livestock samples from 3 testing centers and 37 archived farm-collected samples from the 2018 outbreak were obtained via the Rwanda Veterinary Laboratory for virus sequencing. Overall, two whole genome sequences for the 2018 outbreak and 36, 41 and 38 virus sequences for S, M and L RVFV genome segments, respectively, from the 2022 outbreak were generated. Both Maximum Likelihood and Bayesian-based phylogenetic analyses as well as lineage assignment were performed. The findings demonstrated that viruses belonging to a single lineage C circulated during both outbreaks and shared a recent common ancestor with viral strains isolated in Uganda between 2016 and 2019, which were also genetically linked to the 2006/2007 largest East Africa RVF outbreak reported in Kenya, Tanzania and Somalia. Along-side the wild-type viruses, genetic evidence of RVFV Clone 13 vaccine virus in slaughterhouse animals was also found, emphasizing the importance of reinforcing the protection of public health during RVF outbreak as well as during vaccination campaigns using live-attenuated vaccines. The results provided further evidence of the ongoing cross-border widespread of RVFV lineage C in Africa and underscored the need for efficient national and international multi-disciplinary collaboration to fight and control this emerging hemorrhagic fever.

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SINGLE CELL TRANSCRIPTIONAL PROFILING OF DRY AND WET SEASON *PLASMODIUM FALCIPARUM*

Lasse Votborg-Novél1 Votborg-Novél[1], Martin Kampmann[1], Manuela Carrasquilla[1], Georgia Angeli[1], Hamidou Cisse[2], Safiatou Duombo[2], Didier Doumtabe[2], Gabriela M. Guerra[3], Kassoum Kayentao[2], Aissata Ongoiba[2], Mir-Farzin Mashreghi[3], Thomas D. Otto[4], Peter D. Crompton[5], Boubacar Traore[2], **Silvia Portugal**[1]

[1]Max Planck Institute for INfection Biology, Berlin, Germany, [2]Malaria Research and Training Center, Bamako, Mali, [3]DRFZ, Berlin, Germany, [4]University of Glasgow, Glasgow, United Kingdom, [5]NIH, Rockville, MD, United States

Persistence of malaria parasites in asymptomatic hosts is crucial in areas of seasonal transmission, where *P. falciparum* bridges wet seasons months apart. In the dry season, infected red blood cells (iRBCs) exhibit extended circulation with reduced cytoadherence, promoting splenic clearance of iRBCs and hindering parasitaemia increase. What determines longer circulation of iRBCs and asymptomatic persistence remains unknown. Here, we investigated transcriptional differences between parasites of similar developmental stage infecting asymptomatic Malian children at the transition from the dry to the wet season, and children showing symptoms of clinical malaria in the wet season. We generated a single cell RNAseq reference atlas of a lab-adapted line of *P. falciparum*, containing over 27000 iRBCs, capturing all asexual stages and developing gametocytes, allowing to infer pseudotime along the 48h asexual cycle. Then, we performed single cell RNAseq of iRBCs from 6 asymptomatic carriers at the transition from the dry to the wet season, and from 9 clinical malaria cases in the wet season, obtaining ~3600 and ~23000 *P. falciparum*-iRBCs, respectively, which were grouped by hours post invasion (hpi) through projection to the single cell reference atlas. Pseudotime of *P. falciparum*-iRBCsin clinical malaria samples ranged from 3.9 to 24.3 hpi (mode: 8.7 hpi), while the dry season parasites were 4.4 to 27.1 hpi (mode: 11 hpi). We then compared gene expression of 10 groups of cells with parasites between 6 and 11.25 hpi containing >100 cells of equivalent pseudotime from the dry season and malaria-causing parasites. We found 26 differentially expressed genes (DEGs, $p<0.05$ and fold change >1.5) across groups, which likely contribute to persisting infections in the dry season. DEGs upregulated in the dry season (n=14) encode parasite proteins exported to the host erythrocyte, and are linked to the remodelling of the host cell required for efficient cytoadhesion. Our data will help clarify the molecular mechanisms used by *P. falciparum* to adjust cytoadhesion and present the decreased virulence observed in persisting parasites during the dry season.

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THE DYNAMICS OF PARASITE GROWTH IN *PLASMODIUM FALCIPARUM* AND *P. KNOWLESI* CO-CULTURES

Jeremy S. Goodwin-Gower, Jenny M. Peters, Hayley E. Mitchell, Stacey Llewellyn, Fiona H. Amante, Bridget E. Barber

QIMR Berghofer Medical Research Institute, Herston, Australia

In Malaysia, the incidence of human cases of *Plasmodium knowlesi* malaria has increased alongside the elimination of *P. falciparum* and *P. vivax*. However, whether the elimination of *P. falciparum* and/or *P. vivax* has contributed directly to the increase of *P. knowlesi* is unknown. We therefore utilised co-culture competition assays to investigate the *in vitro* interaction between *P. knowlesi* and *P. falciparum. P. knowlesi/P. falciparum* mono- and co-cultures were established in 6-well plates and maintained under standard conditions for 14 - 28 days. Total parasitemia was monitored by flow cytometry and maintained between 1-8%; samples were taken daily for digital (dPCR) targeting the 18S gene of each species to quantify the parasitaemia of each species. In the co-cultures, *P. falciparum* and *P. knowlesi* were seeded at *P. falciparum*: *P. knowlesi* parasitemia ratios of 90:10, 80:20, 70:30, 60:40 and 50:50. In all cases *P. falciparum* rapidly suppressed growth of *P. knowlesi*, accounting for >95% of parasites by day 10 and maintaining this dominance until the end of the assays. In subsequent experiments *P. falciparum/P. knowlesi* co-cultures were established in Transwell plates to prevent direct contact between the species. Growth rates of *P. falciparum* and *P. knowlesi* in the Transwell co-cultures were comparable to the growth rates of each species in the monocultures, with no inhibitory interaction observed. In summary, we have shown that *P. falciparum* suppresses growth of *P. knowlesi in vitro*, suggesting that *P. falciparum* may play a role in maintaining low prevalence of *P. knowlesi* in co-endemic regions, and that the removal of this suppressive effect may contribute to an increase in cases of knowlesi malaria. Further experiments evaluating the mechanisms of this inter-species interaction are underway, and data will be presented.

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DEFINING THE ROLE OF PIPECOLIC ACID IN THE ENCEPHALOPATHY OF CEREBRAL MALARIA

Cheryl Sachdeva[1], Akua Mensah[1], Maxwell Rubin[2], Kyu Rhee[3], Anas Saleh[3], Terrie Taylor[4], Karina Garcia[1], Stephen Ray[5], Nicole O'Brien[6], Karl Seydel[7], **Johanna Daily**[1]

[1]Albert Einstein College of Medicine, Bronx, NY, United States, [2]Tulane University, New Orleans, LA, United States, [3]Weill Cornell College of Medicine, New York, NY, United States, [4]Michigan State University, Lansing, NY, United States, [5]University of Oxford, Oxford, United Kingdom, [6]Ohio State University, Columbus, OH, United States, [7]Michigan State University, Lansing, MI, United States

Encephalopathy is the hallmark of cerebral malaria (CM), however the mechanism of coma is unknown. Our previous studies demonstrated elevated pipecolic acid (PA) levels in the blood of children with CM compared to children with mild malaria. We also found elevated PA levels in the brains of mice in the experimental cerebral malaria (ECM) model. Here we demonstrate that both *P. falciparum* and *P. berghei* ANKA generate PA using 13C-lysine metabolic tracing studies. To determine if other neuromodulatory metabolites are present in the brain in the animal model, we conducted whole metabolome analysis of ECM brains versus uninfected, and the only neuromodulatory molecule, pipecolic acid was increased ($p=0.0002$). ECM brains also demonstrated an increase in the lactate:pyruvate ratio ($p=0.003$), compared to controls suggestive of ischemia. Cerebral spinal fluid in children with CM compared to non-malaria controls demonstrated distinct metabolomic signatures, without differences in PA levels between the groups. To directly test if PA induces a decline in neurologic function, we administered PA subcutaneously into mice and observed a reversible neurological decline using the rapid murine coma and behavior scale ($p=0.02$). Our studies demonstrate that *Plasmodium* generates PA, brain metabolism during ECM is distinct and that PA induces a rapid and reversible effect on behavior.

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CEREBRAL MALARIA, THE BLOOD-BRAIN BARRIER AND BEYOND. THE IMPACT OF ICAM-1/EPCR DUAL BINDING PARASITES ON BARRIER DYSFUNCTION

Yvonne Adams, Katrine Zeeberg, Nanna Dalgaard, Anja R. Jensen

University of Copenhagen, Copenhagen, Denmark

Cerebral malaria (CM) is characterised by parasite sequestration in the brain microvasculature triggering inflammatory responses, resulting in localised changes in surface receptor expression such (increased ICAM-1/decreased EPCR) culminating in increased barrier permeability and loss of tight junctions. In approximately 15-20% of cases, the disease is fatal with death occurring due to compression of the brain stem via sudden, excessive swelling of the brain. The exact mechanisms which trigger this swelling are not fully understood, nor is the impact of the adhesion of specific subsets of parasites on the blood-brain-barrier (BBB). We previously showed how ICAM-1/EPCR dual binding parasite can cross the blood brain barrier using 3D blood brain barrier organoids to model the human blood brain barrier, and that these dual binding parasites caused the organoids to swell in a PfEMP1 dependent manner. He we present further evidence of the negative impact of the interaction between dual binding PfEMP1s using defined parasites such as HB3VAR03 (ICAM-1/EPCR) and IT4VAR13 (ICAM-1/CD36) where binding impact not only the tight-junctions, but transcellular transport as evidenced by reduced efflux by P-glycoprotein. To investigate the secretory impact of parasites, we challenged the blood-brain barrier organoids with conditioned media, and again found differential responses suggesting heterogeneity in the secretory components of parasites to trigger increased barrier permeability. Taken together, these data highlight the complex nature of barrier dysfunction and interplay between parasite binding, secretory products, and immune responses contribute to barrier dysfunction.

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HETEROGENEITY IN PATHOGENIC BRAIN SEQUESTERED CD8$^+$ T CELLS DURING EXPERIMENTAL CEREBRAL MALARIA REVEALED BY SINGLE CELL SEQUENCING

Miranda Oakley[1], Micah P. Fletcher[2], Victoria Majam[1], Hong Zheng[1], Gregory K. Tharp[2], Mark KuKuruga[1], Sanjai Kumar[1]

[1]FDA, Silver Spring, MD, United States, [2]Emory, Atlanta, GA, United States

Brain sequestered CD8$^+$ T cells play a prominent role in the pathogenesis of experimental cerebral malaria (ECM) in mice and are found in the cerebrovasculature of young children with cerebral malaria. In mice, CD8$^+$ T cells accumulate in the brain during the effector phase of a *Plasmodium berghei* ANKA (*Pb–A*) infection and promote pathogenesis by inducing apoptosis of endothelial cells of the blood brain barrier. However, the molecular events associated with this CD8$^+$ T cell-mediated pathogenesis remain poorly understood. We performed single cell sequencing and bioinformatic analysis of brain sequestered CD8$^+$ T cells isolated from perfused tissue of *Pb–A* infected moribund and non-moribund mice and uninfected mice. We find that 42 genes associate with disease symptoms and 17 genes associate with *Pb–A* infection. Importantly, cluster analysis revealed that the brain sequestered CD8$^+$ T cells consists of two large clusters and ten small but distinct clusters indicating a large degree of heterogenicity in these cell populations during ECM. Furthermore, one of the large clusters is enriched in moribund mice and therefore associates with the symptoms of disease and the other large cluster preferentially expresses IFN–γ, a known biomarker of ECM pathogenesis. Lastly, we have created a transcriptional atlas of brain sequestered CD8$^+$ T cells by plotting the average expression and percentage of CD8$^+$ T cells that express various cytokines, chemokines, transcription factors, cell surface molecules such as checkpoint inhibitors, and other relevant molecules. The results of this study are being used to identify and test promising targets for adjunctive therapy to reduce the high mortality of human CM.

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METABOLITES ASSOCIATED WITH CEREBRAL MALARIA PATHOGENESIS AND PROTRACTED PRO-THROMBOTIC PROPENSITY IN CHILD SURVIVORS OF CEREBRAL MALARIA

Katherine Dobbs[1], Kenzie Birse[1], Sausan Azzam[1], Laura Noel-Romas[1], David Midem[2], Paula Embury[1], Yelenna Skomorovska-Prokvolit[1], Arlene Dent[1], Adam Burgener[1], Sidney Ogolla[2], James Kazura[1]

[1]Case Western Reserve University, Cleveland, OH, United States, [2]Kenya Medical Research Institute, Kisumu, Kenya

The pathogenic mechanisms of cerebral malaria and its sequelae remain poorly understood. Our previous transcriptomic and biomarker studies suggest that survivors of cerebral malaria have a prolonged period of risk for thrombosis, defective fibrinolysis, and vascular endothelium dysfunction, which may underlie post-recovery clinical sequelae. For this project, we aimed to determine the metabolic mediators associated with these biological processes. We collected plasma from Kenyan children aged 1-10 yrs with cerebral malaria (n=12), uncomplicated malaria (n=10), and acute febrile non-malarial illness (n=11) at presentation and 6 wks after clinical recovery. Untargeted metabolomics was performed using reverse phase C18 and hydrophilic interaction liquid chromatography mass spectrometry. MetaboAnalyst was used to analyze differential abundance of 1,612 metabolites. At presentation, children with cerebral malaria had significantly elevated levels of Neuroprotectin D1, a docosanoid metabolite derived from docosahexaenoic acid (DHA) in response to oxidative stress. Acute cerebral malaria was associated with decreased levels of citrulline, a known marker of gut barrier integrity, as well as with increased levels of gut microbial-derived metabolites phenylacetyl glycine (PAGLy) and indole-3-lactic acid (ILA), which directly correlated with Tie-2, a marker of endothelium dysfunction. At 6 wks, children with cerebral malaria had persistently elevated levels of metabolites associated with protracted coagulation and endothelial dysfunction, such as tryptophan pyrolysis product P2 (TrpP2), gamma-glutamylglutamic acid (GGA), and beta-aminoproprionitrile. TrpP2 and GGA directly correlated with markers of platelet activation. In summary, these data suggest that metabolic mediators of cerebral malaria pathogenesis may include gut microbial-derived metabolites and metabolites associated with pro-thrombotic propensity. We are currently validating our findings using quantitative targeted metabolomics and will correlate results with plasma markers of hemostasis, cerebral injury, and gut barrier integrity.

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TRANSCRIPTOMIC DATA ANALYSIS IDENTIFIES ACTIVE HOST UBIQUITIN-PROTEASOME PATHWAY IN KENYAN CHILDREN WITH SEVERE MALARIAL ANEMIA

Samuel B. Anyona[1], Qiuying Cheng[2], Sharley A. Wasena[3], Shamim W. Osata[4], Evans Raballah[5], Ivy Hurwitz[2], Clinton O. Onyango[3], Philip D. Seidenber[6], Benjamin H. McMahon[7], Christophe G. Lambert[8], Kristan A. Schneider[9], Collins Ouma[3], Douglas J. Perkins[2]

[1]Department of Medical Biochemistry, School of Medicine, Maseno University, Maseno, Kenya, [2]Department of Internal Medicine, Center for Global Health, University of New Mexico, Albuquerque, NM, United States, [3]Department of Biomedical Sciences and Technology, School of Public Health and Community Development, Maseno University, Maseno, Kenya, [4]University of New Mexico-Kenya Global Health Programs, Kisumu and Siaya, Kenya, [5]Department of Medical Laboratory Sciences, School of Public Health, Biomedical Sciences and Technology, Masinde Muliro University of Science and Technology, Kakamega, Kenya, [6]Department of Emergency Medicine, School of Medicine, University of New Mexico, Albuquerque, NM, United States, [7]Theoretical Biology and Biophysics Group, Theoretical Division, Los Alamos National Laboratory, Los Alamos, NM, United States, [8]Department of Internal Medicine, Division

of Translational Informatics, University of New Mexico, Albuquerque, NM, United States, [9]Department Applied Computer and Bio-Sciences, University of Applied Sciences Mittweida, Mittweida, Germany

Malaria remains a significant global public health challenge, accounting for 249 million annual cases and 608,000 deaths. In western Kenya, severe malaria manifests primarily as severe malarial anemia [SMA, hemoglobin (Hb)<6.0 g/dL]. Our previous studies reported differential expression of host ubiquitination genes in children with malarial anemia, and temporal changes of ubiquitination gene expression following ingestion of hemozoin. Here, we report on transcriptome analysis of 1,761 genes that constitute the ubiquitin-proteasome system (UPS) in children with non-SMA (Hb≥6.0g/dl, n=41) and SMA (n=25), presenting at a rural Hospital in western Kenya. Total RNA isolated from peripheral blood of every child was sequenced using Illumina® NovaSeq 6000. Reads were mapped to the human genome (GR-Ch38) and differential gene expression analysis was performed using the EdgeR package. Gene Ontology (GO) enrichment analysis was performed to identify key domains, and the MetaCore™ network-building algorithm was used to identify functional interactions of differentially expressed genes (DEGs). A total of 659 UPS genes were differentially expressed in children with SMA versus non-SMA (*p*adj≤0.050): 404 up- and 255 down-regulated. GO enrichment analysis identified proteasome-mediated ubiquitin-dependent protein catabolic process (*p*adj=1.173E-76); ubiquitin ligase complex (*p*adj=1.290E-78); and ubiquitin-like protein ligase activity (*p*adj=3.321E-182) as top pathways in biological process (BP), cellular components (CC) and molecular functions (MF), respectively. Functional enrichment analysis using MetaCore™ identified protein modification by small protein conjugation or removal (*p*adj=3.781E-189) and ubiquitin-like protein transferase activity (*p*adj=7.782E-119) as top GO processes in BP and MF, respectively, with localization of the processes mapped to the cell cytosol (*p*adj=1.024E-94). Notably, the proteolysis ubiquitination pathway emerged as the most significantly enriched map (*p*adj=2.832E-19). Collectively, these results highlight active ubiquitin-proteasome-pathway in SMA pathogenesis.

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TO TEST OR NOT TO TEST: WHAT DETERMINES WHETHER CLIENTS TEST FOR MALARIA IN THE PRIVATE SECTOR IN KENYA AND NIGERIA?

Meley Woldeghebriel[1], Indrani Saran[2], David Arthur[3], Nwamaka Eze[4], Theodoor Visser[5], Jeremiah Laktabai[6]

[1]CHAI, Kampala, Uganda, [2]Boston College School of Social Work, Boston, MA, United States, [3]Duke University Department of Biostatistics & Bioinformatics, Durham, NC, United States, [4]CHAI, Lagos, Nigeria, [5]CHAI, Boston, MA, United States, [6]Academic Model Providing Access to Healthcare, Eldoret, Kenya

Nearly half of suspected malaria clients in Sub-Saharan Africa first seek care in the private informal sector. A randomized controlled trial was conducted among private retail outlets in Lagos, Nigeria and Western Kenya to assess whether anti-malarial price subsidies conditional on testing positive for malaria could improve the targeting of first-line antimalarials (malaria rapid diagnostic tests (mRDTs) were made available for purchase in the outlets during the study period). Although the subsidies did not improve targeting of antimalarials, we observed a large difference in malaria testing rates across study sites: 49% clients in Kenya tested, compared to 23% in Nigeria. We analyzed exit interview data from 2,441 clients in Nigeria and 5,696 clients in Kenya collected between August 2021-February 2023 to assess factors that might explain these differences in uptake of testing. This was supplemented with qualitative data collected from six focus group discussions (FGD) with outlet owners in Nigeria and four FGDs in Kenya. Client demographics were similar across both study sites. Levels of confidence in mRDTs were also similar in both sites: more than 95% of test-positive clients believed the test result was correct, while only 68% of test-negative clients in Kenya and 64% of test-negative clients in Nigeria believed the test result was correct. The proportions of untested clients who believed their illness was malaria were also similar in both countries (87% in Kenya, 83% in Nigeria). In both Nigeria and Kenya, the most common reason for not testing was that the client was sure the illness was malaria (38% in Nigeria and 17% in Kenya). However, one major difference between the countries was that 25% of untested clients in Nigeria said the test was not offered, compared to only 7% in Kenya. Moreover, FGDs indicated that while outlet owners understood the benefit of testing they had concerns about the accuracy and reliability of mRDTs as did many of their clients. Our results suggest that in addition to making testing more widely available, there is a need to increase awareness among outlet owners and clients about the accuracy and value of malaria testing.

6075

PERFORMANCE AND UTILITY OF HIGHLY SENSITIVE MALARIA RAPID DIAGNOSTIC TEST FOR DETECTING INFECTIONS THAT AFFECT HEALTH AND TRANSMISSION IN SCHOOL-AGED CHILDREN IN SOUTHERN MALAWI

Meredith G. Sherman[1], Godfrey Mvula[2], Karl B. Seydel[3], Lauren M. Cohee[4]

[1]Global Health Initiative, Children's National Hospital, Washington, DC, United States, [2]Kamuzu University of Health Sciences, Blantyre, Malawi, [3]Department of Internal Medicine, College of Osteopathic Medicine, Michigan State University, East Lansing, MI, United States, [4]Department of Clinical Sciences, Liverpool School of Tropical Medicine, Liverpool, United Kingdom

Over 325 million African children under the age of 14, including 200 million school-aged children, are at risk of malaria infection in endemic areas. Infections characterized by low levels of parasitemia are traditionally described as 'asymptomatic.' However, such infections can contribute to anemia-related cognitive deficits and decreased educational attainment in children. We aimed to assess the use of a high sensitivity rapid diagnostic test (hs-RDT) for detecting low density infections and to assess for evidence of possible *Plasmodium falciparum* histidine protein 2/3 (pfhrp2/3) gene deletions. We evaluated a hs-RDT (NxTek Eliminate Malaria Pf, Abbott Diagnostics Korea Inc.), a conventional-RDT (co-RDT) (SD Bioline Ag Pf, Abbott Diagnostics Korea Inc.), and a combination RDT (Biocredit Malaria Ag Pf (pLDH/HRP2), RapiGEN Inc.). All RDTs were compared to quantitative polymerase chain reaction (qPCR) as the reference standard. We hypothesized the hs-RDT would detect significantly more *P. falciparum* infections when compared to the other RDTs at the time of screening. Our analysis includes 474 children and 500 household members, of all ages, of children enrolled in a parent study (NCT05244954) evaluating intermittent screening and treatment. Out of 958 participants with a qPCR test result, 43.95% (421/958) tested positive by qPCR with a mean parasite density of 13.96p/uL. The prevalence estimated by NxTek was 54.79%, by SD Bioline was 44.04%, by Biocredit HRP2 was 46.91%, and by BIOCREDIT pLDH was 38.49%. The overall sensitivity of the NxTek RDT was 82.63% (195/236), SD Bioline was 73.27% (307/419), Biocredit HRP2 was 78.15% (329/421), and Biocredit pLDH was 68.33% (287/420). For parasite densities over 200p/uL, the sensitivity of the NxTek RDT was 100% (29/29), SD Bioline was 98.21% (55/56), Biocredit HRP2 was 94.64% (53/56), and Biocredit pLDH was 92.86% (52/56). The specificity of the NxTek RDT was 72.20%, SD Bioline was 79.55%, Biocredit HRP2 was 78.17%, and Biocredit pLDH was 85.45%. Participants that were positive by qPCR but negative by RDT are being analyzed for potential pfhrp2/3 gene deletions.

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WIDESPREAD PFHRP2/3 DELETIONS AND FALSE NEGATIVE RESULTS ASSOCIATED TO HRP2-BASED RDTS IN SOUTHERN ETHIOPIA

Lemu Golassa[1], Bacha Mekonen[1], Sisay Dugassa[1], Sindew M. Feleke[1], Boja Dufera[2], Bedasa Gidisa[3], Aderaw Adamu[4], Aynalem Mandefro[1], Geremew Tassew[5]

[1]Addis Ababa University, Addis Ababa, Ethiopia, [2]alaria and NTDs research team, Bacterial, Parasitic, and Zoonotic Diseases Research, Addis Ababa, Ethiopia, [3]rmeur Hansen Research Institute, Malaria and NTDs Research Team, Addis Ababa, Ethiopia, Addis Ababa, Ethiopia, [4]Wollo University

College of Medicine and Health Science, Department of Medical Laboratory Science, Wollo, Ethiopia, [5]Malaria and NTDs research team, Bacterial, Parasitic, and Zoonotic Diseases Research, Addis Ababa, Ethiopia

HRP2-based RDTs have been widely used to diagnose malaria although the evolution and spread of *Plasmodium falciparum* parasite strains with deleted HRP2/3 genes have compromised it causing false-negative results. This study aimed to assess the prevalence of pfhrp2/3 deletions among symptomatic patients seeking malaria diagnosis at selected health facilities in southern Ethiopia. A cross-sectional study was conducted from July to September 2022. A purposive sampling strategy was used to enroll patients with microscopically confirmed P.falciparum infection. A capillary blood sample was obtained to prepare a blood film for microscopy and test the SD Bioloine TM Malaria Pf/Pv RDT. Dried blood spot samples were collected for further molecular analysis. Of 279 P. falciparum PCR-confirmed samples, 249 (89.2%) had successful msp-2 amplification, which was then genotyped for hrp2/3 gene deletions. The study revealed that pfhrp2/3 deletions were common in all health centers, and it was estimated that 144 patients (57.8%) across all health facilities had pfhrp2/3 deletions, leading to false-negative PfHRP2 RDT results. Deletions spanning exon 2 of hrp2, exon 2 of hrp3, and double deletions (hrp2/3) accounted for 68 (27.3%), 76 (30.5%), and 33 (13.2%) of cases, respectively. While the HRP2 RDT false-negative rate due to pfhrp2 exon-2 deletion was 27.3% (68/249), the population-level prevalence estimate of pfhrp-2 exon-2 deletion leading to HRP2 RDT false negatives was 24.3% (68/279). This study also showed that the sensitivity of the SD Bioline PfHRP2-RDT test was 76.5% when PCR was used as the reference test. In conclusion, this study confirmed the existence of pfhrp2/3 gene deletions, and their magnitude exceeded the WHO-recommended threshold (>= 5%). False-negative RDT results resulting from deletions in Pfhrp2/3 affect a country's attempts at malaria control and elimination. Therefore, the initiation of non-HRP2-based RDTs as an alternative measure is required to curb the grave consequences associated with the continued use of HRP-2-based RDTs in the study area in particular and in Ethiopia in general.

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PREVALENCE OF *PFHRP2/3* DELETIONS IN SOUTH SUDAN: RESULTS OF A 10-SITE NATIONAL SURVEY

Désiré Ndisabiye[1], Constantino Doggale[2], Olivier Denis[3], Pascale Chaillet[3], Letizia Di Stefano[3], Erwan Piriou[4], Valérie Briand[5], Jane Cunningham[6], Qin Cheng[7], **Matthew Coldiron**[5]

[1]Médecins Sans Frontières, Juba, South Sudan, [2]National Malaria Control Programme, Juba, South Sudan, [3]Médecins Sans Frontières, Brussels, Belgium, [4]Médecins Sans Frontières, Amsterdam, Netherlands, [5]Epicentre, Paris, France, [6]WHO, Geneva, Switzerland, [7]Australian Defence Force Malaria and Infectious Disease Institute, Brisbane, Australia

pfhrp2/3 deletions are recognized as a major threat to malaria control, particularly in the Horn of Africa. Data from South Sudan are lacking. We conducted a cross-sectional survey at 10 geographcially distinct sites across South Sudan to estimate the prevalence of *P. falciparum* with *pfhrp2/3* gene deletions. Patients under 15 years with suspected uncomplicated malaria were eligible for enrolment. After informed consent was obtained, capillary blood was taken, and a short questionnaire was administered. HRP2 and PfpLDH-based RDTs were performed in parallel and dried blood spots prepared. A positive test on either RDT triggered malaria treatment. Multiplex quantitative PCR will amplify *pfhrp2*, *pfhrp3*, *pfldh* and human tubulin genes simultaneously. Samples with ΔCq (Cq*pfhrp2* - Cq*pfldh* and Cq*pfhrp3* - Cq*pfldh*) values ≥ 3 will be classified as *pfhrp2* and *pfhrp3* deleted. Using the standard WHO protocol for surveillance of *hrp2/3* deletions, we targeted enrolling 200 suspected cases per site in order to have at least 80 pLDH-positive cases per site. From January 22 to March 27, 2024, a total of 1842 participants (53% males) were enrolled at the 10 sites. The median age of participants was 3 years (IQR 1-8). Overall HRP2 RDT positivity was 729/1842 valid tests (40%), and site-specific positivity rates ranged between 13% and 65%. Overall pLDH RDT positivity was 584/1839 valid tests (32%), and site-specific positivity ranged between 12% and 56%. A total of 15 of 584 (2.6%) pLDH-RDT positive samples were HRP2-RDT negative, a proportion that ranged between 0 and 5.4% by study site. To our knowledge, this is the first large-scale evaluation of *pfhrp2/3* deletions in South Sudan. Results of molecular testing will be available in September 2024 and will help inform policymaking around the continued use of HRP2-based RDTs in South Sudan.

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COUNTRYWIDE PFHRP2 GENE DELETION SURVEILLANCE IN MALI

Hinda DOUCOURE[1], Mahamadou S. Sissoko[1], Mamadou M. Tekete[1], Antoine Dara[1], Maimouna Dembele[1], Bintou Diarra[1], Boi Koné[1], Aïssata Koné[2], Mady Cissoko[2], Vincent Sanogo[2], Abdoulaye Djimde[1]

[1]Pathogens genomics Diversity Network Africa, Bamako, Mali, [2]National Malaria Control Program of Mali, Bamako, Mali

Malaria remains a public health concern with approximately 249 million cases in 2022 mainly in Sub-Saharan Africa. To prevent malaria-related death each malaria-endemic country should ensure that every suspected malaria case is tested, and confirmed cases are treated with quality-assured antimalarial medicine. The rapid, simple, and easy to use tests are the rapid diagnostic test (RDT). More than 90% of current available RDTs are based on the *Plasmodium falciparum* histidine-rich protein 2 (PfHRP2). Strain with PfHRP2 deletion may have serious consequences for malaria case management and threaten the use of such RDTs. WHO recommends switching to non-PfHRP2 RDTs when the prevalence of PfHRP2 deleted parasites reaches 5%. The objective of this study is to evaluate the countrywide prevalence of PfHRP2 deletion in Mali. We conducted a prospective, cross-sectional study including patients with suspected malaria based on clinical symptoms from September 2023 to January 2024 in 78 health centers from 13 health districts representing the 4 malaria transmission strata of the country. Dried blood spotted onto filter paper (DBS), HRP2-based RDT, and non-HRP2-based RDT (pLDH-based RDT) were used to collect samples from patients. RDT results were used directly to screen for discordant diagnoses. Real-time qPCR will be performed on DNA extracted from DBS to detect HRP2 deletion. The protocol has been approved by the Ethics Committee of the University of Science, Techniques and Technologies of Bamako, Mali. We have collected 22,778 samples out of the 28,080 planned (81.1%). The malaria prevalence by RDT was approximately 30%. The discordant samples i.e. Pfhrp2 (-) but PfLDH (+) ranged from 0.2% to 13.4% (average 2.9%). Molecular detection of pfhrp2 and pfhrp3 deletion is ongoing and results will be available by November 2024 and presented at the ASTMH annual meeting. This study will provide and update on the prevalence of pfhrp2/3 gene deletions across Mali, which will be critical data for malaria case management in the Country.

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ANALYTICAL PERFORMANCE ASSESSMENT OF THE AUTOMATED AND ARTIFICIAL INTELLIGENCE-ENABLED MILAB™ MAL MALARIA SYSTEM FOR THE DETECTION OF *PLASMODIUM FALCIPARUM* IN SUSPECTED MALARIA PATIENTS IN LAGOS, NIGERIA

Wellington A. Oyibo[1], Chinonye Anabike[1], Oladipo O. Oladosu[1], Michael O. Kusimo[1], Rita O. Urude[2], Chinyere Okoro[1]

[1]Centre for Transdisciplinary Research for Malaria and Neglected Tropical Diseases (CENTRAL-NTDS)/ANDI Centre of Excellence for Malaria Diagnosis,College of Medicine of the University of Lagos, Nigeria, Idi-Araba, Nigeria, [2]NTD Division, Federal Ministry of health, Abuja, Nigeria

Accurate malaria diagnosis is a critical requirement of malaria case management for mandatory parasitological confirmation of all suspected cases before treatment. Progress in malaria testing is so far inadequate especially in Nigeria where cases tested is less than 40%. Poor quality of malaria microscopy, confidence in malaria rapid diagnostic tests and access to testing remain a challenge. Malaria microscopy has several limitations including high skills, long turn-around-time (TAT) that delay test results for patient management, work-load, and inadequate manpower, emerging and expanding histidine-rich protein 2/3 (HRP-2/3) deletions are challenges

that limit testing. We present preliminary analyses of the analytical clinical performance evaluation of the MiLab™ Malaria diagnosis system compared with expert microscopy among suspected malaria patients in Lagos, Nigeria. A total of 400 patients were assessed in this study. The MiLab™ diagnostic device is an automated optical malaria diagnostic system that detects *P. falciparum (Pf)* and *P. vivax (Pf)* using 5ul of blood in an automated thin film slide preparation by staining with modified Romanowsky stain and an artificial intelligence-enabled slide reading that provides results of the tests within about 15-25 minutes when set at 200,000 red blood cells (RBCs) or 300,000 RBCs. Expert Malaria microscopy was performed using standard protocol. Of 400 patients' preliminary analyses of 399 patients show that *parasite* detection by MiLab™ and expert microscopy was 96(24.1%) and 103 (25.8%) respectively. Performance of MiLab™ at 200,000 RBC among 251 patients was: sensitivity: 94.4% (95% CI:87.6;97.6); specificity (95% CI:94.7;99.4); positive predictive value (PPV):96.9% (95% CI: 90.5;98.8); negative predictive value (NPV): 96.9% (95% CI:93.0;98.7%); False positive (FP): 1.9% and False negative (FN): 5.6%. MiLab™ malaria diagnosis automated artificial intelligence (AI) capabilities for faster TAT and high-performance is a potential game changer in accelerating access to parasitological confirmation and an asset to Malaria control programs.

6080

END-USERS PERCEPTIONS ON THEORETICAL NON-INVASIVE MALARIA TESTING TOOLS

Serafina Calarco[1], Vanessa Fargnoli[1], Catherine Thomas[2], Caroline Thomas[2], Claudius Mone Iye[2], Valerie Paz Soldan[3], Amy Aegypti[3], Janvier Serumondo[4], Ladislas Nshimiyimana[4], Yvonne Delphine Nsaba Uwera[4], Sonjelle Shilton[1], Kevin KA Tetteh[1]

[1]Foundation for innovative New Diagnostics (FIND), Geneva, Switzerland, [2]Yayasan Peduli Hati Bangsa, Jakarta, Indonesia, [3]Asociación Benéfica PRISMA, Lima, Peru, [4]Rwanda Biomedical Center, Kingali, Rwanda

Malaria remains a global health challenge in tropical and subtropical regions. Prompt and accurate diagnosis is crucial for effective disease management. Common diagnostic approaches include microscopy, lateral flow tests, and polymerase chain reaction. These methods rely on invasive sampling via venous or fingerpick blood draw, which can pose a level of risk to healthcare workers due to the handling of potentially infectious body fluids. Non-invasive tests based on saliva exhaled volatile organic compounds, and transdermal detection have the potential to revolutionize malaria diagnostics, increasing care linkage and case detection, and reducing bio-safety risks. Knowledge gaps exist on the feasibility and acceptability of these tools. This qualitative study aimed to generate evidence directly from end-users in the adoption of non-invasive diagnostic technologies to determine if these technologies are fit for purpose. This study was conducted across endemic and non-endemic areas in Indonesia, Rwanda, and Peru. Between October and November 2023, a total of 24 interviews were conducted with stakeholders and professionals working at borders, and 16 focus groups conducted with teachers, caregivers of children under five years old, healthcare workers, pregnant women, and community members (140 total participants). The comfort provided by non-invasive approaches to service recipients stands out, especially when compared to blood draw which is considered painful for children. Ease-of-use and the rapid diagnostic capabilities enabling real-time disease diagnosis were perceived as particularly beneficial in remote areas with limited healthcare infrastructures. Device portability was seen as a game-changer. The major concerns across the three countries were the lack of information on the accuracy of these non-invasive tools compared to established methods, the lack of reliance on tests targeting samples other than blood products, and the inability of the tools to differentiate between malaria species. This pioneering study shows the importance of engaging with end users early in the diagnostic development process.

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ANTIBODY-OMICS REVEALS DISTINCT HUMORAL PROFILES AND BIOMARKERS IN HIV/TB COINFECTION

Sarah M. Ali[1], Abhipsa Panigrahi[1], Marwou de Kock[2], Willem A. Hanekom[3], Cheryl L. Day[4], Aniruddh Sarkar[1]

[1]Georgia Institute of Technology, Atlanta, GA, United States, [2]South African Tuberculosis Vaccine Initiative, University of Cape Town, Cape Town, South Africa, [3]Africa Health Research Institute, Durban, South Africa, [4]Emory University School of Medicine, Atlanta, GA, United States

Despite the prevalence of antiretroviral therapy, HIV remains the strongest risk factor for developing Tuberculosis (TB), which is the leading cause of death among people living with HIV (PLHIV). Lack of accurate yet rapid and inexpensive diagnostics is a critical bottleneck in control of TB. Key challenges in TB diagnostics include difficulty in discrimination of the heterogenous spectrum of TB disease, which is further complicated in HIV/TB co-infection, and difficulty in obtaining and processing sputum samples for point-of-care (POC) diagnosis. Earlier, antibody (Ab)-based tests have failed due to poor specificity in discriminating past infection from current infection as well as latent TB infection (LTBI) from active TB (ATB). Recent work has shown that the inflammatory state of *M. tuberculosis* (Mtb)-specific Abs, driven by changes in Fc-glycosylation, differs across LTBI and ATB. Here we report the discovery of a Mtb-specific Ab Fc profile-based biomarker to distinguish ATB from LTBI in PLHIV. We have developed a multiplexed 'Ab-omics' platform for deep biophysical characterization (Fab and Fc) of a broad set of antigen-specific Abs including their isotype, subclass, glycosylation, and Fc receptor binding. We apply the Ab-omics pipeline to plasma from adults in South Africa with HIV and ATB (n=17) and LTBI (n=17) using multiple Mtb antigens (PPD, LAM, Ag85A, ESAT6, CFP10, HspX, PstS1) and non-Mtb antigens. Briefly, antigen-coated barcoded beads were incubated with plasma and probed with fluorescently labeled isotype probes, Fc receptors and lectins. With a total of 56 measured features (8 Ab Fc features 7 antigens) from each participant, machine-learning based analytics (LASSO-SVM) applied to this high-dimensional dataset revealed a minimal Ab Fc profile biomarker (AuRoC>0.9) distinguishing ATB vs LTBI. This included increased specific Ab isotypes (IgM-PPD, IgA-HspX), and Fc receptor binding (FcR3b-CFP10) and decreased Ab galactosylation (PstS1), in ATB vs LTBI. Our findings suggest that a non-sputum-based, purely Ab-based biomarker can achieve accurate diagnosis of ATB vs LTBI in PLHIV in endemic areas.

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DEVELOPMENT OF AMPLICON-BASED WHOLE-GENOME SEQUENCING OF *MYCOBACTERIUM TUBERCULOSIS*

Chaney C. Kalinich[1], Freddy Gonzalez[2], Mallery I. Breban[3], Isabel Distefano[3], Ted Cohen[3], Nathan D. Grubaugh[3], Seth Redmond[3]

[1]Yale School of Medicine, New Haven, CT, United States, [2]Department of Ecology and Evolutionary Biology, Yale University, New Haven, CT, United States, [3]Department of Epidemiology of Microbial Diseases, Yale School of Public Health, New Haven, CT, United States

Tuberculosis (TB) is a leading cause of infection-related mortality worldwide, causing an estimated 1.13 million deaths in 2022. The majority of infections can be successfully treated with antibiotics, but the prevalence of drug-resistant TB and a lack of rapid and cost-effective drug susceptibility tests for many key antibiotics is a significant barrier to treatment. Whole-genome sequencing of *Mycobacterium tuberculosis* can be used for drug susceptibility testing and can provide valuable insight into transmission patterns. However, *M. tuberculosis* is slow to grow, meaning traditional sequencing methods which require culture can take many weeks to return results, greatly limiting the potential clinical benefits. Tiled amplicon sequencing is a low-cost method of amplifying target nucleic acid which has been used widely to sequence viruses such as SARS-CoV-2 and MPox directly from clinical samples. Extending this approach to *M. tuberculosis* would significantly reduce the cost, labor, and turnaround time for whole-genome sequencing, enabling more rapid determination of drug susceptibility and insight into transmission. We designed a tiled

amplicon panel consisting of 5128 primers, the largest tiled amplicon sequencing panel we are aware of to date, and employed the widely-used Illumina COVIDSeq protocol to enable sequencing of the full *M. tuberculosis* genome from minimal input samples. Compared to the same sample without amplification, we achieved >80% genome coverage with 500-1000x lower input DNA with our primer scheme. These findings indicate that tiled amplicon sequencing can be extended to bacterial pathogens to enable whole-genome sequencing from input DNA concentrations typical of clinical samples with minimal additional cost and laboratory effort. Using this approach to sequence *M. tuberculosis* could revolutionize TB control programs, enabling genomic epidemiology to be performed in resource-limited settings and reducing the time needed for comprehensive drug susceptibility testing from weeks to days.

6083

TWO DECADES OF MOLECULAR SURVEILLANCE OF MULTIDRUG-RESISTANT TUBERCULOSIS IN ARGENTINA: LATEST TRENDS AT THE DAWN OF THE GENOMIC ERA

Roxana Paul, Federico Lorenzo, Francisco Delvento, Eduardo Mazzeo, Ingrid Wainmayer, Beatriz López, Norberto Simboli, **Noemi Kaoru Yokobori**

INEI, ANLIS "Dr. C. G. Malbrán", Ciudad Autónoma de Buenos Aires, Argentina

Multidrug resistant (MDR) tuberculosis (TB) poses a challenge to the global TB control. Argentina is a mid-incidence country for TB with a persistent 1% of MDR cases (~100 cases/year). Herein, we analyzed the genotypes obtained through an in-house PCR (5.9%), MIRU-VNTR15 (52.7%) or WGS (41.4%), of 1249 MDR isolates (of which 77.6% were newly diagnosed cases) collected in 2012-2022, compared to 2003-2009. East-Asian isolates were rare (1 %). Clustering rate remained high (76.3 % in 50 clusters) among new cases. Of the clustered cases, 442/739 (59.8%) were due to four major strains. Particularly, the Callao2 strain imported from Peru, caused a local outbreak, and displaced the historically dominant M strain. We classified 123 cases as preXDR and 9 cases as XDR, of which 76.1% and 44.4% belonged to a cluster, respectively, but were not associated to a particular genotype. PreXDR cases due to direct transmission were sporadic. The median time of persistence of the clusters was of 7 years (range: [0 - 30]) and median cluster size was 3 (range: [1 - 132]). Resistance conferring mutations in positions other than the most frequently found (*rpoB* codons 450, 445 or 435, and *katG*315 or -15*inhA*) were more common among unique isolates compared to clustered isolates (for *rpoB*: 16.7% and 3.6% respectively, Chi-sq test, $p < 0.01$; for isoniazid resistance: 31.2% and 11.2% respectively, Chi-sq test, $p < 0.001$; major strains excluded). Interestingly, no associations with cluster persistence time or size were observed (Chi-sq test, $p > 0.05$). Three provinces only had unique cases, while other three low incidence provinces only had clustered cases, mostly caused by strains circulating in the hot spots of the country. As expected, WGS revealed the intrinsic diversity of certain strains defined by classical genotyping methods, such as the O strain, which included four different subclusters. We conclude that underneath the relatively stable number of MDR-TB cases, epidemiologically relevant changes took place. This work provides the basis for the implementation of a real-time WGS-based surveillance which is expected to allow timely and fine-tuned interventions.

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PRELIMINARY OUTCOMES FROM A PROSPECTIVE OBSERVATIONAL COHORT OF ADULTS WITH DRUG-SUSCEPTIBLE CAVITARY TUBERCULOSIS IN HAITI

Nancy Dorvil[1], Marc A. JeanJuste[1], Nao Haba[2], Joissaint Guy[1], Kathleen Walsh[2], Jean W. Pape[1], **Daniel Fitzgerald**[2]

[1]GHESKIO, Port-au-Prince, Haiti, [2]Weill Cornell Medicine, New York, NY, United States

Standard therapy for drug-susceptible pulmonary tuberculosis (TB) lasts 6 months due to the ability of *M. tuberculosis* (Mtb) to persist in a non-replicating state. Trials of treatment-shortening regimens often fail due to high rates of treatment failure and recurrence. Patients with cavitary TB or extensive disease involving >50% of lung are at increased risk of failure or recurrence with standard therapy and even higher risk in treatment-shortening trials. We are conducting a prospective observational study of adults with cavitary or extensive TB in Port-au-Prince, Haiti to seek biomarkers and immune correlates that may predict failure or recurrence in this population. Since May 2022, we have enrolled 137 adults without HIV with chest radiographic evidence of cavitary or extensive disease, with medium or high Xpert MTB/RIF (Cepheid, Sunnyvale, CA, USA) and with no evidence of rifampin resistance, and who have not yet started treatment. Participants are treated according to World Health Organization (WHO) and Haitian national guidelines with 2 months of rifampin, isoniazid, pyrazinamide, and ethambutol followed by 4 months of rifampin and isoniazid. Of the 137 participants enrolled, median age is 27 (interquartile range (IQR) 21, 34). Fifty-six (41%) are female. Median BMI is 17.7 (IQR 16.3, 19.0). Twenty-five participants (18%) were sputum culture positive at month 2. Of n=114 participants who reached end of treatment, 95 (83%) were cured, 5 (4%) failed, 2 (2%) died and 1 (1%) had recurrence. In this prospective cohort of adults with cavitary or extensive TB, there was 18% sputum culture positive at 2 months and a high rate of failure. According to the WHO, globally <1% of HIV-negative people with drug-susceptible TB have treatment failure. Treatment failure among people with cavities may be due to several factors, such as inadequate drug penetration into the cavity or ineffective host immune response. People with cavitary TB represent a unique population who are at very high risk of poor outcome and are likely driving the need for 6 months of therapy.

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TUBERCULOSIS DRUG SUSCEPTIBILITY TEST WITH SNP-RESOLUTION USING SINGLE SAMPLE MELT ANALYSIS

Nicole A. Malofsky, Dalton J. Nelson, Megan E. Pask, Frederick R. Haselton

Vanderbilt University, Nashville, TN, United States

The global burden of tuberculosis (TB) disease is compounded by the emergence of drug-resistant strains. Determining TB drug susceptibility is essential to match disease-infected patients with effective drug regimens. High resolution melt (HRM) analysis offers a rapid and cost-effective screening solution for confirming susceptibility of polymerase chain reaction (PCR)-amplified nucleic acid products. However, a single base change can be difficult to detect by standard HRM. These limitations are particularly detrimental for TB drug susceptibility testing in which a single nucleotide polymorphism (SNP) can be sufficient to make a first-line TB drug ineffective. In this study, a reagent-based calibration strategy based on synthetic (L)-left-handed DNA, designated LHRM, was developed to confirm the drug-susceptible sequence of a PCR product with single base resolution. To test our LHRM approach, a constant amount of double-stranded L-DNA was used as a within-sample melt standard. LHRM and standard HRM were used to classify PCR products as drug-susceptible or not drug-susceptible with a test bed of nine synthetic *katG* variants, each containing single or multiple base mutations that are known to confer resistance to the first-line TB drug isoniazid (INH). Using a state-of-the-art calibrated instrument and multiple sample classification analysis, standard HRM performed at 33.3% sensitivity and 97.5% specificity. For this small data set, incorporating L-DNA for reagent-based calibration into every sample improved overall sensitivity to 77.8% and maintained high specificity of 98.7%. This improvement was due to improved classification of the most difficult S315T variant containing only a single base change. Notably, LHRM achieved sample classification only relying on within-sample melt differences between L-DNA and the unknown PCR product. LHRM shows promise as a high-resolution single sample method for validating PCR products in applications where the expected sequence is known and highly calibrated instruments are unavailable.

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RISK FACTORS ASSOCIATED WITH POST-TUBERCULOSIS SEQUELAE: A SYSTEMATIC REVIEW AND META-ANALYSIS

Temesgen Yihunie Akalu[1], Archie C.A Clements[2], Alemneh Mekuriaw Liyew[1], Beth Gilmour[3], Megan B. Murray[4], Kefyalew Addis Alene[1]

[1]Curtin University, Perth, Australia, [2]Queens University Belfast, Belfast, United Kingdom, [3]Teleth, Perth, Australia, [4]Harvard University, Boston, MA, United States

Post-tuberculosis (TB) sequelae pose a significant challenge in managing TB survivors, often leading to persistent health issues post-treatment. This systematic review and meta-analysis aim to identify risk factors linked with long-term physical sequelae among TB survivors. We systematically searched Medline, Embase, PROQUEST, and Scopus for studies on long-term physical sequelae among TB survivors up to December 12, 2023. Included were all forms of TB patients experiencing long-term physical sequelae (respiratory, hepatic, hearing, neurological, visual, renal, and musculoskeletal). Narrative synthesis was used for risk factors reported once, and random-effect meta-analysis for primary outcomes with two or more studies. The review included 73 articles from 28 countries representing 31,553 TB-treated patients in the narrative synthesis, with 64 studies included in the meta-analysis. Risk factors associated with post-TB lung sequelae included older age (OR=1.62, 95% CI: 1.07-2.47), previous TB treatment (OR=3.43, 95% CI: 2.37-4.97), smoking (OR=1.41, 95% CI: 1.09-1.83), alcohol consumption (OR=1.84, 95% CI: 1.04-3.25), bacteriologically positive TB diagnosis (OR=3.11, 95% CI: 1.77-6.44), and presence of pulmonary lesions in radiology (OR=2.04, 95% CI: 1.07-3.87). Risk factors associated with post-TB liver injury included pre-existing hepatitis (OR=2.41, 95% CI: 1.16-6.08), previous TB treatment (OR=2.64, 95% CI: 1.22-6.67), hypo-albuminemia (OR=2.10, 95% CI: 1.53-2.88), and HIV co-infection (OR=2.72, 95% CI: 1.66-4.46). Risk factors linked with post-TB hearing loss included baseline hearing problems (OR=1.72, 95% CI: 1.30-2.26) and HIV co-infection (OR=3.02, 95% CI: 1.96-4.64). In conclusion, this review underscores that long-term physical post-TB sequelae, including respiratory, hepatic, and hearing issues, are linked with diverse socio-demographic, behavioural, and clinical factors. Identifying these risk factors is vital for targeting interventions to alleviate the post-TB treatment burden.

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TUBERCULOSIS TRENDS AMONG INDIGENOUS PEOPLE IN BRAZIL BEFORE, DURING, AND AFTER THE SARS-COV-2 PANDEMIC

Ida Kolte[1], Eunice Atsuko Totumi Cunha[2], Paulo Cesar Basta[1]

[1]Oswaldo Cruz Foundation (FIOCRUZ), Rio de Janeiro, Brazil, [2]Laboratório Central de Saúde Pública do Estado de Mato Grosso do Sul, Campo Grande, Brazil

Despite some advances in tuberculosis control in recent decades, hotspots of transmission persist in some Indigenous communities. Delivery of healthcare to Brazil's Indigenous population was severely interrupted by the SARS-CoV-2 pandemic. The impact of this on tuberculosis transmission in these key vulnerable communities has not yet been investigated. We analyzed data from 2012-2022 from the Brazilian Ministry of Health database of notifiable disease (SINAN) to map the level of laboratory-confirmed diagnostics, multidrug-resistant TB, and disease outcome among the Indigenous population compared to the general population of Brazil and at state level. 964 cases of TB in Indigenous people were reported in 2022, the highest level since 2015. An increase in laboratory-confirmed TB diagnoses was registered for both the non-Indigenous and Indigenous population, but the level was significantly lower for the Indigenous population throughout the period (63.9% vs 68.4% in 2022). At the state level, the percentage of laboratory-confirmed diagnosis ranged widely - from 20-100% in 2022. Levels of cure were consistently higher, and level of fatality lower, among the Indigenous versus the non-Indigenous population. the percentage of Indigenous cases with multidrug-resistant TB has increased since the pandemic, from 0.44% (2019) to 1.94% (2021) and 1.11%(2022). In 2022, 5,91% of Indigenous cases of TB were recurrent episodes, and another 5.91% were re-treated after treatment abandon. Drug sensitivity testing was done for 9.02% of all Indigenous cases, and 14.04% of recurrent cases and people who had previously abandoned treatment. The dramatic increase in MDR-TB among the Indigenous population in Brazil demands urgent action considering the hyperendemic transmission levels in some communities. Diagnostics must be improved to ensure that TB treatment is given only to TB patients, and drug sensitivity testing must be carried out as a minimum for all patients returning to treatment after treatment abandon and for all recurrent episodes.

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COVID-19 COMMUNITY 'BANTABA': RAISING AWARENESS AND REDUCING MISINFORMATION ON COVID-19 A TWO URBAN LOCALITIES IN THE GAMBIA

Omar Ceesay

Medical Research Council@lshtm, Banjul, Gambia

Omar Ceesay, Ebrima Manneh, Bakary Dibba, Effua Usuf.The COVID-19 pandemic has posed a significant challenge to global health, with over 3 million deaths worldwide as of April 2023. The Gambia, a small West African country, has not been immune to the impact of the pandemic, with over 10,000 confirmed cases. One of the challenges in the Gambia has been the spread of misinformation about COVID-19, which has contributed to low compliance with public health measures and hampered efforts to mitigate the spread of the disease. Community engagement employs participatory communication, a community development initiative that utilizes a bottom-up approach rather than a top-down approach in health interventions. Data collection was carried out between 17th February to 1st March 2022, when we conducted the Pre-test questionnaire. A post-community engagement was conducted nine months after the first community engagement and the result shows significant improvement in knowledge and understanding of the disease.The result shows that 79% of the participants know the signs and symptoms of covid. This shows engaging with the community with help in raising knowledge and awareness of the disease. 76% of the respondent shows fear of the covid 19 virus, while 90% shows feelings toward family member affected by the virus, because they think they can die anytime with the virus has it doesn't have a treatment.Community engagement is an effective strategy for raising awareness and reducing misinformation about COVID-19 within communities. Community leaders' engagement, public spaces for sensitization sessions, and dissemination of accurate information through various media channels are essential components of successful community engagement initiatives.

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THE EFFECT OF PANDEMICS ON DECENT WORK AND TASK PERFORMANCE AND ITS INFLUENCE ON THE LEADERS' EMOTIONAL INTELLIGENCE

Emmanuel Boateng Fosu

University Of Ghana, Accra, Ghana

Pandemics poses unprecedented challenges for healthcare workers, impacting their work conditions, performance, and the role of leaderships emotional intelligence. Decent work, as defined by the International Labor Organization, encompasses opportunities for productive work with fair income, security in the workplace, social protection, and better prospects for personal development and social integration. The aim of this research was to assess the effect of the relationship between decent work and the task performance, the mediational role of leaders emotional intelligence, and the moderating effect of pandemics on health workers. This study, adopted a quantitative approach and utilized a cross-sectional survey design, collecting data from 2,000 healthcare workers. The data, obtained through stratified random sampling, were analyzed using the Structural Equation Modeling. We observed that pandemic dampens the positive relationship that exists between decent work and task performance of healthcare workers. Furthermore, the study identified that leaders emotional

intelligence played an appreciable mediating role in the relationship between decent work and task performance of health workers. Our data suggests and recommends that policies and practices to implement regulations to promote decent work standards (fair wages, safe conditions, work-life balance) across healthcare organizations should be developed. Additionally, training and assessment on emotional intelligence for healthcare workers, especially the leaders should be mandated. Further to this we encourage the development of emergency preparedness policies to address work conditions and employee support during pandemics/crises.

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QUANTIFYING THE IMPACT OF MODIFIABLE RISK AND PROTECTIVE FACTORS ON MORTALITY AMONG CHILDREN AND YOUNG ADOLESCENTS RECEIVING ANTIRETROVIRAL THERAPY

Jiawei He, Hmwe Kyu, Amanda Novotney, Edmond Brewer, Austin Carter, Kemal Oumer

Institute for Health Metrics and Evaluation, Seattle, WA, United States

Heterogeneity of mortality estimates of children who have received antiretroviral therapy (on-ART) exist across regions, with HIV mortality remaining much higher in resource-limited countries compared to resource-rich countries. An understanding of the factors influencing the risk of on-ART mortality could help explain the variation in mortality across locations and inform priorities for interventions. This research explores how modifiable risk factors influence mortality rates in children and young adolescents who have been initiated with ART. We perform a systematic review and meta-analysis to synthesize the existing literature on mortality among on-ART children using all global data sources. A list of biomedical, behavioral, and structural factors associated with HIV mortality are identified through literature review. We then perform a systematic procedure of covariates selection through the application of LASSO technique, which identifies the most statistically significant covariates. We also quantify the impact of these covariates on the mortality through counterfactual scenario simulation, which models and evaluates the potential effects of changes in these covariates on mortality. Variable selection has identified key covariates for the mortality among under-5 and over-5 age groups. For under-fives, PCV and antibiotics coverage for LRI are crucial, while for over-fives, hepatitis B vaccine, antibiotics, and PCV coverage are significant. A 10% increase in PCV and antibiotic coverage is associated with 5% and 15% mortality odds decrease for under-5 age groups. For the 5-14 age group, hepatitis B vaccine coverage shows pronounced effectiveness, reducing mortality odds by 8% with a 10% increase and 15% with a 20% increase. In conclusion, our study demonstrates that modifiable risk factors play a significant role in on-ART mortality among children and adolescents. The outcomes of this study are expected to contribute valuable knowledge to pediatric and adolescent healthcare and shape future health policies aimed at reducing mortality of children and adolescents living with HIV/AIDS.

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CHALLENGES AND LESSONS LEARNED WHILE COMPLETING/INITIATING VACCINE CLINICAL TRIALS DURING THE COVID-19 PANDEMIC IN A DEVELOPING COUNTRY: EXPERIENCE FROM NEPAL

Pragya Thapa[1], Tarun Saluja[2], Pranodan Poudel[3], Shanti Bogati[3], Naveena Aloysia D'Cor[2], Birendra Prasad Gupta[1]

[1]International Vaccine Institute, Kathmandu, Nepal, [2]International Vaccine Institute, Seoul, Korea, Democratic People's Republic of, [3]Dhulikhel Hospital-Kathmandu University Hospital, Dhulikhel, Nepal

The global burden of vaccine-preventable infectious diseases is comparatively higher in developing countries. However, the safety and efficacious studies of vaccines in diseases prevalent countries are limited. Nevertheless, COVID-19 pandemic added many challenges in conducting clinical trials worldwide, especially in resource limited countries. This is an educational study where researchers from Nepal have shared their experiences while completing ongoing clinical trial and initiating the new clinical trial in Nepal using real time events, records, and secondary data. Lock down for rising case of COVID-19 affected the ongoing clinical trial for typhoid vaccine, sponsored by International Vaccine Institute (IVI). This clinical trial was first large scaled phase III clinical trial conducted in Nepal at 4 different sites. Out of 6 visits, 4th visits was almost completed and completion of remaining visits with retention of subjects in the study was challenging. The swift response from the IVI and proactive response from site staffs towards provided guidelines and study activities completed the study on time by fulfilling both government & protocol safety requirements. Similarly, conducting a clinical trial by Nepal for a novel vaccine during pandemic was quite more challenging than we presume. However, making necessary changes as per the local requirements, collaborating with local stake holders, conducting community engagement program, pre-screening activities, continuous subjects counselling, affiliating with various hospital departments, involving hospital staffs, etc. we were able to initiate & conduct the study amid the COVID-19 pandemic with highest enrolling sites among other countries & advance to continue the study. Conducting a clinical trial in Nepal during health crisis might be challenging, but it is possible. High subject enrollment rate, safety monitoring plans, rapid adaptability to new technologies, biological sample handling, active & passive surveillance of the subjects, etc. shows the optimal proficiency of sites and health professionals that needs to be explored more in Nepal.

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ADVANCING GEOSTATISTICAL METHODS FOR FUTURE STRATEGIES IN NEGLECTED TROPICAL DISEASE PROJECTS

Luke EW Klein

FHI 360, Washington, DC, DC, United States

Neglected Tropical Diseases (NTDs) disproportionately affect the world's most vulnerable populations. The adoption of georeferenced survey results and recent advancements in geostatistical methods offer promising avenues for refining NTD project strategies, leveraging the growing inventory of GIS datasets. This presentation highlights the evolving landscape of geostatistical modeling, emphasizing its critical role in informing targeted interventions. Traditional methods often fail to capture spatial patterns inherent in NTDs. Cutting-edge geostatistical models, however, offer nuanced insights by accounting for spatial autocorrelation and heterogeneity. These models, such as Bayesian spatial approaches and geographically weighted regression, facilitate more accurate risk mapping and resource allocation. The integration of diverse GIS datasets, including environmental, demographic, and healthcare infrastructure layers, enhances the precision of NTD risk assessments. From high-resolution satellite imagery to crowd-sourced data, these resources provide invaluable context for optimizing intervention delivery. Despite significant progress, several challenges remain. Funds to develop geospatial datasets vital for effective geostatistical analysis of NTDs, such as accurate evaluation unit boundary files and georeferenced sampling frames, are needed. Further development of current tools, and strategies to extend them to more users, are also imperative. By harnessing the full potential of geostatistical methods, NTD projects can achieve greater impact with limited resources. From evaluating intervention effectiveness, to prioritizing endemic hotspots, or informing survey design strategies, these tools offer a paradigm shift in how we conceptualize and address NTDs. As we navigate the evolving landscape of global health, investing in robust geostatistical frameworks will be paramount to achieving sustainable progress in NTD control and elimination.

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FROM RESEARCH TO POLICY - LEVERAGING SCIENCE AND STRATEGIC COMMUNICATION TO TACKLE DENGUE IN BANGLADESH

A K M Tariful Islam Khan, Mohammad Shafiul Alam, Tahmeed Ahmed

International Centre for Diarrheal Disease Research, Bangladesh (icddr,b), Dhaka, Bangladesh

In 2017-18, icddr,b scientists documented significant resistance to permethrin in Aedes aegypti mosquitoes across several districts in Bangladesh, including Dhaka, and recommended alternative insecticide malathion and its combinations. The findings were disseminated to relevant stakeholders, including the Institute of Epidemiology, Disease Control and Research (IEDCR), Bangladesh in May 2018; however, resistance to these recommendations persisted and led to no immediate changes in vector control interventions. The dengue situation escalated into a severe outbreak in 2019, with cases dramatically increasing from 1,884 in June to a historical high of 101,354 for the year, the death toll was 164. icddr,b launched a strategic media advocacy campaign in mid-July, producing over 500 media outputs, including reports, talk shows, opinion articles. This campaign also involved targeted advocacy with entomologists and decision-makers to reinforce the scientific validity of the recommendations. The campaign gained momentum with support from the Honourable Prime Minister, leading to the Dhaka City Corporations adopting the recommended insecticides in the second week of August. This led to a temporal decline in cases, decreasing to 16,856 in September, and finally 4,011 in November. Despite these successes, in 2023, a staggering 321,179 dengue cases were reported across all 64 districts with a death toll of 1,705. This coincided with increased intermittent rainfall patterns, where average rainfall exceeded 120 mm from April to October, suggesting a link between the soaring dengue cases and the impacts of climate change. The severe dengue burden demands a multi-faceted response, combining public awareness and action, robust vector control, effective vaccines, and enhanced case management, supported by ongoing research. Engaging the mass media is crucial, as evidenced by icddr,b's 2019 advocacy success. This approach offers a proven model for implementing effective dengue control and prevention strategies throughout the year.

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Documentation And Analysis of the Social Contact Patterns Using Standardized Diaries Across Different Ages in Low-Income Settings in Vellore District, Tamil Nadu, Southern India

RAJAN SRINIVASAN[1], Ayyappan V R[1], Janani Rathi[1], Agil Somasundaram[1], Meenakshi N[1], Moses Chapa Kiti[2], Noureen Ahmed[3], Ben Lopman[2], Saad Omer[3], Venkat Raghava Mohan[1]

[1]Christian Medical College Vellore, Vellore, India, [2]Rollins School of Public Health, Emory University, Atlanta, GA, United States, [3]O'Donnell School of Public Health, UT Southwestern, Dallas, TX, United States

Infectious diseases have a greater impact in low- and middle-income countries (LMICs), as evident from the COVID-19 pandemic. Human behaviors play an essential role in the transmission of infections. A study was conducted in urban and rural areas of Vellore district, Tamil Nadu, Southern India, to document and analyze social contact patterns using standardized social contact diaries across different ages in low-income settings. The study also documented symptoms of respiratory infections in this population. Using a symptomatic approach. The study identified acute respiratory infections and associated factors among 1257 index participants, of which 631 were from urban and 626 from rural Vellore. The mean number of contacts over two days was 15.86 ± 5.82 and 15.61 ± 6.9 for urban and rural areas, respectively. In both regions, the age group of 5-19 years ($p<0.001$) and school-goers ($p<0.001$) had significantly more contacts than all other age groups. On the other hand, the least number of contacts was observed among people of extreme ages (<6 months and ≥60 years). Overall, 10.8% of individuals experienced acute respiratory infections during a 3-month follow-up. The risk of respiratory infections was highest among children under 5 years of age, with other age groups having a significantly lower risk of ARI (OR 2.9, 0.21 – 0.47). Those without any education had a higher risk of ARI than those with any education (OR 2.8, 0.21 – 0.47). Pre-existing respiratory conditions like asthma increased the risk of ARI compared to those without them (AOR 6.07, 1.56 – 21.7). Participants from houses using biomass as fuel were also at a higher risk (OR 2.9, 1.96 – 4.48) of ARI than households using non-biomass fuel. Findings from this study will contribute to developing context-specific and targeted preventive and control strategies during infectious disease outbreaks. Understanding and quantifying social mixing patterns within communities will provide much-needed data for infectious disease modeling in LMICs.

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ENHANCING COMMUNITY HEALTH DIGITIZATION IN BURKINA FASO WITH ENTERPRISE ARCHITECTURE: ACHIEVEMENTS AND LESSONS.

Alain Kabore[1], Fatou Fall[2], Salif Traore[3], Jean Serge Dimitri Ouattara[3]

[1]PATH, Ouagadougou, Burkina Faso, [2]PATH, Dakar, Senegal, [3]Ministry of health and public hygiene, Ouagadougou, Burkina Faso

Malaria remains the leading cause of morbidity and mortality in Burkina Faso. In response, the government has implemented a 2019-2023 community health strategy with the digitization of service delivery as a priority intervention. To leverage the use of digital solutions, they must be developed, deployed, and scaled up in a coherent scheme and an optimum level of security to ensure a sustainable implementation. Thus, the Ministry of Health and Public Hygiene with support from Digital Square at PATH and the US Presidential Malaria Initiative , has developed an enterprise architecture to sustain the scale up of the community digital health solution (eSanteCom). The Open Group Architecture Framework (TOGAF) was implemented in a participative and collaborative way, involving relevant stakeholders from MoH, implementing organizations, civil society organizations, community health workers in an iterative process to describe the current state of the community health system through business, data, applications, technologies, and security domains, and envision the future state expected for a coherent scale up. The six-months intervention included stakeholders' engagement, framing workshop,10 key informants' interviews including five MoH information systems experts, and technical directors. Those actions resulted in a design of a general architecture document and a blueprint. Main outcomes include the stakeholder's awareness raising about enterprise architecture as an evidence-based approach to address the siloed and fragmented digital health interventions. Additional findings include a low maturity architecture capacity within MOH (<1/5), the lack of architecture governance framework. Some gaps and requirements were also highlighted to enhance the system security for all domains, leading to formulating recommendations and scenarios that will inform the new 2024-2028-health strategy. Overall, the lessons learned were endorsed by MOH who, accordingly, expressed interest in undertaking the extension of architecture from community health to the whole health system.

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CHIKUNGUNYA VIRUS RISK OF ACQUISITION, DIFFERENTIAL DIAGNOSIS AND VACCINE DEVELOPMENT: IMPACT OF INDEPENDENT ONLINE MEDICAL EDUCATION ON PHYSICIAN KNOWLEDGE AND CONFIDENCE

Julia C. Duffey[1], Iwona Misiuta[2], Lin H. Chen[3]

[1]Manifold Medical, Matlock, United Kingdom, [2]Medscape Education Global, New York, NY, United States, [3]Division of Infectious Diseases and Travel Medicine, Mount Auburn Hospital and Harvard Medical School, Cambridge and Boston, MA, United States

Chikungunya virus (CHIKV) has a wide geographical spread and effective mosquito control is difficult to adopt for all individuals at risk. As previously reported, several vaccine approaches are being evaluated in clinical trials or are approved. Here, we assessed whether a short global online summary of an independent medical education symposium at the American Society

of Tropical Medicine and Hygiene 2023 meeting could improve primary care physicians' (PCPs) and infectious disease (ID) physicians' knowledge and confidence. Educational effect was assessed using a repeated-pairs design with pre-/post-assessment. Three multiple choice questions assessed knowledge, and one question assessed confidence. Statistical tests to assess significance included: Paired samples t-test for overall average number of correct responses and confidence. McNemar's test for individual questions and learning objectives (P<.05). Cohen's d estimated the effect size impact on number of correct responses (<.20 modest, .20-.49 small, .59-.79 moderate, ≥.80 large). From a total audience of 1115, there were 315 assessment completers. Overall, there were significant knowledge gains for ID physicians (P<.001; Cohen's d = 0.98) and PCPs (P<.001; Cohen's d = 0.78). Significant knowledge gains regarding symptoms that differentiate CHIKV from other arboviruses were found (PCPs P<.001; ID physicians P<.01). Very high knowledge gains regarding vaccine data were seen with a relative percentage change in knowledge of 1225% for ID physicians (P<.001; 31% improved) and of 1033% for PCPs (P<.001; 51% improved). A considerable proportion of PCPs (56%; confidence shift 101%; P<.001) and ID physicians (56%; confidence shift 87%; P<.001) increased their confidence regarding their ability to advise travellers about their risk of CHIKV acquisition. Online medical education significantly improved physicians' knowledge and confidence regarding diagnosis, vaccine data and their ability to advise travellers of the risk of CHIKV. As a CHIKV vaccine development continues, it is critical that physicians are aware of the need for a vaccine and to optimally advise individuals.

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AN EXPLAINABLE MACHINE LEARNING APPROACH FOR PREDICTING LINEAR GROWTH FALTERING FOLLOWING A DIARRHEAL ILLNESS AMONG CHILDREN AGED 6-35 MONTHS IN WESTERN KENYA

Billy Ogwel[1], Vincent H. Mzazi[2], Alex O. Awuor[1], John B. Ochieng[1], Stephen Munga[1], Kirkby D. Tickell[3], Patricia B. Pavlinac[3], Karen L. Kotloff[4], Richard Omore[1]

[1]Kenya Medical Research Institute, Kisumu, Kenya, [2]University of South Africa, Pretoria, South Africa, [3]University of Washington, Seattle, WA, United States, [4]University of Maryland, Baltimore, MD, United States

Stunting affects one-fifth of children globally with diarrhea accounting for an estimated 13.5% of stunting. Identifying risk factors for its precursor, linear growth faltering (LGF), is critical to designing interventions. Moreover, developing new predictive models for LGF using more recent data offers opportunity to improve model performance and capture new insights. We employed machine learning (ML) to derive and temporally validate a predictive model for LGF among children enrolled with diarrhea in the Vaccine Impact on Diarrhea in Africa (VIDA) study and the Enterics for Global Heath (EFGH) *Shigella* study in rural western Kenya. We used 7 ML algorithms to retrospectively build prognostic models for the prediction of LGF (≥0.5 decrease in height/length for age z-score [HAZ]) among children 6-35 months. We used de-identified data from the VIDA study (n=1,473) combined with synthetic data (n=8,894) in model development, which entailed split-sampling and K-fold cross-validation with over-sampling technique, and data from EFGH-Shigella study (n=655) for temporal validation. Potential predictors included demographic, household-level characteristics, illness history, anthropometric and clinical data chosen using an explainable model agnostic approach. The champion model was determined based on the area under the curve (AUC) metric. The prevalence of LGF in the development and temporal validation cohorts was 187 (16.9%) and 147 (22.4%), respectively. The following variables were associated with LGF in decreasing order: age (16.6%), temperature (6.0%), respiratory rate (4.1%), SAM (3.4%), rotavirus vaccination (3.3%), breastfeeding (3.3%), and skin turgor (2.1%). While all models showed good prediction capability, the gradient boosting model achieved the best performance (AUC% [95% Confidence Interval], 83.5 [81.6-85.4] and 65.6 [60.8-70.4]) on the development and temporal validation datasets, respectively. Our findings accentuates the enduring relevance of established predictors of LGF whilst demonstrating the practical utility of ML algorithms for rapid identification of LGF among at-risk children.

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ANALYSIS OF CARE-SEEKING PATHWAY AND FACTORS INFLUENCING EARLY AND APPROPRIATE CARE-SEEKING FOR MALARIA PATIENTS IN THE REPUBLIC OF GUINEA, 2022-2023

Elhadj Marouf Diallo[1], Mateusz M Plucinski[2], Fatoumata Bintou Traore[1], Bienvenu Salim Camara[3], Alice Langlet[4], Alexandre Delamou[1], Ousmane Oumou Diallo[5], Laurent Gerbaud[4], Alioune Camara[6]

[1]African Center of Excellence for the Prevention and Control of Communicable Diseases, Faculty of Health Sciences and Techniques, University of Conakry, Dixinn, Conakry, Guinea, [2]Malaria Branch, Centers for Disease Control and Prevention, Atlanta, GA, USA; President's Malaria Initiative, Atlanta, GA, United States, [3]Centre National de Recherche et de Formation en Santé Rurale de Maferinyah Guinée (CNRFSR), Maferenya, Forecariah, Guinea, [4]CHU Clermont-Ferrand, UFR Medicine & Paramedical Professions, University Clermont Auvergne, CNRS, Sigma Clermont Institute Pascal, Clermont-Ferrand, France, [5]Department of Preventive Medicine and Institute for Global Health, Northwestern University, Chicago, IL, United States, [6]National Malaria Control Program, Dixinn, Conakry, Guinea

The main aim of this study was to analyze the care pathway and the factors associated with early and appropriate care-seeking for malaria patients in the Republic of Guinea. A cross-sectional study was carried out between November 2022 and March 2023 among 3300 patients diagnosed with malaria in nine health districts. Axel Kroeger's conceptual framework was used. The conventional recourse was defined as the use of a public or private health facility or at the community services. Then an early and appropriate care-seeking as seeking care within 24 hours of the onset of symptoms in the conventional recourse. Sankey's alluvial diagrams were used to represent patients' pathways and logistic regression models identified factors associated with early and appropriate care-seeking. A total of 1632 (49.45%) was female and 1132 (34.30%) were under 5 years of age with mean age of 27.46 months. For those aged 5 years and older, the mean age was 27.03 years. At time of interview, 1337 (40.52%), 1423 (43.12%), 437 (13.85%) of patients were respectively in their first, second and third recourse. Of all patients, 1757 (53.25%) had sought care within 24 hours and 28.55% had sought care within 24 hours at a conventional recourse. Individually and as a first intention, self-medication was the main modality with 1214 (37.30%). In 1992 (60.36%) patients had a conventional care pathway. Overall, the health districts of Boffa (Lower Guinea, coastal region) OR = 0.48 95% CI 0.33 – 0.70 (p <0.001), Dabola (Savanna region) OR = 0.43 95% CI 0.30 – 0.63 (p<0.001), and Labe (Mountain region) OR = 0.43 – 0.91 (p=0.016) were at risk of delaying appropriate care seeking (final ORs) for all group, regarding Dixinn district in Conakry. Low rates of early and appropriate care-seeking were observed. Patients generally sought care through multiple means, often resulting in a delay in adequate management. The risk associated with certain health districts in the care-seeking behavior shows the need to deploy strategies adapted to the needs of communities through an in-depth community diagnosis of health service utilization.

6099

SCREENING, VACCINATION, AND AWARENESS CREATION FOR HEPATITIS B VIRUS INFECTION IN ACCRA, GHANA

Rawdat Baba-Adam, Kwadwo Asamoah Kusi Lab, Linda Eva Amoah, Kwadwo Asamoah Kusi, Joseph Humphrey Kofi Bonney

Noguchi Memorial Institute for Medical Research, University of Ghana, Accra, Ghana

Viral hepatitis remains a major public health threat, affecting millions globally. For over a decade now, 28th July is observed as World Hepatitis Day (WHD), with the goal of raising awareness of viral hepatitis, intensifying screening efforts, increasing vaccination coverage, and mobilizing global efforts towards control. We present a report of our 2021 and 2022 WHD medical outreach activities in Accra. The activities took place in the Legon and Maamobi communities in 2021 and 2022 respectfully. The Advanced

quality one-step multi-HBV test was used to test for HBV surface and envelope antigens, as well as surface, core and envelope antibodies. Positive persons were recommended for management while negative persons were encouraged to take up vaccination. In 2021, 297 participants were screened at Legon – 19 (6.4%) tested positive for HBsAg, and 278 (93.6) were negative. Of these, 246 (82.8%) were offered free vaccination. In the end, 66% (163/246) completed all three vaccinations whereas 34% (83/246) were lost to follow-up after either the first or second vaccination. In 2022, 388 participants from 2 communities (Legon, Maamobi) were screened and 25 (6.4%) tested positive for HBsAg - 21 (5.4%) from Maamobi and 4 (1.0%) from Legon. A total of 204 (64.8%) received all 3 vaccine doses while 111 (35.2%) were lost to follow-up. Our activities align with the WHO's agenda to reduce the global burden of viral Hepatitis by 2030 through active case search, improved clinical care, and increased access to HBV vaccination. Additionally, generated data shed light on participants commitment to full dose completion which could be useful for HBV screening and vaccination activities in other areas across the continent.

6100

SUSTAINING MALARIA CONTROL THROUGH WARD DEVELOPMENT COMMITTEES IN NIGERIA

Ekpo Edet[1], Olusola Adeoye[1], Oluseyi Akintola[1], Oluyemi Abodunrin[1], Aderonke Popoola[1], Foyeke Oyedokun-Adebagbo[2], Veronica Momoh[2], Jules Mihigo[2], Samuel Owoya[3], Angela Acosta[4], Bolatito Aiyenigba[1]

[1]Breakthrough ACTION Project, John Hopkins University Center for Communication Programs, Abuja, Nigeria, [2]U.S. President's Malaria Initiative, USAID, Abuja, Nigeria, [3]National Malaria Elimination Programme, Federal Ministry of Health, Abuja, Nigeria, [4]Breakthrough ACTION Project, John Hopkins University Center for Communication Programs, Baltimore, MD, United States

Nigeria has a high malaria burden. Ward Development Committees (WDC) are government-constituted governance structures to encourage community participation and increase access to primary health care services at the ward level. However, there is little documentation of their potential contributions to malaria programs. In 2022, the USAID-funded Breakthrough ACTION-Nigeria Project (BA-N) trained 219 WDC representatives from 219 wards in 5 states to conduct community discussions about malaria prevention and treatment; refer, and follow up with eligible community members for fever care and antenatal (ANC) services; address barriers to malaria service provision and uptake; and mobilize resources for community health. WDC representatives are respected community members who volunteer their time and only receive transport stipends for activities. They submitted monthly reports on the number of community members in attendance and numbers referred and participated in monthly review meetings with BA-N and the State Malaria Elimination Programme officers, who provide supportive supervision. Between October 2022 and September 2023, WDCs conducted 5,748 community discussions and reached 108,098 participants (47,527 males (46%) and 60,571 females (54%)). Of these numbers, 26,218 were referred to health facilities for fever care and ANC services (24,320 and 1,898 respectively), with 22,800 (21,206 fever and 1,594 ANC) completing these referrals. Completed referrals were equivalent to 1% and 2% of the total number of cases seen at their focal facilities for fever and ANC. The overall referral completion rate was 87% for fever and 84% for ANC. The results suggest that WDCs can reach community members with malaria messages and are successful at engaging both males and females. While their contribution to overall service uptake for both services was minimal, they achieved high referral completion rates, suggesting high trust in WDCs. Future initiatives should measure WDCs' influence on community perceptions and other malaria behaviors and explore ways to cost-effectively scale the use and reach of WDCs for malaria social and behavior change.

6101

CALL FOR A FAIRER APPROACH TO AUTHORSHIP PRACTICE IN THE REPORTING OF BIOMEDICAL RESEARCH

Phaik Yeong Cheah[1], Michael Parker[2]

[1]Mahidol Oxford Tropical Medicine Research Unit, Bangkok, Thailand, [2]The Ethox Centre, University of Oxford, Oxford, United Kingdom

Authorship of academic publications confers credit, responsibility, ownership, and accountability for published research work. However, significant inequities currently exist in the research landscape concerning authorship practices. Current guidelines and practices disadvantage field workers, early career researchers (ECRs), those whose English is not their first language, and researchers from low-resource settings who do not have protected time for research or training in academic writing. For biomedical researchers, the most commonly used guidelines are the 'International Committee of Medical Journal Editors' (ICMJE) guidelines. However, the ICMJE guidelines and current practice do not adequately reflect the reality of contributions in such research and could exacerbate existing inequalities among researchers. Many contemporary teams are multidisciplinary and consist of individuals with varying abilities and existing inequalities. As such, not all individuals, such as ECRs, can or should be 'accountable for all aspects of the work' (criterion 4) as required by the ICMJE. Additionally, not all intellectual contributions meriting authorship require 'drafting the article or reviewing it critically for important intellectual content' (criterion 2). Our paper gives arguments for calls for a revision in the ICMJE guidelines, particularly in criteria 2 and 4, as well as current practices, to enable deserving individuals to be given the opportunity to be authors. For the latter, we urge lead and senior researchers to engage and support less privileged members of the team, ECRs, those who face language barriers, and those with limited experience to enable their enhanced contributions. We are not calling for a loosening of authorship requirements but rather, for recognition of those who have substantially contributed to be given the opportunity to be authors.

6102

UNDERSTANDING THE VIEWS OF PREGNANT AND LACTATING WOMEN ON CHILD BREASTFEEDING. A QUALITATIVE STUDY IN EASTERN ETHIOPIA.

Ketema Begna

Haramaya University, Harar, Ethiopia

Malnutrition is a common cause of death among children in Ethiopia. Exclusive breastfeeding is crucial for children survival, but fewer than half of children under six months old are exclusively breastfed. Child malnutrition can result from early weaning from breast milk. In Ethiopia, little is known about how socio-cultural factors impact breastfeeding. The paper explores how mothers conceptualise breastfeeding practices and strive to prevent child malnutrition. The study was conducted from May 2023 to July 2023 using ethnographic and phenomenological approaches, including in-depth interviews, focus group discussions, and participant observations. The study was conducted in the mother's home and nearby health facilities, where confidentiality was maintained. All the data was collected with the consent of the participants. The study found misalignment between biomedical and community understandings of breastfeeding. The three main obstacles to breastfeeding were: mothers' assumptions that breastmilk alone does not cover their children's nutritional needs; social and cultural burden to wean children from breastfeeding quickly; and the perception that breastfeeding during pregnancy could lead to child malnutrition, particularly kwashiorkor malnutrition. This dilemma is made worse by the impact of this misalignment because breastfeeding is not understood by communities or health workers in the same way. The study conceptually explores what is at stake when mothers and healthcare professionals don't speak the same language when it comes to breastfeeding. Open dialogue and engagement between socio-cultural and biomedical perspectives could lessen pervasive misconceptions and support mothers in breastfeeding.

6103

LESSONS FROM THE FIELD: MINIMUM SERVICE STANDARDS ASSESSMENT TOOL AND THE HOSPITAL STRENGTHENING PROGRAM: A NOVEL FIRST STEP TOWARDS THE QUALITY IMPROVEMENT OF NEPAL'S GOVERNMENT HOSPITALS

Rita Pokhrel[1], **Abigail Knoble**[2], Pratibha Gautam[3], Mohammad Kashim Shah[1], Archana Amatya[1], Ruma Rajbhandari[2]

[1]Nick Simons Institute, Kathmandu, Nepal, [2]Mass General Birgham, Boston, MA, United States, [3]Harvard School of Public Health, Boston, MA, United States

District hospitals in Nepal, as in other LMIC settings, struggle to provide quality care due to inadequate investments in equipment, human resources, and hospital infrastructure. To address these challenges, the novel Minimum Service Standards (MSS) assessment tool was developed by the Nick Simons Institute in partnership with the Ministry of Health and Population to routinely assess hospital readiness through a detailed checklist evaluating governance, clinical services, and support services. The Hospital Strengthening Program (HSP) then provides a mechanism to close the identified gaps through a small annual grant, thus together providing knowledge and resources to improve healthcare at the district hospital. Nepal is a mountainous, low-income country in South Asia which faces significant health challenges, including high maternal and under-five mortality rates. Since its inception in 2014, MSS/HSP has expanded to 127 government hospitals as of 2023. The MSS/HSP program provided a blueprint for hospitals to pursue excellence and has tracked and motivated substantial improvements in services, such as 24hr emergency (+14%), X-ray (+23%) and Cesarean service (+31%). Additionally, the program has profoundly impacted policy and management within the healthcare sector, influencing key areas such as budget allocation, insurance payments, and hospital upgrade criteria. The MSS/HSP program has given hospitals in Nepal a blueprint towards success through the use of the novel MSS assessment tool and the financial support to address gaps in the LMIC setting. Due to the close collaboration with the MoHP, the program has secured support at all levels of government, thereby ensuring its sustainability, and impact.

6104

PHYSICIAN KNOWLEDGE, ATTITUDES, AND PERCEPTIONS OF FACILITY-WIDE ANTIBIOGRAMS IN SOUTHERN SRI LANKA: A PRE-IMPLEMENTATION STUDY

Lorenna Cristal Garcia-Bochas[1], Sherine Nanayakkar[2], Perla Medrano[1], Ajith Nagahawatte[1], Lakmal Fonseka[2], Armstrong Obale[1], Lana Abusalem[1], Melissa Watt[3], Christopher Woods[1], Truls Ostbye[1], Champica Bodinayake[2], Gayani Tillekeratne[1]

[1]Duke University, Durham, NC, United States, [2]University of Ruhuna, Galle, Sri Lanka, [3]University of Utah, Salt Lake City, UT, United States

Antimicrobial resistance (AMR) poses significant challenges to global public health. Critical drivers of AMR are the misuse and overuse of antimicrobials for human health. Antibiograms are paper-based or electronic tools that display summary data of local antibiotic susceptibility trends, aiding physicians in selecting empirical antimicrobial therapies for a specific patient when microbiologic culture data are unavailable. However, scant literature exists on the feasibility and challenges of developing and implementing antibiograms in low- and middle-income countries (LMICs) with limited microbiological and antibiogram implementation capabilities. This qualitative study, conducted at the most prominent public tertiary care hospital in southern Sri Lanka from June to August 2023, explored physicians' knowledge, attitudes, and perceptions towards antibiograms. Through convenience sampling, 31 critical informant physicians were recruited from pediatric and adult medical wards. Interviews were conducted in English, audio recorded, transcribed, and analyzed using thematic analysis. Most physicians were unaware of antibiograms. However, almost all (96%, 29/31) physicians expressed enthusiasm for using antibiograms in their facility, citing the potential to refine antibiotic prescriptions, curb antibiotic resistance, and improve patient care. One-third (33%,10/31) expressed skepticism about antibiogram implementation, citing time and resource constraints. The physicians recommended that a multidisciplinary team in small, discussion-based groups conduct antibiogram training. These findings offer insights for developing and implementing antibiograms in an LMIC setting to optimize antibiotic prescribing practices and combat AMR globally.

6105

EFFECTS OF A SCHOOL-BASED PHYSICAL ACTIVITY PROGRAM AND MULTI-MICRONUTRIENT SUPPLEMENTATION ON BODY COMPOSITION AMONG SCHOOLCHILDREN IN THE KILOMBERO DISTRICT, TANZANIA

Elihaika Gilbert Minja[1], Emmanuel C. Mrimi[1], Winfrida Mponzi[2], Johanna Beckmann[3], Marceline F. Finda[2], Christin Lang[3], Fredros O. Okumu[2], Markus Gerber[3], Juerg Utzinger[1], Kurt Z. Long[1]

[1]Swiss Tropical and Public Health, CH-4123 Allschwil, Switzerland, [2]Ifakara Health Insitute, Ifakara, Tanzania, United Republic of, [3]University of Basel, CH-4052 Basel, Switzerland

Over the past decade, there has been a sharp rise in childhood obesity and overweight in low- and middle-income countries, including Tanzania, mainly governed by rapid changes in lifestyle and dietary patterns. We examined the effects of a school-based physical activity program and multi-micronutrient supplementation on body composition among schoolchildren in the Kilombero district, Tanzania. Children aged 6-12 years were cluster-randomized by class into one of four groups: (i) physical activity (PA) (n=236); (ii) a multi-micronutrient supplementation (MMNS) (n=263); (iii) physical activity plus multi-micronutrient supplementation (PA+MMNS) (n=257); and (d) control group (n=248). Children were followed over 2 years and assessment done at 12 and 24 months post-intervention. Generalized estimated equations (GEE) with random intercepts for school classes were employed to examine the intervention groups association with fat mass (FM), fat-free mass (FFM), truncal fat mass (TrFM), and truncal fat-free mass (TrFFM) during the second (T2) and third assessment (T3). A secondary set of GEE analyses were conducted, adjusting for children's sex, age, and height-for-age z score (HAZ) at the respective data assessments. In the unadjusted model, both boys and girls had decreased TrFM associated with PA promotion. In the adjusted model PA promotion among boys was significantly associated with reduced TrFM and FM. Boys engaged in the PA+MMNS arm also notably reduced FM. Girls in the MMNS arm had significant FM reductions, whereas those in PA arm had significant decreases in FM, TrFM and increases in FFM at T2. Girls in PA arm showed decreased in TrFM in the unadjusted model, while boys in PA+MMNS exhibited declined TrFFM. However, after adjustment, boys in the PA+MMNS arm had significant FM and TrFM declines. Furthermore, significant TrFM reduction in females assigned to the PA intervention at T3. Our research indicates that among Tanzanian schoolchildren in the Kilombero district, micronutrient supplementation and school-based physical activity programs were associated with decreased FM and enhanced FFM.

6106

CHARACTERIZATION OF MICROBIAL ISOLATES IN ANTIMICROBIAL STEWARDSHIP PROGRAM (ASP) OF A TERTIARY HEALTHCARE FACILITY IN SOUTHEAST NIGERIA - THE MONITORY PROJECT

Perpetua Onyinye Nnemelu[1], Chukwuanugo N. Ogbuagu[1], Ikemefuna N. C Onyeyili[1], Grace A. Elemuo[1], Irene U. Okeke[1], Ngozika U. Anazoba[1], Debra U. Okeh[2]

[1]Nnamdi Azikiwe University Teaching Hospital Nnewi Anambra state Nigeria, Nnewi, Nigeria, [2]Federal Medical Centre Umuahhia Abia state Nigeria, Umuahia, Nigeria

The emergence of antimicrobial resistance (AMR) poses a significant threat to global public health, challenging the effectiveness of traditional antibiotics. This section introduces the MONITORY project, which investigates AMR

within an Antimicrobial Stewardship Program (ASP) at a tertiary healthcare facility in Southeast Nigeria. The study employed a hospital-based descriptive approach, analyzing 235 clinical isolates collected from the microbiology laboratory of Nnamdi Azikiwe University Teaching Hospital. Isolation techniques, microbial identification, and antimicrobial susceptibility testing methods are described, alongside molecular analysis procedures. Gram-negative organisms predominated among clinical isolates, with notable resistance patterns observed. Staphylococcus aureus displayed significant methicillin resistance (MRSA), and molecular analysis confirmed the presence of ermC genes associated with macrolide resistance in the majority of S. aureus isolates. The findings highlight the urgent need for comprehensive ASPs to address AMR, particularly in resource-limited settings. Challenges in ASP implementation and the importance of strategic policy interventions, interdisciplinary collaboration, and international cooperation are discussed. Recommendations for responsible antimicrobial use, surveillance, and research efforts are provided. The MONITORY project underscores the critical importance of addressing AMR through collaborative efforts across healthcare sectors and global partnerships. Responsible antimicrobial stewardship, surveillance, infection control measures, and research and development initiatives are essential to mitigate the growing threat of AMR and safeguard public health.

6107

PRECARIOUS HUMANITARIAN SITUATION RISKING INFECTIOUS DISEASES OUTBREAKS FOR INTERNALLY DISPLACED PERSONS IN PORT-AU-PRINCE, HAITI

Fritznel C. Pierre[1], Saurel Doirin[1], Anthony Meme[1], Matthew H. Howard[2], Louise C. Ivers[3]

[1]Combite Pour la Paix et le Développement, Port-au-Prince, Haiti, [2]Milton Public Schools, Milton, MA, United States, [3]Harvard Global Health Institute, Cambridge, MA, United States

Since 2018, the humanitarian situation in Haiti has deteriorated through a series of escalating sociopolitical crises. This includes gang violence, internal displacement, migration, fuel stockouts, closing of schools, businesses and hospitals, and rising living costs. As of Dec 2023, 310,000 people were reported as forcibly displaced in Haiti due to violence and insecurity. A cholera outbreak resurged in Haiti in late 2022. Displaced persons are at particular risk of exacerbation of chronic diseases and of acute illnesses such as infectious diseases. We sought to better understand the living situations, motivations for displacement, and health risks of a population of displaced persons in a spontaneous camp in Carrefour Feuille, Haiti, a neighborhood of Port-au-Prince. We interviewed 100 camp residents in Sept 2023. The mean age of respondent was 37.5 years (range 19-78), 53% were male. More than half had been property owners. 69% were displaced due to gangs taking over their neighborhood, at least 20% experienced their home being set on fire. Regarding their home neighborhood, 88% reported never seeing a government representative, 75% were unaware of any support or services there. Only 18% reported a visit from any official to the camp, however 61% did receive some assistance. Regarding health and hygiene, 10 respondents (10%) reported being aware of someone with a diarrheal illness that resembled cholera. 84% reported the presence of latrines. Only 43% reported having access to a place to bathe, 44% had access to water, but only 16% had access to potable water in the camp. 42% reported being aware of someone who was ill, yet only 2 people reported that there had been a mobile clinic at the camp. Displaced persons in greater Port-au-Prince, Haiti in Sept 2023 were in precarious humanitarian conditions, vulnerable to infectious diseases outbreaks and other ill-health as a result of lack of appropriate shelter, water and hygiene facilities, health care and potable water access. In the context of an ongoing cholera epidemic and worsening socio-political crisis there is an urgent need for appropriate humanitarian support for displaced persons in Haiti.

6108

AWARENESS AND PRACTICE OF MEDICAL WASTE MANAGEMENT AMONG HEALTHCARE PROVIDERS AT SALAVANH AND SEKONG PROVINCIAL HOSPITAL, LAO PDR

Vannyda Namvongsa[1], Chathaya Wongrathanandha[2]

[1]Sethathirath Hospital, Vientiane, Lao People's Democratic Republic, [2]Faculty of Medicine, Ramathibodi Hospita, Mahidol Universityl, Bangkok, Thailand

Globally, medical waste management is a crucial public health concern. This descriptive cross-sectional study aimed to explore the amount of medical waste, and healthcare providers' awareness, knowledge, and practice in handling medical waste at Salavanh and Sekong provincial hospitals, from September 1st to December 31st, 2022. Every days waste was weighed and record from 2016-2022 for Salavanh Provincial Hospital, and from 2018-2022 for Sekong Provincial Hospital. All nurses (115), doctors (64) and the garbage collectors (2) for the inpatient departments were interviewed. Interviews were face to face using a Lao Language adaption of Zimba Letho's (2021) waste management surveys including their demographics questionnaire and medical waste management awareness and knowledge questionnaire previously used in Bhutan. Waste management practices were observed during the busiest hour of the day from 9:00-10:00 AM on seven consecutive workdays (Monday-Friday). Zimba Letho's (2021) observation check list was adapted to Lao Language and used in inpatient departments and at medical waste collection point to document the condition of waste receptacles, segregation of waste and how waste was transported. STATA version 13 was used for analysis. Salavanh Provincial Hospital produced was 0.68 kg/bed/day, and 0.80 kg/bed/day for Sekong Provincial Hospital with increasing amount of waste during time tracked. Most of the respondents had not received prior medical waste management training (71.8%). They knew some hazards of medical waste (blood-borne pathogens, sharps disposal, personal protective equipment, and waste disinfection). Most of them had difficulties following the medical waste management guidelines due to insufficient equipment for waste collection, storage, and disinfection. The observation results corresponded well with the interviews. Both hospitals lacked medical waste management training and Hepatitis B virus vaccination for their staff. There were several opportunities for improvement in medical waste management, especially in disinfection of transport vehicles.

6109

UNVEILING LIVES: EXPLORING THE DAILY ROUTINES OF LEPROSY-AFFECTED INDIVIDUALS IN MALAYSIA THROUGH VIDEO ETHNOGRAPHY

Norana Abdul Rahman[1], Vaikunthan Rajaratnam[2], Ruth M.H. Peters[1], Marjolein B.M. Zweekhorst[1], Karen Morgan[3], Mohamed Rusli Abdullah[4]

[1]Athena Institute, Vrije University, Amsterdam, Netherlands, [2]Khoo Teck Puat Hospital, Yishun, Singapore, Singapore, [3]RCSI & UCD Malaysia Campus, Penang, Malaysia, [4]Universiti Sains, Kubang Kerian, Kota Bharu, Malaysia

The study employed video ethnography, utilising the "walk and talk" technique, to invite individuals affected by leprosy to lead the researcher through their daily routines as part of a broader research on the lived experiences of individuals affected by leprosy in Malaysia. This method allowed participants to share their stories on their own terms, capturing spontaneous moments and insights that might not emerge in structured settings. The research aimed to understand the everyday routines and needs of leprosy-affected individuals and how they navigated challenges, societal expectations, and personal aspirations, using visual and auditory elements to provide nuanced exploration. Video ethnography typically involves smaller sample sizes, and participants were selected through purposive and convenience sampling based on leprosy diagnosis. Six participants volunteered, three each from Kelantan and Sungai Buloh Leprosarium, ensuring diverse data and some generalisability.

Participants were not choreographed but were given some instructions and information, and they were free to select their study locations. The walkabout resulted in 3 to 4 hours of video footage each, capturing verbal and nonverbal expressions. The videos, audio recordings, and field notes were transcribed and analysed using NVivo 12 software, revealing themes of resilience, economic engagement, stigma, supportive family dynamics, and community support. Despite challenges, participants demonstrated determination to lead productive lives, emphasising the importance of work for sustaining families and communities. Self-imposed stigma hindered confidence and societal participation, but strong family bonds and community support promoted social inclusion and combatted stigma. Overall, the study sheds light on the daily struggles, aspirations, and resilience of individuals affected by leprosy. It advocates tailored interventions for their overall well-being.

6110

EMPOWERING WOMEN AND GIRLS: A PATH TO GENDER EQUITY IN HEALTH AND WELLBEING

Elvis Safary[1], Angela Nguku[2], Vanessa Fargnoli[1], Debashish Das[1], Angela Muriuki[1]

[1]FIND, Geneva, Switzerland, [2]White Ribbon Alliance Kenya, Nairobi, Kenya

Women's health remains a critical global challenge, with significant disparities in access to healthcare, and diagnostic services especially in less-resourced settings. Empowering women and girls is not only a moral imperative but also a strategic investment in achieving gender equity in health and wellbeing. Despite significant progress in recent decades, gender disparities persist in access to healthcare, education, and decision-making power, disproportionately affecting women and girls in many parts of the world. Building upon the success of the "What Women Want" (WWW) campaign initiative conducted between 2018 and 2023 across eight countries, primarily in South-East Asia and Sub-Saharan Africa, FIND and White Ribbon Alliance (WRA), Kenya would like to embark on an initiative to understand how women and girls define health and wellbeing in Kenya. This endeavour aims to explore how women and girls define health and wellbeing in Kenya, with the goal of reshaping the understanding and response to their needs aiming to increase their decision-making rights agency for their health and wellbeing. Employing the WRA Kenya programming framework of 4Ps (people-practice-policy-products) within the Ask-Listen-Act approach, the initiative will ensure that women and girls receive quality, dignified, and equitable health services tailored to their needs at all levels. Key informant interviews and 'listening sessions' with adolescent girls, and women across the life continuum will be conducted. Additionally, the initiative will capture and document the lived experiences of women and girls through digital and multimedia storytelling to gain insights into their needs. The findings will inform community engagement, advocacy with duty bearers, and collaboration with key stakeholders to raise awareness among women and girls about their right to health, wellbeing, and self-care. Furthermore, the initiative aims to empower women and girls to demand and access quality, equitable, and dignified healthcare while advocating for policy reforms and resource allocation to prioritize women and girls' health and wellbeing throughout their lives.

6111

EXPLORING RESILIENCE AND WELL-BEING AMONG COMMUNITY HEALTH WORKERS: AN EXPLORATORY STUDY IN THE UPPER EAST REGION, GHANA

James Kotuah Sakeah[1], David Addiss[2], Cindy Feng[3], John Bosco Aburiya[4], Norbert Achibase[5], Margaret Gyapong[6], Ian Colman[1], Alison Krentel[1]

[1]University of Ottawa, Ottawa, ON, Canada, [2]The Task Force for Global Health, Atlanta, GA, United States, [3]Dalhousie University, Halifax, NS, Canada, [4]SD Dombo University of Business and Integrated Development Studies, Wa, Ghana, [5]Navrongo Health Research Centre, Navrongo, Ghana, [6]University of Health and Allied Sciences, Ho, Ghana

Community Health Workers (CHWs) play a pivotal role in the execution of health interventions for neglected tropical diseases (NTDs) and routine health services in low-resource settings. However, they encounter challenges and stressors that may impact their resilience and well-being. While the importance of resilience and well-being is increasingly recognized, most quantitative research can not fully capture how these concepts are expressed in the African context, identify factors that shape and maintain them or account for the diversity and complexity of experiences. To address this gap, a phenomenological approach was used to explore the meaning of resilience and well-being of CHWs in the Upper East Region of Ghana and to identify contextual factors that contribute to their ability to cope with challenges and serve their communities. Twenty in-depth interviews were conducted: 16 with CHWs and 4 with community stakeholders. Interviews were transcribed verbatim into English and analyzed in NVivo (Version 14). Findings showed that resilience emerges as expressions of reciprocal altruism, personal drive, community solidarity, and spirituality amidst adversity. Well-being is perceived by participants as holistic, encompassing physical health, mental state, spirituality, and social harmony. Resilience and well-being among CHWs are shaped and maintained by how they navigate individual strengths, relationships, resources, beliefs, and seasonal challenges. For instance, heavy rains disrupt CHWs' daily routines, create competing demands, and introduce stressors into their work. Resilience and well-being are essential aspects of the human experience, yet their expression and interpretation differ globally. To boost the resilience and well-being of CHWs, interventions must be context-specific. Among CHWs in Sub-Saharan Africa, understanding their unique needs and experiences is essential to fostering resilient CHWs and ensuring that they can effectively serve their communities for NTDs and routine healthcare interventions.

6112

THE COST OF ACCESS TO HEALTH CARE FOR CHILDREN UNDER-FIVE YEARS WITH SEVERE ANAEMIA - A COSTING STUDY OF REFERRAL HOSPITALS IN MALAWI KENYA AND UGANDA

Grace Wezi Mzumara[1], Melf-Jakob Kühl[2], Jobiba Chinkhumba[3], Oddvar Martin Kaarbøe[2], Bjarne Bjarne Robberstad[2]

[1]Training Research Unit of Excellence, Blantyre, Malawi, [2]University of Bergen, Bergen, Norway, [3]Kamuzu University of Health Sciences, Blantyre, Malawi

Severe anaemia is a major cause of hospital admissions in African children under five years of age and the condition bears a high cost of access to health care. This study estimates the household costs of accessing care for severe anaemia at referral hospitals in Malawi (Zomba and Kamuzu Central Hospitals), Kenya (Kisumu and Busia Referral Hospitals) and Uganda (Jinja and Kitgum Hospitals). We prospectively collect data on the costs incurred when accessing services for severe anaemia alongside the randomized controlled trial, titled: Dihydroartemisinin-piperaquine and azithromycin for the post-discharge management of children with severe anaemia in Malawi, Kenya and Uganda. After enrolling children under five with severe anaemia, their guardians report direct and indirect costs encountered during their hospital admission. Since August 2023, 192 participants have been enrolled across the six study sites. Among these, subsistence farming 79/192 and informal trading 50/192 were the main sources of income of guardians supporting children with severe anaemia while 129/192 paid for part of the services accessed. About 88/192, needed a loan and 29/192 had to sell their assets to cover costs for the illness. Motorcycle taxis, 104/192 and ambulances, 25/192 were the main modes of transport used to get to a referral facility. Across all study sites, the major costs incurred were for transportation $2.9 (on average) and food $3.8. Medication cost was a common cost in Uganda with 58/64 of participants paying an average of $6.31. Opportunity costs were high as primary guardians did not work for 6 days (inter-quartile range: 3-7 days) during the illness and a secondary guardian was commonly involved during the hospital stay for 4 (3-5) days. This amounts to a mean productivity loss of $21 in Malawi, $38 in Kenya and $11 in Uganda during an illness with severe anaemia. The mean total cost for access to care was $30 in Malawi, $45 in Kenya and $36 in Uganda. In conclusion, transport, medication and nursing care are major sources of catastrophic health expenditure in this population.

6113

MORTALITY ESTIMATES IN SOUTH AND SOUTHEAST ASIA BY ELECTRONIC VERBAL AUTOPSIES

Nan Shwe Nwe Htun[1], Elizabeth Ashley[2], Koukeo Phommasone[3], François Nosten[4], Aung Pyae Phyo[4], Moul Vanna[5], Md Akramul Islam[6], Shayla Islam[6], Aninda Sen[6], Nawrin Kabir[6], Carlo Perrone[1], Sue J Lee[7], Yoel Lubell[8], Thomas J Peto[8]

[1]Mahidol-Oxford Research Unit, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand, [2]Lao-Oxford-Mahosot Hospital-Wellcome Trust Research Unit, Vientiane, Lao People's Democratic Republic, [3]Lao-Oxford-Mahosot Hospital-Wellcome Trust Research Unit,, Vientiane, Lao People's Democratic Republic, [4]Shoklo Malaria Research Unit, Mae Sod, Thailand, [5]Action for Health Development (AHEAD), Battambang, Cambodia, [6]Communicable Diseases Programme, BRAC, Dhaka, Bangladesh, [7]Department of Infectious Diseases, Monash University and Alfred Hospital, Melbourne, Australia, [8]Centre for Tropical Medicine and Global Health, Nuffield Department of Medicine, University of Oxford, Oxford, United Kingdom

In low- and lower-middle income countries in Asia most deaths happen outside of the healthcare system with no cause of death (COD) assigned. Cause-specific mortality data are crucial for designing public health policies. Alongside a rural febrile illness etiology study of Southeast Asian patients, we sought to understand the underlying major COD in these underserved populations using electronic verbal autopsies (VA). Between 2021 to 2023, mortality surveys were conducted at rural sites in Bangladesh, Myanmar, Lao PDR, Thailand and Cambodia using standardized WHO VA questionnaires and ICD-10 codes, ascertained by the physicians' review. Over 3,000 adult deaths were identified across the sites with 80-95% completeness of death reporting. Following a death, most interviews were scheduled between three and six months in order to minimize recall bias. In all countries, men accounted for about 1,748 (60%) of the recorded deaths. The majority of cases died at home 2,290 (76%) and the deceased individuals used a variety of healthcare facilities, primarily government hospitals and health centers. At all sites non-communicable diseases predominated and the three leading COD among adults were conditions relating to the digestive system, cardiovascular disease, and neoplasms. Our research highlights the necessity of regional and country-specific strategies to address the growing burden of non-communicable diseases. To monitor changes over time, verbal autopsies could be integrated into countries' civil registration and vital statistics systems at the time of national surveys.

6114

UNDERSTANDING THE IMPACT OF WORKING HOURS ON MEDICAL DOCTORS IN NIGERIA. A STUDY ON MENTAL HEALTH AND DECISION-MAKING

Alamin M. Usman, Aisha J. Suleiman

Dalhatu Araf specialist Hospital, Lafia, Nasarawa state., Nigeria

This study seeks to comprehensively examine the impact of working hours on the mental health and decision-making of medical doctors in Nigeria. The hypothesis is that prolonged working hours may lead to increased stress, and impaired decision-making among medical doctors, ultimately influencing the quality of healthcare delivery. A quantitative approach was employed, utilizing a survey questionnaire to gather data from 58 medical doctors in a tertiary hospital in Nigeria. The questionnaire encompassed items related to expected working hours, self-reported mental health status, and perceived impact of long working hours on decision-making. Participants were selected through convenience sampling. Preliminary findings indicate that 86% of doctors start work by 8 am, with 55% only closing when they are done with work. 53% reported working 5 days a week, 26% work 7 days a week, and 50% reported working even on weekends. Additionally, 95% reported not having scheduled breaks during their workday, and 97% reported taking multiple in-hospital calls a week. 97% believe the impact of long working hours on them affects their patients care and 86% believe it drives their decision-making. A significant number, 60% reported their hospital not having policies guiding their working hours. Notably, 100% believe that revising and regulating working hours would positively impact their physical and mental well-being, as well as improve healthcare delivery to patients. These findings underscore the urgency of addressing working hour policies to safeguard the mental and physical well-being of medical practitioners and enhance patient care. Further analysis of the data is underway to provide a comprehensive understanding of the impact of working hours on medical doctors in Nigeria.

6115

THE ROLE OF GENDER IN MALARIA HEALTHCARE PROVIDER PERFORMANCE

Elizabeth Arlotti-Parish[1], Katherine Wolf[1], Lolade Oseni[1], Annie Ciceron[2], Patricia Gomez[1], Thierno Ba[2], Jadmin Mostel[2]

[1]Jhpiego, Baltimore, MD, United States, [2]Population Services International, Washington, DC, United States

Gender dynamics shape interpersonal interactions between healthcare providers and supervisors and influence provider recruitment, retention, and performance. Providers of different genders receive different professional development opportunities and face different constraints which impact their performance. PMI Impact Malaria conducted a secondary analysis of malaria service delivery training data and supervisory data to examine how the gender of training participants, supervisors, and supervisees influenced training participation, and training and supervisory performance. The study examined 491 supervisory observations across three countries, and 23,671 training observations across 11 countries using descriptive statistics, multivariate regression, and qualitative data validation with country teams. The study found that women improve more between pre- and post-test when trainings are at parity with regard to participant gender; for example, case management trainings in Rwanda had roughly equal numbers of female and male participants, and women had greater gains than men by 3.58% (p=0.02). Country team validation meetings noted that parity is difficult to achieve due to gender barriers to women entering the cadres targeted for the trainings, or preference by predominantly male managers to select male participants even within predominantly female cadres (e.g. nursing). Teams noted that women often make fewer training gains and perform worse overall due to household and childcare responsibilities (such as bringing infants to trainings or leaving early to complete household tasks) and having less experience or seniority in the field than male counterparts. Analysis of supervisory data did not indicate significant differences in competency scores based on supervisor or supervisee gender; however, this analysis was limited due to sample size, and it is recommended that supervisory checklists include gender of supervisor and supervisee to further this analysis. Both global and country-level analysis of these results can help programs understand and address gaps in malaria provider performance.

6116

EVALUATION OF PHYSICAL ACTIVITY AND DIET INTERVENTIONS IN PREVENTING CHILDHOOD OBESITY IN THE UNITED STATES OF AMERICA: A SYSTEMATIC REVIEW

Christopher I. Chukwu

University of Oxford, Oxford, United Kingdom

In the United States (US), ethnic/racial minorities and poor socioeconomic status children are disproportionately affected by childhood obesity. Among Organization for Economic Cooperation and Development member states, the US has the highest obesity rate in the world. In an investigation of the prevalence of childhood obesity in the US, no age group showed indications of a decrease. Obesity prevention in children is a global priority. Diet and physical activity are considered to be changeable behavioural factors that affect overweight and obesity. A significant body of research on behavioural risk factors associated with childhood obesity implies that physical activity and eating behaviours are related and likely bidirectionally causal, and hence should be explored simultaneously in this study. A narrative synthesis for quantitative studies was chosen to answer the research questions in this systematic review. The search utilised specified

combinations of MeSH words, Boolean operators, and Truncation and was conducted using three databases: PubMed, MEDLINE, and Child Development & Adolescent Studies and covered between the year 2016 and 2022. The Critical Appraisal Skills Programme for Randomised Control Trial (RCT) research appraisal instrument was utilised to evaluate the study's quality. Included were ten studies from across the US between the year 2016 to 2022. These studies were undertaken primarily in two distinct settings: the school and the community. The review found that childhood obesity is still pervasive in the US, especially among racial/ethnic minorities and low-income groups, and will continue to rise if not adequately addressed. The current behavioural interventions, which include physical activity and nutrition education, are capable of positively influencing weight-related outcomes and BMI among 5 to 18-year-old children in the US, but an integrated multicomponent strategy will achieve better results. Nonetheless, future RCT research should focus a greater emphasis on systemic therapies, such as policy and socioeconomic interventions.

6117

NAVIGATING THE LOW COVID-19 VACCINATION RATE NEXUS: BIBLICAL INTERPRETATIONS AND PRACTICES OF PENTECOSTAL CHRISTIANS IN DMV

Patricia Mbah Epse Nchamukong

Walden University, Minneapolis, MN, United States

The development and release of a vaccine during an epidemic or pandemic is aimed at mitigating the adverse effects of infection, transmission, and spread of the disease. However, the development and availability of a vaccine does not always guarantee the acceptance of the vaccine by the entire population. This is the case of COVID-19 vaccine that was approved and released by FDA in August 2021. The low COVID-19 vaccine rate within the Black Pentecostal Christian community in the DMV area is a public health concern. The decision by many in this subpopulation to reject the vaccine would continue to adversely impact the development of herd immunity and may likely extend the spread of the disease. Some factors that might have influenced the decision of this population to accept or reject the vaccine have been linked to politics, anti-COVID-19 vaccine sentiments, and religion. Though research findings have reported that belief in God has led to a delay or refusal of COVID-19 vaccine, the research focused on only one aspect of the image of God - how individuals conceptualize God and how this could influence the acceptance of the vaccine. This calls for further research on other aspects of the image of God. This study therefore aimed at exploring and elucidating the convoluted relationship between religious beliefs, practices, and leadership guidance in the context of COVID-19 vaccination within the Black Pentecostal Christian community in the Washington D.C. Metro area. The qualitative study uses the phenomenological approach with in-depth interviews and the theory employed is the health belief model. Findings of this study are significant in bridging the gap in knowledge with regards to health, religious studies, and community engagement. The findings would also have the potential to enact positive social change both at personal and community level. Moreso, the findings would help policy makers, public health professionals, and researchers to make informed decisions. The author is finalizing on the interview and wishes to present the results and findings of this study in November 2024 in New Orleans, USA during the ASTMH annual conference.

6118

MASS CYTOMETRY DATA INTEGRATION METHODS REVEAL RURAL-URBAN GRADIENT OF IMMUNE PROFILES ACROSS GEOGRAPHY

Koen Stam[1], **Marloes M.A.R. Dorst**[1], Mikhael D. Manurung[1], Jeremia J. Pyuza[2], Dicky L. Tahapary[3], Taniawati Supali[4], Ahmed Mafouz[5], Mohammed Charrout[6], Simon P. Jochems[1], Marion H. König[1], Yvonne C.M. Kruize[1], Moustapha Mbow[7], Wouter A.A. A.A. de Steenhuijsen Piters[1], Maria Yazdanbakhsh[1]

[1]Leiden University Center for Infectious Diseases (LUCID), Leiden University Medical Center, Leiden, Netherlands, [2]Department of Pathology, Kilimanjaro Christian Medical Centre, Moshi, Tanzania, United Republic of, [3]Division of Endocrinology and Metabolism, Department of Internal Medicine, Dr. Cipto Mangunkusumo National General Hospital, Faculty of Medicine Universitas Indonesia, Jakarta, Indonesia, [4]Department of Parasitology, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia, [5]Leiden Computational Biology Center, Leiden University Medical Center, Leiden, Netherlands, [6]Delft Bioinformatics Lab, Delft University of Technology, Delft, Netherlands, [7]Department of Immunology, Faculty of Medicine, Pharmacy and Odontology, Cheikh Anta Diop University of Dakar, Dakar, Senegal

The human immune system strongly varies across populations and is impacted by a range of host and environmental factors. As such, a rural, compared to urban lifestyle, has previously been associated with baseline differences in immune profiles and reduced vaccine responsiveness. Here, using three mass cytometry datasets, we studied shared and population-specific immune characteristics of healthy rural- or urban-living Indonesian, Senegalese and Tanzanian adults and urban-living Europeans. After harmonized preprocessing and quality control, 75.4 million cells were integrated using in total four data-integration methods, including CytoNorm, Harmony, CytofIn and quantile-normalisation. Using the best performing integration method (CytoNorm), we were able to assess in detail cellular immune profiles associated with rural- or urban-lifestyle shared across geography. Generally, rural-living individuals showed an immune profile that showed an overall highly differentiated and activated state. Using machine learning models, we were able to discriminate rural- and urban-living individuals based on these profiles with moderate to-high accuracy. The current study serves is an example on the integration of different large mass cytometry datasets. The findings presented here may guide future studies on the shared or population-specific environmental drivers of baseline immune profiles and how this impacts vaccine immunogenicity in low-responding populations.

6119

SUCCESSFUL RECRUITMENT STRATEGIES FOR ENGAGING PREGNANT WOMEN IN CLINICAL TRIALS: LESSONS LEARNED FROM TWO INDIVIDUALLY RANDOMIZED CONTROLLED TRIALS CONDUCTED IN KENYA

Everline Delylah Ondieki

Kenya Medical Research Institute, Kisumu, Kenya

Experiencing a low recruitment and high attrition rate among participants has been a persistent challenge in numerous clinical trials involving pregnant women and may introduce bias into the trial's findings. Although barriers to effectively recruiting pregnant women to clinical trials are well documented, little is known about how to succeed. The Improving Pregnancy Outcome (IMPROVE) trials were individually randomized clinical trials targeting HIV-negative and HIV-positive pregnant women between 16-28 weeks gestation. Women gave their consent at enrolment, and an obstetric ultrasound was performed to estimate gestational age. Afterward, participants attended regular scheduled study clinic visits every four weeks up to six or eight weeks after delivery. Venous blood and urine samples were collected at each visit. Overall, we enrolled 1925 participants, with 96.7% of scheduled visits attended and 99.0% of participants contributing to the primary analysis. Recruitment strategies reported included: home pregnancy testing by community health workers and referral to the study clinics; establishing group antenatal care tailored towards the studies' inclusion criteria; utilizing key community stakeholders to spread messages about the study; establishing community advisory board (CAB) to help disseminate information about the study and support to curb negative perceptions about the study in the community; site networks, which made it possible for the study to get referrals from other facilities. Retention strategies included: proper consenting using a language a participant is comfortable with and assessing comprehension after consenting; involving guardians and spouses during consenting; regular phone calls to remind the participants about visits; realistic trial timelines; a wide scheduled visit window; free obstetric ultrasound at enrolment; compensation of participants for their time and transport. IMPROVE clinical trials experiences

provide additional evidence of successful recruitment and retention of pregnant women and reinforce more of what has previously been documented.

6120

ESTABLISHING A NATIONAL DEEP VEIN THROMBOSIS NETWORK IN GHANA: RESULTS FROM A PROSPECTIVE MULTI-CENTER STUDY

Lena Huebl*[1], Isaac Kofi Owusu*[2], Collins Kokuro[2], Yaw Adu-Boakye[2], Bernard C. Nkum[2], Isaac Nana Ogyefo[2], Emmanuel Acheamfour-Akowuah[2], Musah Labarau[2], Samuel Frimpong Odoom[2], Richard King Nyamekye[3], Daniel Ohemeng Minkah[4], Henry Kofi Andoh[5], Alfred Doku[6], Francis Agyekum[6], Seth Yao Nani Jnr[6], Allen Steele-Dadzie[6], Klenam Dzefi-Tettey[6], Yaw Asante Awuku[7], Ama Gyadua Boadu[8], Adam Atiku[9], Joan Aguyire[9], Jameela Afriyie Khalid[10], Kirsten Eberhardt[11], Michael Ramharter[1], Christina Rolling[12], Thierry Rolling[13], Christof V. Vinnemeier #[1], Melina Heinemann #[1]

[1]Department of Tropical Medicine, Bernhard Nocht Institute for Tropical Medicine & I Department of Medicine, University Medical Center Hamburg-Eppendorf, Hamburg, Germany, [2]Komfo Anokye Teaching Hospital, Kumasi, Ghana, [3]Agogo Presbyterian Hospital, Agogo, Ghana, [4]Kumasi South Hospital, Kumasi, Ghana, [5]Bono Regional Hospital, Sunyani, Ghana, [6]Korle-Bu Teaching Hospital, Accra, Ghana, [7]Ho Teaching Hospital, Ho, Ghana, [8]Cape Coast Teaching Hospital, Cape Coast, Ghana, [9]Tamale Teaching Hospital, Tamale, Ghana, [10]Effia Nkwanta Regional Hospital, Takoradi, Ghana, [11]Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany, [12]Department of Oncology and Hematology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany, [13]I. Department of Medicine, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

Venous thromboembolism (VTE), including deep vein thrombosis (DVT) and pulmonary embolism (PE), is a leading cause of morbidity and mortality worldwide. Nevertheless, data on VTE in sub-Saharan Africa are scarce. This observational study was carried out in a national capacity development project aiming to improve the diagnosis and treatment of patients with DVT in Ghana. Between 2018-2022, we established a “National DVT Network” comprising nine hospitals across Ghana. “DVT teams” were trained and equipped with technical infrastructure to enable DVT diagnosis. A total of 1422 adult patients with suspected DVT were screened. DVT was confirmed by ultrasound in 626 patients (44%), including 619 patients with lower extremity DVT (LEDVT) and 10 patients with upper extremity DVT. Among the 619 cases of LEDVT, 77% were inpatients. Among 240 patients with suspected DVT and PE, PE was confirmed in 59 (25%) by computed tomography. Anticoagulants were administered to 223 out of 1422 patients (16%) with suspected DVT prior to the onset of suspicious symptoms. This included 25% (37/146) of inpatients with active cancer, 23% (57/244) of inpatients with lower extremity immobilization, and 27% (8/30) of inpatients with a history of DVT. Study phase 2 (2020-2022) comprised 930 patients, including 379 with VTE. Out of 930 patients, 81 died (9%) primarily while hospitalized. Anticoagulation was received by 96% (365/379) of patients with confirmed VTE. The fatality rate was significantly higher among patients with confirmed VTE (17%, 65/379 versus 3%, 16/551, $p<0.001$). Excluding individuals who were not deceased, only 16% (45/310) of patients diagnosed with DVT received follow-up after 6 months. Among these, DVT was completely resolved in 71% (32/45). The prevalence of DVT among Ghanaians with clinical suspicion is high, suggesting that DVT is a common disorder in Ghana. Increased awareness among healthcare professionals for indicated prophylactic anticoagulation is needed. The follow-up of patients with DVT was insufficient and needs to be improved. However, in most patients receiving treatment and follow-up, DVT might be completely resolved after 6 months.

6121

THE FIRST AFRICAN CENTER OF EXCELLENCE IN BIOINFORMATICS & DATA SCIENCE (ACE-MALI): TEN-YEAR ACCOMPLISHMENTS

Mamadou Wele[1], Jeffrey G. Shaffer[2], Cheickna Cisse[1], Mahamadou Diakite[1], Jian Li[3], Abdoulaye Djimde[1], Seydou O. Doumbia[1], Darrell Hurt[4], Mike Tartakovsky[4], Christopher Whalen[4], Alia Benkahla[5], Doulaye Dembele[6]

[1]University of Sciences, Techniques and Technologies of Bamako, Bamako, Mali, [2]Tulane University,, New Orleans, LA, United States, [3]University of Sciences, Techniques and Technologies of Bamako, New Orleans, LA, United States, [4]NIH, Bethesda, MD, United States, [5]NIH, Tunis, Tunisia, [6]NIH, Strasbourg, France

The April 2, 2015, was the opening ceremony of the First African Center for Excellence in Bioinformatics and Data Science at University of Sciences, Techniques and Technologies of Bamako, (USTTB) Mali. The goal of the ACE initiative is to leverage in-kind donations to provide a sustainable, reliable, and local infrastructure of high-performance computing hardware coupled with bioinformatics tools, training, and mentorship designed to improve the quality of infectious disease research in Africa by Africans. ACE aims to carry out relevant research based on high-quality training in Bioinformatics and Data sciences at MSc and PhD levels that takes into consideration the needs of Public health and industry in west Africa. The program has obtained different Research and training grants from NIH, H3Abionet and USTTB to support research and Msc and PhD. Some Students were supported by different programs such us NIH-Fogarty training Grant, H3ABioNet and Welcome Trust funded DELGEM program.In collaboration with Tulane University (USA), we organized annual bioinformatics and data science symposia and workshops, student and faculty exchange program at Tulane University, careers enhancement, grant writing and submission. ACE hosts different short-term training from H3ABionet such us IBT, NGS, AGMT, etc.Today 42 students are graduated in Msc in Bioinformatics from Mali, Gabo, Burundi and Nigeria, 12 of them are enrolled in PhD programs in Mali, in UK or USA and 10 of them have position of Research Assistants in different national Research institutions and research program in Mali. A couple of PhD students are ready to defend their thesis by the end of this year, Their works were focused on malaria vaccine and drug target development in silico, proposing some good targets with their inhibitors. Faculty and student have produced up to twenty scientific publications in international reviews. This work summarizes the highlights of main achievements of the program and developed approaches can serve as a model to build or strengthen capacity in research training program in Africa.

6122

EXAMINING THE PRESENCE OF MONKEYPOX IN A GHANAIAN COMMUNITY: A CASE STUDY AT PENTECOST HOSPITAL

Mary Boadu

Ghana Health Service, Accra, Ghana

The Greater Accra region of Ghana has historically been regarded as nonendemic for monkeypox. Nonetheless, the evolving interplay of factors such as climate change, and urbanization raises uncertainties regarding its current transmission status. The La-Nkwantanang Madina Municipal Health Directorate was notified of a suspected monkeypox case involving a 13-day-old infant, Priceless Danso, at Pentecost Hospital, Madina. Upon admission, the infant presented with a temperature of 37.2°C, pulse rate of 155 bpm, respiration rate of 36 cpm, SpO2 of 64%, and RBS of 9.7 mmol/L. Multiple vascular rashes (approximately 0.5 cm × 0.5 cm) were distributed evenly over the body, and the child was dehydrated. Diagnostic tests confirmed impetigo/monkeypox and sepsis, with a positive result for monkeypox. The infant was treated with oxygen, intravenous antibiotics (ampicillin, flucloxacillin), and intravenous fluids. The mother and grandmother received counseling and reassurance, and health education was provided on the disease condition, signs, and symptoms. A cross-

sectional design involving interviews with a structured questionnaire was used for contact tracing. The survey purposefully sampled individuals who got contact with the infant. Tragically, the infant, Priceless Danso, passed away the following morning. Contact tracing and preventive measures were initiated, including self-observation for involved staff and the provision of a contact number for follow-up information or clarification. The La-Nkwantanang Madina Municipal Health Directorate collaborated with various specialties, including Disease Control, Community Health Nurses, Nutrition Management, Nursing Administration, Clinical Coordinators, and other districts, to break the chain of transmission and avoid a public health emergency.

6123

INVESTIGATING THE INFLUENCE OF HUMAN MILK OLIGOSACCHARIDES ON CHILD GROWTH DEVELOPMENT

Luis E. Zambrana[1], Fredman Gonzalez[2], Lester Gutierrez[2], Nadja Vielot[3], Christian Toval-Ruiz[2], Rebecca Rubinstein[1], Lars Bode[4], Filemon Bucardo[5], Samuel Vilchez[3], Sylvia Becker-Dreps[3]

[1]Department of Epidemiology, UNC, Chapel Hill, NC, United States, [2]Institute for Global Health and Infectious Diseases, UNC, Chapel Hill, NC, United States, [3]Department of Family Medicine, UNC, Chapel Hill, NC, United States, [4]University of California, San Diego (UCSD)., San Diego, CA, United States, [5]Department of Family Medicine and Department of Microbiology and Immunology, Chapel Hill, NC, United States

Detecting growth deficiencies in children early on is imperative for implementing effective interventions and, averting potential long-term health complications. Human Milk Oligosaccharides (HMOs) are vital for infant nourishment, fostering a healthy gut microbiome and strengthening the immune system. Research indicates they positively influence infant growth, leading to healthier outcomes by 24 months of age. Their role in promoting optimal growth and development underscores the significance of breastfeeding in early infancy. Our investigation constitutes a supplementary study to the SAGE birth cohort, an established research initiative comprising 444 mother-infant pairs located in Leon, Nicaragua. Breastmilk collected from mothers after childbirth at one month was analyzed using fluorescent high-performance-liquid-chromatography, where the composition of 19 HMOs was quantified. Linear Regression Models were conducted to examine the relationship between HMO composition and infant growth using the rate of change in the child's Z scores between one and 24 months of age. The assessment of HMO levels revealed distinct patterns between secretor and non-secretor mothers, with varying HMO abundances. In the crude analysis, higher concentrations of 3-sialyllactose (3'SL) were significantly associated with positive changes in Weight-for-Age Z-score (WAZ) and Length-for-Age Z-score (LAZ) over time in infants, underscoring the importance of this HMO in infant growth. Additionally, positive associations were found between difucosyllactose (DFLac), difucosyllacto-N-tetrose (DFLNT), disialyllacto-N-tetraose (DSLNT), and fucosyllacto-N-hexaose (FLNH) concentrations and WAZ rate changes, while promising trends were observed in fucodisialyllacto-N-hexaose (FDSLNH) and sialyl-lacto-N-tetraose c (LSTc) concentrations in relation to WAZ and LAZ rate changes, respectively. These findings underscore the significance of understanding the specific HMOs' impact on infant growth and development, advocating for increased breastfeeding to optimize children's health outcomes.

6124

DETERMINANTS FOR EARLY CARE SEEKING FOR MALARIA AMONG CAREGIVERS OF CHILDREN UNDER FIVE YEARS IN UGANDA

Judith Nalukwago[1], Leonard Bufumbo[1], Anna Passaniti[2], Pallen Mugabe[1], Musa Kimbowa[1], Moses Sembatya[1], Elli Leontsini[3], Jane Alaii[4], Emmanuel Kayongo[1], Pearl Kobusingye[1], Arzum Ciloglu[2], Leanne Wolff[2], Glory Mkandawire[1], Joel Kisubi[5], Sheila Nyakwezi[5], Jimmy Opigo[6], Sharminah Kawuma[6], Richard Kabanda[7], Suruchi Sood[2]

[1]Johns Hopkins University Center for Communication Programs, Kampala, Uganda, [2]Johns Hopkins University Center for Communication Programs, Baltimore, MD, United States, [3]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [4]Simba Educational Consultants, Kampala, Uganda, [5]US President's Malaria Initiative, USAID, Kampala, Uganda, Kampala, Uganda, [6]National Malaria Control Division, Ministry of Health, Kampala, Uganda, Kampala, Uganda, [7]Department of Health Promotion, Education and Communication, Ministry of Health, Kampala, Uganda, Kampala, Uganda

Following decades of implementing malaria prevention and treatment interventions, the Demographic Health and Malaria Indicator Surveys showed a positive trend in prompt care-seeking for children under five years in Uganda from 75% in 2016 to 87% in 2019. A cross-sectional study conducted in West Nile and Karamoja regions in November-December 2022 assessed the determinants for early care seeking practices. The mixed methods study with caregivers included 803 surveys, 18 focus group discussions, 24 in-depth interviews. STATA 17 and Atlas.ti 23 were used for analysis. From the survey, 54% of the caregivers had children under five who had a fever in the past 30 days, 76% sought treatment and advice from providers, 84% indicated a preference for government health care rather than traditional healers. 69% reported being happy with malaria services for children under five at the visited health facility, and the odds of practicing positive malaria behaviors such as prompt care-seeking for children with a fever in 24 hours of onset were significantly higher among those who reported being satisfied with the services and provider interactions (OR 5.9, 95% CI: 2.86 - 12.28). The reasons for prompt care seeking described in focus group discussions and interviews included previous encounters with malaria among children, the need to avoid complicated illnesses, saving the child, fear of treatment costs, the need to avert malaria deaths, and positive results from completing a full course of malaria treatment. The perceived motivators of health facility utilization included advice on malaria prevention, ability to provide diagnostic and appropriate treatment services, availability of free services, proximity of health facilities, availability of community health workers. Accompanying one another to the hospital and taking care of the neighbor's children were common ways of practicing shared compassion. Service delivery programs need to improve client experience by making services more accessible, building provider-client relationships, and encouraging compassionate actions to improve malaria case management.

6125

TRADITIONAL HEALERS REFERRING FOR MALARIA IN UGANDA: RESULTS FROM RAPID ETHNOGRAPHIES

Leonard Bufumbo[1], Judith Nalukwago[1], Anna Passaniti[2], Pearl Kobusingye[1], Pallen Mugabe[1], Musa Kimbowa[1], Paul Odeke[1], Emmanuel Kayongo[1], Nanah Claren[1], Fiona Amado[1], Venansio Ahabwe[1], Glory Mkandawire[1], Moses Sembatya[1], Edward Kagguma[1], Allan Mukumbwa[1], Ivan Ireeta[1], Elli Leontsini[3], Jimmy Opigo[4], Joel Kisubi[5], Sheila Nyakwezi[6], Suruchi Sood[2]

[1]Johns Hopkins University Center for Communication Programs, Kampala, Uganda, [2]Johns Hopkins University Center for Communication Programs, Baltimore, MD, United States, [3]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [4]National Malaria Control Division, Ministry of Health, Kampala, Uganda, Kampala, Uganda, [5]US President's

Malaria Initiative, USAID, Kampala, Uganda, Kampala, Uganda, [6]United States Agency for International Development, Kampala, Uganda, Kampala, Uganda

Available evidence on Uganda suggests that traditional healers are common, however, few studies document the emergence of acquiescence for, and collaboration with, modern medical services. This study investigated traditional healer practices which promote malaria prevention and treatment. Between 27 November and 10 December 2022, a cross-sectional design comprising seven key informant interviews (KIIs) and six rapid ethnographic observations was conducted with traditional healers from the West Nile and Karamoja regions of Uganda. Participants were purposively selected using snowball sampling in areas of high malaria positivity. KIIs were used to collect individual perspectives while ethnographic observation triangulated with contextualized data. Results illustrated that traditional healers were appreciative and supportive of ongoing referrals and linkages with the health facilities. Traditional healer skills were used by health facilities under supervision, to address shortages of staff, for example in the maternity ward where they observed pregnant women progression towards delivery and alerted the midwife. Observation data revealed; a traditional healer tendency for testing for malaria from the facility before complimentary herbal healing was commenced; that traditional healers do not treat conditions they do not understand because they want to avoid wasting herbs and time and fear the consequences of adverse client outcomes. Traditional healers request clients to first get a test at a health facility to ensure the specificity of their treatment methods. KII data revealed that traditional healers were mostly confident of facility-based test results and medicines, which they perceived as fast in healing compared to "slow" herbs. From this study, traditional healers were receptive towards a referral role to health facilities for confirmation of malaria before herbal treatments commence. Potential interventions among traditional healers may include; engagement as partners and referral agents.

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ENHANCING CHILD MORTALITY SURVEILLANCE AND PREVENTION STRATEGIES IN LOW MIDDLE-INCOME COUNTRIES: THE CHAMPS NETWORK APPROACH IN PAKISTAN

Asefa S. Ansari[1], Sameer Belgaumi[2], Saima Jamal[1], Raheel Allana[1], Dr. Saad B. Omer[2], Dr. Zeeshan Uddin[1], Dr. Abdul Momin Kazi[1]

[1]Aga Khan University, Karachi, Pakistan, [2]UT Southwestern, Dallas, TX, United States

Child mortality is a pressing issue in countries, like Pakistan, where under-five mortality rates are nearly double the global average. Many more deaths, particularly those at home, go unaccounted for—with incomplete determination of the cause of death (CoD). The Child Health and Mortality Prevention Surveillance (CHAMPS) site in Pakistan aims to establish mortality surveillance of children under five years in three peri-urban areas in Karachi. The goal is to collect robust data on CoD through lab testing of post-mortem minimally invasive tissue samples (MITS) alongside clinical and verbal autopsy (VA) data. Implementation began in July 2023 with a target of 100 MITS samples in a year. Preliminary socio-behavioral science (SBS) investigations informed our strategy. On-going community engagement and liaison with stakeholders helped establish a network of key informants to provide timely death alerts. Mobile vans, specifically designed for MITS procedures, are deployed in catchment areas for flexible sample collection outside households or hospitals, addressing the gap in accounting for home deaths. A rapid response time and collection outside households also help reach the target population within the narrow window between death and shrouding/burial common to Muslim communities—after which the body cannot be disturbed. Grief support for bereaved parents and a CoD report are provided as incentive for study participation. The van can also be used to aid families in ritual bathing, shrouding and transport of the body following collection. A total of 180 microbiology, histology and molecular tests are conducted on samples. Lab data is reviewed in conjunction with available child and maternal clinical and VA data by a panel of physicians to determine CoD. To date, 31 MITS samples have been collected. 45% were collected from home deaths. MITS data on community deaths should provide an in-depth understanding of pediatric CoD in Pakistan to help tailor interventions and policies to reduce under-five mortality. Our approach may benefit others looking to overcome challenges in community-oriented surveillance and sample collection.

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ASSESSING TENSION AND ALIGNMENT OF COMMUNITY VALUES AND CHILD HEALTH AND MORTALITY PREVENTION SURVEILLANCE (CHAMPS) IN URBAN NEIGHBORHOODS OF KARACHI, PAKISTAN

Shaheen Sarfraz[1], Nazia Ahsan[1], Hannah Melchinger[2], Sameer Belgaumi[2], Iqbal Sheikh[3], Sultana Mukhtar[1], Momin Kazi[1], Saad Omer[2], John Belvins[4], Fauzia Malik[2]

[1]Aga Khan University, karachi, Pakistan, [2]UT Southwestern, Dellas, TX, United States, [3]Aga Khan University, Karchi, Pakistan, [4]Emory University, Atlanta, GA, United States

The Child Health and Mortality Prevention Surveillance (CHAMPS) networks conducts mortality and pregnancy surveillance in Africa and South Asia to understand and prevent child mortality. A key data component is the collection of minimally invasive tissue samples (MITS) to determine cause of death. We assessed the feasibility, acceptability, and implementation of CHAMPS through a series of participatory workshops within the communities of three peri urban areas of Karachi, Pakistan. Participatory Inquiry into Community Knowledge for Child Health and Mortality Prevention (PICK-CHAMP) workshops introduce CHAMPS to target communities and assess how well study activities align with community perceptions and priorities. Ten workshops were conducted from May to June 2023 with both community members and leaders, selected to participate through purposive and snowball sampling. PICK-CHAMP workshop exercises identified participants' health concerns and explored their attitudes towards mortality and pregnancy surveillance. Data was thematically analyzed and the degree of alignment or tension between community priorities and CHAMPS goals was scored using the CHAMPS tool. Results demonstrated strong alignment with CHAMPS pregnancy surveillance and moderate alignment, with minimal tension, with CHAMPS MITS. Most antenatal and child health concerns expressed by participants were alleviable through medical/public health interventions, highlighting compatibility with study aims. 99.1% of respondents believed pregnancy surveillance fit with community priorities, and 56.1% believed the same for MITS. We found moderate-high alignment for pregnancy surveillance (81.5%) and MITS (76.1%) when participants were asked about community supportiveness. Understanding the alignment and misalignment of community priorities and CHAMPS aims allowed us to tailor advocacy strategies to meet community values. Moreover, community engagement facilitated through PICK-CHAMP activities improved our connections with stakeholders and spread valuable awareness about the study.

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ENHANCING THE IDENTIFICATION OF CAUSES OF DEATH THROUGH COMMUNITY-BASED VERBAL AUTOPSY METHODS DURING THE COVID 19 OUTBREAK

Raheel Allana[1], Chrisitina Arif[1], Saima Jamal[1], Sehrish AmirAli[1], Abdul Momin Kazi[1], Saad B. Omer[2]

[1]Aga Khan University, Karachi, Pakistan, [2]UT Southwestern Medical Center, Dallas, TX, United States

In a resource constrained settings like Pakistan, obtaining accurate mortality data can be challenging due to limited autopsy practices and a significant proportion of deaths occurring outside of hospital facilities. This study utilized a community-based verbal autopsy approach in two peri-urban sites in Karachi, Pakistan to obtain cause of death (CoD) data at the household level. The two sites, Ali Akbar Shah and Bhains Colony, are part of a health demographic site surveillance (HDSS) system that was established in 2003. Rigorous interviewer training ensured sensitive data collection using the

WHO 2016 Questionnaire. Verbal autopsy (VA) data underwent physician review to determine causes of death in accordance with ICD-10 codes. VA was conducted on 1500 deaths, with a male: female ratio of 1.12:1. 41.6% of deaths occurred at home. For children under 5 years, 31.5% of deaths occurred at home. Non-communicable diseases were most common, accounting for 39.3% of deaths with acute cardiac disease (12.6%), liver cirrhosis (7%), and stroke (4.3%) being the three most common causes. Major causes of death associated with communicable diseases included diarrheal disease (6.4%), pneumonia (4.1%), and sepsis (3.4%). Among adults (>18 years) acute cardiac disease (25.1%) and liver cirrhosis (13.4%) were top causes of mortality. There was a higher prevalence of liver cirrhosis among females (14%) compared to males (10.1%). Neonatal sepsis (12.8%) and perinatal asphyxia (11.7%) were prominent CoD in children under five. Pneumonia (8.8%) and road traffic accidents (8.8%) were the two major CoD in children between 5-18 years. The considerable proportion of deaths, particularly in children, taking place at home underscores a potential gap in health access and utilization which needs to be explored. Understanding trends in disease burdens across varying demographic groups can help tailor interventions to improve public health outcomes in resource-constrained settings.

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COMMUNITY BEHAVIORS AND PRACTICES TOWARDS ROUTINE IMMUNIZATION IN POLIO HIGH RISK UNION COUNCILS OF PAKISTAN

Ahmad Khan, Imtiaz Hussain, Muhammad Umer, Sajid B. Soofi
Aga Khan University, Karachi, Pakistan

Behavioral and Social Drivers of Vaccination (BeSD) are people's beliefs, experiences, andcircumstances influencing vaccination decisions. BeSD are categorized into four domains: attitudes towardsvaccines, social influences, motivation (or hesitancy) to vaccinate, and practical considerations. This studypresents the BeSD findings about essential childhood vaccinations from seven districts over three provincesthat hold the polio super-high-risk union councils (SHRUCs) in Pakistan. A cross-sectional survey was conducted in 2023 in 39 SHRUCs over 7 districts using a two-stage stratified cluster sampling technique. Altogether, 7,829 caregivers across the SHRUCs districtsresponded to the BeSD childhood vaccination survey for caregivers, designed to assess the drivers ofvaccination for children under age 5. Of the respondents, 43.3% wanted their child to receive all vaccines according to Pakistan'sschedule, with notable differences across SHRUC districts: 84.9% in Sindh, 33.2% in Balochistan, and only2.6% in Khyber Pakhtunkhwa. Nearly two-thirds of respondents (65%) considered vaccines are veryimportant for their child's health, but opinions varied by district, ranging from 84.6% in Sindh to 41.5% inBalochistan. Altogether, 91.8% reported encouragement from family and friends to vaccinate their child,and 93.4% knew where to access vaccination services. Affordability varied greatly, with 59.3% finding itvery easy, ranging from 35.9% in Balochistan to 81.2% in Sindh.Challenges in accessing vaccination services were reported by 1,644 respondents, primarily due to difficultyreaching clinics (73.4%) and long waiting times (63.9%). Dissatisfaction with vaccination services wasexpressed by 2,434 respondents, centred on long waiting times (62.7%), insufficient staff interaction(41.0%), and vaccine unavailability (38.0%). Despite caregivers' willingness, access and affordability barriers remain, includingtransportation challenges and long wait times. Targeted interventions addressing these issues are crucial toimproving vaccination coverage.

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OPTIMIZING COMMUNITY HEALTH RESOURCES FOR UNIVERSAL ITN COVERAGE IN THE DRC: OUTCOMES OF A *TRINÔME* TO*BINÔME* PILOT IN LUALABA PROVINCE, 2023-2024

Jeanine D. Musau[1], Yibayiri Osee Sanogo[2], Clara Harb[1], Ferdinand Ntoya[3], Eric Mukomena[4], Mulamuli Mpofu[1]
[1]Chemonics International Inc, Washington, DC, United States, [2]U.S. President's Malaria Initiative, U.S. Centers for Disease Control and Prevention, Kinshasa, Congo, Democratic Republic of the, [3]U.S. President Malaria Initiative, USAID, Kinshasa, Congo, Democratic Republic of the, [4]National Malaria Control Program, Ministry of Health, Kinshasa, Congo, Democratic Republic of the

The USAID End Malaria Project has supported the National Malaria Control Program in the Democratic Republic of the Congo (DRC) in distributing insecticide-treated nets (ITNs) through door-to-door mass campaigns since 2021, employing teams of three community health workers (CHWs), known as *trinômes.* The *trinôme* approach, consisting of a designated sensitizer, carrier, and investigator, became national policy for ITN campaigns during the COVID-19 pandemic. In response to financial constraints, the project piloted a transition in August 2023 to a *binôme* model that combined the roles of sensitizer and carrier in seven health zones in Lualaba province before scaling up to Lualaba's remaining seven health zones. Feasibility and cost-effectiveness were assessed by evaluating time taken to distribute an ITN to a household and the number of households covered per day against national targets set based on *trinôme* performance in previous campaigns. Cost savings were also calculated comparing the *binôme* and *trinôme* approaches. Instead of the 8,175 expected *trinôme* CHWs, 5,450 CHWs formed the *binôme* teams. Across the pilot and scale-up phases, similar outcomes and outputs were achieved. Teams successfully reached 97% of targeted households with a total of 2,132,389 ITNs benefitting 3,959,639 individuals. Teams spent between 12-15 minutes distributing in each household against the national target of 10-15 minutes. Daily *binôme* output was between 25-30 households per day in rural areas against a target of 30, and between 40-50 households in urban areas against a target of 50. There were direct cost savings of 30% ($221,997) on both labor and training. The operational distribution cost (which excludes the physical ITN cost) in the province also dropped to $1.53 per ITN distributed against the planned target of $1.81. The *binôme* approach for door-to-door mass distribution campaigns proved viable in the DRC; significant cost savings were realized without impacting the target output coverage. This approach also frees up CHWs to concentrate on delivery of other health services, allowing for better delivery of routine care during ITN campaign periods.

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INSIGHTS FROM CHILD HEALTH & MORTALITY PREVENTION SURVEILLANCE (CHAMPS) NETWORK - PAKISTAN SITE

Farah Jabeen[1], Sameer Belgaumi[2], Asefa Shariq[1], Saima Jamal[1], Tehreem Maqsood[1], Saad B. Omer[2], Zeeshan Uddin[1], Momin Kazi[1]
[1]Aga Khan University, Karachi, Pakistan, [2]UT Southwestern Medical Center, Dallas, TX, United States

Child mortality is a global public health concern. It disproportionately affects emerging nations in Sub-Saharan Africa and South Asia, accounting for over 80% of five million under-5 deaths per year. In Pakistan, the under-five child mortality rate is 63.3 deaths per 1,000 live births. Pakistan was added to the Child Health and Mortality Prevention Surveillance (CHAMPS) network to investigate and address this high rate of under-five mortality. Study implementation began in July 2024 at three peri-urban sites in Karachi aiming to enumerate all stillbirths and under-5 deaths and collect data on cause of death (CoD). Data collected from consenting families includes minimally invasive tissue samples (MITS) and available (non-MITS) data from clinical records and verbal autopsy (VA). Our target is to collect 80 MITS this year. Following lab testing, data is reviewed by a panel of physicians to determine CoD. We are presenting preliminary findings from our study. As of March 31st, 2024, we documented 302 under-five deaths. We completed 31 MITS cases, consented 166 non-MITS cases, and conducted 156 VA. Of 31 MITS cases, 12 (38.7%) were stillbirths, 10 (32.2%) neonates, 7 (22.5%) post-neonates, and 2 (6.4%) were 1-5yrs. 45% of samples were collected from deaths occurring at home. 58% of MITS cases were male. Blood microbial testing showed eight positive cases of *K. pneumonia*, four for *Staphylococcus species* and two for *P. aeruginosa*. CSF microbial testing showed six positive cases for *Acinetobacter baumannii* and four positive for *E. Coli*. Pathology reports showed presence of aspiration pneumonia in neonatal and 1-5yrs groups. Intrauterine fetal distress was reported among stillbirths. MITS has been helpful in providing a concrete

understanding of child mortality causes. Following case- review by physician panels and determination of CoD, data trends will help inform interventions and policies aiming to reduce regional child mortality.

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ENHANCING DISEASE SURVEILLANCE AND RESPONSE SYSTEMS IN THE GAMBIA AND SENEGAL: A CROSS-BORDER COLLABORATION

Haddijatou Allen, Sergio Torres Rueda, Hilary Bower, Melisa Martinez-Alvarez, Anna Vassall

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

Effective disease surveillance is crucial for early detection and response to disease threats. However, resource constraints, including infrastructure and financing gaps, can create hurdles, particularly in low- and middle-income countries. These difficulties are compounded by increased population mobility in border areas, which exacerbates the risk of infectious disease spread. Cross-border collaboration offers an opportunity to enhance disease surveillance and response, particularly in countries such as The Gambia and Senegal, which have long open borders. In these contexts, communication, data sharing and mapping population movement, which may require substantial investment, are critical for minimizing disease transmission. This study evaluates the costs and financial implications of a cross-border surveillance strategy between The Gambia and Senegal, for earlier detection and response to disease outbreaks. Employing a mixed methods approach, the study assesses existing surveillance systems and identifies changes needed in resource allocation, infrastructure, and institutional arrangements. A micro-costing assessment estimated implementation costs, with a focus on identifying sustainable co-financing models. A multi-criterion mapping exercise will facilitate the co-development of a cross-border collaboration strategy. Initial findings indicate suboptimal performance of the Integrated Disease Surveillance and Response systems at facility levels, with weaknesses in outbreak investigation and timeliness of reporting. Qualitative inquiry revealed that inadequate laboratory capacity hampered early detection and confirmation of outbreaks, compounded by insufficient funding. Recommendations for enhancing cross-border collaboration included: improving funding and timely access to funds and implementing district to district collaboration. This research identifies barriers and enablers to cross-border surveillance, and proposes strategies for effective implementation, informing analyses on the financial implications of operating a cross-border surveillance system in the subregion.

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IDENTIFYING DISTRICT-LEVEL RISK FACTORS FOR DELAYS IN YELLOW FEVER SPECIMEN COLLECTION AND ARRIVAL FOR TESTING IN GHANA

Seth Judson[1], Lee Schroeder[2], Franklin Asiedu-Bekoe[3], Dennis Laryea[3], Gifty Boateng[3], Horlali Gudjinu[3], David Dowdy[1], Ernest Kenu[4]

[1]Johns Hopkins University, Baltimore, MD, United States, [2]University of Michigan, Ann Arbor, MI, United States, [3]Ghana Health Service, Accra, Ghana, [4]University of Ghana, Accra, Ghana

Delayed diagnosis of yellow fever (YF) leads to increased morbidity and mortality during outbreaks, but the steps in the YF diagnostic pathway which cause the greatest delays in case detection are not well known. Ghana is classified as a high-risk country for YF by the WHO Eliminate Yellow Fever Epidemics strategy. When a patient meets the case definition for suspected YF, a specimen is collected and sent to the National Public Health and Reference Lab (NPHRL) for testing. Our interviews with 148 clinicians at 53 health facilities in Ghana revealed perceived delays in YF testing. We therefore explored district-level risk factors contributing to differences in specimen collection and arrival at the NPHRL for YF testing. We conducted a cross-sectional sample of 12 districts in Ghana with patients tested for suspected YF from 2018-2022. Districts were stratified by ecological zone, patient volume, and distance of highest tier health facility from a reference zonal laboratory. A total of 298 patients were tested for YF from 190 health facilities in the sampled districts. We used descriptive statistics to compare time from symptom onset to specimen collection and time from specimen collection to arrival at the NPHRL. We used multivariable linear regression to identify district-level risk factors associated with diagnostic delays including distance from districts to NPHRL, population density, and poverty level. The mean duration from symptom onset to specimen collection was 5.1 days (range 0-30, standard deviation 4.4), and from collection to arrival at NPHRL was 8.9 days (range 0-94, standard deviation 11.8). There was a significant relationship between distance and time to specimen arrival at the NPHRL, with arrival time delayed by 1.5 days for every 100 kilometers from the NPHRL. We conclude that the timing of specimen collection and arrival for YF testing differs across districts in Ghana. Improving access to YF testing, including through decentralized testing and improved specimen transport, could lead to earlier detection of YF outbreaks.

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SEROPREVALENCE OF ELEVEN NEGLECTED DISEASES OF PUBLIC HEALTH INTEREST IN NAURU

Ashley Simon[1], **Zachary M. Reynolds**[2], Sarah Gwyn[1], Diana Martin[1], John Kaldor[3], Sue Chen Apadinuwe[4], Susana Vaz Nery[3]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Synergy America Inc., Atlanta, GA, United States, [3]The Kirby Institute, University of New South Wales, Sydney, Australia, [4]Ministry of Health and Medical Services, Denig, Nauru

Nauru is a small island nation in the western Pacific region, approximately 1800 miles northeast of Australia. Here we share the results of a 2019 integrated serosurvey conducted using dried blood spots collected during a trachoma survey in Nauru. Antibody data are presented for six neglected tropical diseases (NTDs) and five additional parasitic diseases of public health interest to the Nauru Ministry of Health. This was a nationally representative, randomized, two-stage cluster survey, with 769 samples analyzed from children ages 1-9 years. For NTDs, less than 5% of children were antibody positive to any of three lymphatic filariasis (LF) antigens included, with two children double positive and none triple positive. No children tested antibody positive to both yaws antigens. For trachoma, 32.5% (95% CI, 29.2% - 35.8%) of children were antibody positive. One quarter (25.6%, 95% CI, 22.5% - 28.7%) of children were seropositive for *Strongyloides stercoralis*. For the parasite *Taenia solium*, 6.6% (95% CI, 4.9% - 8.4%) were seropositive for cysticercosis marker T24H and 1.2% (95% CI, 0.4% - 1.9%) were positive for the taeniasis infection marker ES33. Toxoplasmosis seropositivity was significantly high, at 44.3% (95% CI, 40.8% - 47.9%) as was toxocariasis at 28.9% (95% CI, 25.7% - 32.1%). Approximately a quarter of children surveyed were seropositive for giardiasis, 23.2% (95% CI, 20.3% - 26.3%) and cryptosporidiosis, 26.8% (95% CI, 23.7% – 29.9%) respectively. Less than 5% of children were seropositive for amebiasis, 4.3% (95% CI, 2.9% - 5.7%). In addition to confirming the need for trachoma interventions, the use of the multiplex bead assay identified high seroprevalence to several parasitic and water-borne diseases that can guide programmatic intervention. The serological data lends support to epidemiological data indicating LF and yaws are not present in Nauru. Taking advantage of specimens collected during a vertical trachoma survey provided added public health benefits by providing data to guide programs that would have required additional funding to collect otherwise.

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COVID-19 VACCINE HESITANCY: A GLOBAL SURVEY ON KNOWLEDGE, EXPERIENCE, ATTITUDE, AND PSYCHOLOGICAL STRESS

Le Van Truong[1], **Van Thanh Nguyen**[2], *Vu Thi Thu Trang[3], Thi Thu Thao Le[2], Chern Choong Thum[4], Dang Nguyen[5], Yen Jun Wong[6], Minh Nguyen Le Thi Binh[7], Dao Huynh Anh[8], Fatima Ezzahraa EL IDRISSI[9], Nityanand Jain[10], Khoa Le Anh Huynh[11], Abdelrahman M Makram[12], Nguyen Tien Huy[13], TMGH Online Research Club Global Team[14]*

[1]Traditional Medicine Hospital, Ministry of Public Security, Hanoi, 100000, Vietnam, [2]Oxford University Clinical Research Unit, Ho Chi Minh City,

700000, Vietnam, [3]National Hospital of Traditional Medicine, Hanoi, 100000, Vietnam, [4]Ministry of Communications, Putrajaya, 62100, Malaysia, [5]Massachusetts General Hospital, Corrigan Minehan Heart Center, Harvard Medical School, Boston, MA, United States, [6]School of Pharmacy, Monash University Malaysia, Subang Jaya, 47500, Malaysia, [7]University of Medicine and Pharmacy at Ho Chi Minh City, Ho Chi Minh City, 700000, Vietnam, [8]Faculty of Medicine, Can Tho University of Medicine and Pharmacy, Can Tho, 900000, Vietnam, [9]Faculty of Medicine, Pharmacy and Dental Medicine of Fez - Sidi Mohammed Ben Abdellah University, Morocco, Morocco, [10]Statistics Unit, Riga Stradiņš University, Riga, LV-1007, Latvia, [11]Department of Biostatistics, Virginia Commonwealth University, Richmond, VA, United States, [12]School of Public Health, Imperial College London, London, United Kingdom, [13]School of Tropical Medicine and Global Health, Nagasaki University, 1-12-4 Sakamoto, Nagasaki, 852-8523, Japan

The COVID-19 pandemic has underscored the urgency of rapid vaccine development and availability. However, vaccine hesitancy remains a significant barrier to global efforts to curb the pandemic, despite extensive public health campaigns. The present study aimed to explore the factors that may contribute to COVID-19 vaccine hesitancy. A global online cross-sectional survey was conducted in 2023, involving 102,481 adult participants from 189 countries. The 48-question survey, translated into 33 languages, examined the effects of various factors such as demographics, knowledge, attitude, experience, and psychological stress (egocentricity, callousness, and antisociality) on COVID-19 vaccine hesitancy. The cohort's mean age was 27.3±9.9 years, with about 58% of respondents identifying as female. A notable proportion of respondents, 18%, reported being unvaccinated against COVID-19, while over 31% expressed disagreement with COVID-19 vaccination. Furthermore, the optimal structural equation model (RMSEA=0.032/CFI=0.955/TLI=0.950) incorporated gender, level of education, and family member's COVID-19 severity. The model elucidated 45.34% of the observed variance, suggesting that female participants and those with family members experiencing more severe COVID-19 conditions tended to express lower levels of hesitancy related to vaccine reactions (HRVR), in contrast to individuals with higher levels of education. Regarding psychological stress, antisociality was significantly associated with increased concern about COVID-19 vaccine information (CVI) and HRVR (OR=1.19 and 1.08, 95%CI:1.17-1.2 and 1.06-1.09, p<0.001, respectively). Conversely, both egocentricity (OR=0.97, 95%CI:0.96-0.98, p<0.001) and callousness (OR=0.76, 95%CI:0.75-0.77, p<0.001) were associated with reduced concern about CVI. Egocentricity was also associated with increased HRVR (OR=1.12, 95%CI:1.10-1.14, p<0.001). These findings offer global evidential insights to enable policymakers to develop multitiered interventions to combat COVID-19 vaccine hesitancy that could be scaled up to other vaccine-preventable diseases.

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COMPARATIVE ANALYSIS OF STATE-LEVEL POLICY RESPONSES IN GLOBAL HEALTH GOVERNANCE: COVID-19 AS A CASE

Fengyuan Tang, Hengjin Dong
Zhejiang University, hangzhou, China

States are key actors in global health governance, particularly in the prevention and control of infectious diseases. The emergence and re-emergence of infectious diseases in recent decades pose profound challenges to global health security. As the first coronavirus pandemic, the COVID-19 caused significant damage worldwide, but responses and outcomes varied greatly among states. Using COVID-19 as an example, this study aims to compare the policies and measures implemented by different states during the COVID-19 pandemic and to synthesize experiences to strengthen global health governance for future infectious disease crises. We selected the United States, Sweden, India, and Nigeria as the representative states with different economic development and impact of COVID-19 prevention and control strategies. We systematically collated data on the policies adopted by these states to control COVID-19 from literature, reports, authoritative media and official websites. A comparative analysis was then conducted to analyze the differences, rationale, and challenges of the approaches taken by these states. The management of COVID-19 by states is divided into domestic and international governance. Domestically, the United States and India have taken more measures, yet notable disparities in infection source control, transmission interruption, vulnerable population protection, collaborative governance, and so on were observed among all four states. Internationally, the United States and Sweden were more proactive in international governance, and all four states have variations in their adherence to global regulations, information sharing, resource distribution, and cooperative engagement. Significant disparities occurred during the response to early COVID-19 in four states, which may be due to differences in politics, economy, and culture. To prevent and mitigate the impact of infectious diseases, states should prioritize solidarity and cooperation, and improve governance domestically and internationally based on national contexts and global health principles in the future.

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SEARCH FOR ACTIVE CASES OF YAWS IN PARTS OF IMO STATE, SOUTHEAST NIGERIA

Treasure Njideka Njoku-Obi[1], Chiinyere Nneka Ukaga[2], Chika Maureen Ezenwa[1], Innocentia Ogechi Nwachukwu[1]
[1]Imo State University Owerri, Imo State Nigeria, Owerri, Nigeria, [2]Wigwe University Isiokpe, Port Harcount, Nigeria

Yaws was previously eradicated in Nigeria but Nigeria belongs to the WHO Yaws epidemiological category B countries. This study focused on active search for cases of Yaws amongst adults in two communities in 2 local government areas of Imo State respectively in Ehime Mbano and Mbaitoli. Informed written consents and appropriate ethical clearance were duly obtained from relevant authorities. Sampling method was purposive and medical screening were conducted among the participants using the WHO SkinNTD toolkit. Blood samples (2mls) of participants were collected and subjected to serological and VDRL test. Pre-tested questionnaires were used for demographic data as well as data on their knowledge and practices. A total of 125 adults (75 adults from Ehime Mbano, 50 adults from Mbaitoli) participated. From Ehime Mbano, despite high presumptive cases of yaws 49(65.3%), only 28(37.3%) was reactive to serological test strip and 47(62.7%) showed no symptoms. Clinical examination of the seropositive participants showed that 5(17.9%) papiloma virus, 8(28.6%) ulcer, 3(10.7%) swelling bones and 12(42.9%) hyper keratosis on feet and palms. Findings from Mbaitoli LGA showed that despite high presumptive cases of yaws 35(70%), only 3(6%) was reactive to serological test strip and 47(94%) showed no symptoms. Knowledge on Yaws from the data collected from the total 125 participants, indicated that 14(11.2%) insect bites caused the disease, 4(3.2%) bad drinking water source, 7(5.6%) bathing cold water, 10(8%) enemies and 90(72%) unidentified causes. On their practices, 40(32%) among participants visit the clinic for their treatments, 17(13.6%) visit herbalist, 5(4%) and 3(10.7%) consults oracle and spiritualists respectively while 30(24%) use more than one treatment method. Active search for Yaws yielded positive results in Ehime Mbano which require further confirmation and follow up. In conclusion, this study confirmed that there had been considerable progress in the treatment of yaws in the last decade, but the danger of re-emergence exists. Thus the need for more attention on yaws in Nigeria is advocated.

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IDENTIFICATION OF ANTHRAX AS THE CAUSE OF A CLUSTER OF UNEXPLAINED DEATHS, UGANDA, 2023: THE ROLE OF METAGENOMIC NEXT GENERATION SEQUENCING AND POSTMORTEM SPECIMENS

Sonja Weiss[1], Nicholas Bbosa[2], Deogratius Ssemwanga[2], Sam Kalungi[3], Anatoli Mawanda[3], Richard Ssentudde[3], Emmanuel Ssekyeru[4], Alfred Ssekagiri[2], Ronald Kiiza[2], Cleophous Rwankindo[2], Joshua Buule[2], Hamidah Suubi Namagembe[2], Stella Esther Nabirye[2], Justine Priscilla Nassolo[2], Robert Downing[2], Julius Lutwama[2], Tom Lutalo[2], Henry Kyobe Bosa[5], Michael Berg[1], Mary Rodgers[1], Francisco Averhoff[1], Gavin Cloherty[1], Pontiano Kaleebu[2]

[1]Abbott Laboratories, Abbott Park, IL, United States, [2]Uganda Virus Research Institute, Entebbe, Uganda, [3]Mulago National Referral Hospital, Kampala, Uganda, [4]Kalisizo General Hospital, Kyotera, Uganda, [5]Ministry of Health, Kampala, Uganda

Several deaths of unknown etiology were reported in Kyotera District of Southern Uganda in August of 2023. Patient symptoms included fever, shortness of breath, abdominal pain, vomiting, loss of appetite, profuse sweating, body aches, swelling of limbs, and blisters with a black center. Field response teams deployed by the Uganda Ministry of Health (MOH) collected clinical and epidemiological data and specimens. The Mortuary Surveillance Program, a collaborative effort between the MOH, the Abbott Pandemic Defense Coalition (APDC) and Uganda Virus Research Institute (UVRI), analyzed postmortem specimens to explore potential infectious causes of death. Blood specimens from deceased individuals with unknown cause of death from Kyotera District were PCR screened for viral hemorrhagic fevers: Ebola, Marburg, Rift Valley and Crimean Congo. Metagenomic Next Generation Sequencing (mNGS) was performed on PCR-negative specimens using the Illumina DNA Prep kit on the MiSeq NGS platform. Data was analyzed with an Abbott-internal bioinformatics pipeline, DiVir. Results were confirmed by an alternate NGS library prep method, the Respiratory Pathogen ID/AMR Enrichment Panel (RPIP). Deep sequencing identified the presence of *Bacillus anthracis* reads in only one of the index patients from August, and thus cases were deemed unrelated. By November 2023, a cumulative total of 27 human and 22 animal deaths had been reported in the Kyotera District for which symptoms were consistent with anthrax infection. For six additional cases subjected to mNGS and RPIP enrichment, an anthrax diagnosis was confirmed by UVRI and DiVir pipelines. Notably, only 3/7 were positive for anthrax using an in-house PCR assay. Utilizing mNGS of postmortem specimens through the Mortuary Surveillance Program, was a powerful tool for identifying an otherwise unrecognized Anthrax outbreak in Uganda. Building and sustaining the infrastructure for mortuary surveillance and NGS should be prioritized for control of emerging and re-emerging pathogens and integrated into public health programs in sub-Saharan African countries.

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SARS-COV-2 TRANSMISSION POTENTIAL AND CONTROL MEASURES IN ZIMBABWE: AN ECOLOGICAL ANALYSIS

Emmanuela Nwaonumah[1], Jing Kersey[1], Gerardo Chowell[2], **Isaac Chun-Hai Fung**[1]

[1]Georgia Southern University, Statesboro, GA, United States, [2]Georgia State University, Atlanta, GA, United States

This ecological study aimed to investigate the SARS-CoV-2 transmission potential in Zimbabwe from Mar 20, 2020, through Mar 9, 2023, and its ten provinces from Mar 20, 2020, through May 11, 2022. The association between the transmission potential and policy interventions was accessed until Feb 28, 2021, before the introduction of vaccines. Datasets from Johns Hopkins University, African Surveyors Connect, and the Zimbabwe Ministry of Health and Child Care were analyzed. Negative and zero incident case counts were imputed, infection dates were estimated from report dates via deconvolution, and infection counts were estimated via a Poisson-distributed multiplier of 4. The time-varying reproduction number (Rt) was estimated by the R package 'EpiEstim' using the 7-day sliding window and non-overlapping time windows. Between Mar 2020 and Dec 2022, Zimbabwe and its ten provinces experienced three case surges corresponding with the Beta (Nov 2020), Delta (Jun 2021) and Omicron (Dec 2021) waves. In alignment with the waves, Rt>1 was observed during case surges, and Rt<1 was observed during case declines between waves. Secondary to low incident cases (Mar-Jun 2020), Rt estimates showed greater uncertainty. On the national level, Zimbabwe's 'dusk-to-dawn' daily curfew on July 21, 2020, was associated with a decrease in Rt (-11.39%, 95%CrI: -17.00%, -6.02%). On the provincial level, the daily curfew was associated with a statistically significant decrease in Rt in Harare (-16.89%, 95%CrI: -25.38%, -10.04%), Manicaland (-10.01%, 95%CrI: -19.03%, -1.17%), Midlands (-10.40%, 95%CrI: -19.94%, -1.60%), Matabeleland South (-13.54%, 95%CrI: -22.88%, -5.65%), and Bulawayo (-16.20%, 95%CrI: -24.49%, -8.04%). This study highlighted how non-pharmaceutical interventions impacted the SARS-CoV-2 transmission in Zimbabwe. It emphasizes the importance of public health interventions implemented at national and sub-national levels in different countries. Tailoring interventions to specific locations and populations is crucial, as various factors can affect their effectiveness.

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ONE-YEAR PATTERN OF ANTIMICROBIAL RESISTANCE IN *ESCHERICHIA COLI*, *KLEBSIELLA PNEUMONIAE* AND *PSEUDOMONAS AERUGINOSA* ISOLATES IN OSOGBO, NIGERIA

Oluwabunmi Bola Olajide[1], Olubunmi O. Alaka[2], Joshua S. Olajide[3], Isaac O. Oni[4], Idara E. Inyang[1], Titilayo Olaoye[1]

[1]Department of Public Health, Babcock University, Ilishan-Remo, Nigeria, [2]Department of Medical Microbiology and Parasitology, Obafemi Awolowo University Teaching Hospital Complex, Ile-Ife, Nigeria, [3]Department of Medical Microbiology and Parasitology, Obafemi Awolowo University, Ile-Ife, Nigeria, [4]Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria

Escherichia coli, Pseudomonas aeruginosa and *Klebsiella pneumoniae* are gram-negative bacteria that have been linked with various healthcare-associated and community-acquired infections. These three organisms have been identified by MAAP survey as critical multi-drug resistant pathogens that require new drugs to combat their infections in humans. Therefore, antimicrobial resistance patterns of *E. coli*, *K. pneumoniae* and *P. aeruginosa* isolates from clinical specimens submitted for microbial culture and sensitivity test were analyzed between January and December 2022. 286 bacterial isolates including 171, 83 and 32 of *E. coli*, *K. pneumoniae* and *P. aeruginosa* respectively were identified. All *P. aeruginosa* isolates exhibited resistance to at least one antibiotic. Essentially, 75% of *P. aeruginosa*, 66.1% of *E. coli* and 71.1% of *K. pneumoniae* demonstrated multidrug resistance pattern. *E. coli* 63.69% and *P. aeruginosa* 14.65% showed highest resistance to ZEM, and least resistance to LBC. Whereas, *K. pneumoniae* showed highest resistance to IMP 30.19% and least resistance to GEN 23.99%. AUG-CIP-CRO-CTX-CXM-GEN-IMP-LBC-ZEM antimicrobial resistance pattern occurred in urine, wound specimens for *P. aeruginosa* isolates. The same pattern was also found in *E. coli* isolated from vagina swab. The 3 bacteria isolates demonstrated a considerable high prevalence of resistance to antibiotics under study. The resistance to Imipenem is noteworthy as it is often reserved for the treatment of multidrug resistant pathogens. *P. aeruginosa* has always shown higher rate of multidrug resistance (MDR) and this was also established in this study. ZEM and CIP are often used in the treatment of *P. aeruginosa* infections but this study indicated that the resistance by *P. aeruginosa* to both antibiotics is very high. This study shows that antimicrobial resistance among these organisms is on the increase. As such, there is need for community-based sensitization on proper use of antibiotics as well as regulated sale of antibiotics to lower the rate of antimicrobial resistance.

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COMMUNITY PERCEPTIONS OF HEALTH-RELATED RISK FACTORS, HEALTH STATUS, AND HEALTHCARE SERVICE IN RURAL SOUTHEAST ASIA: INSIGHTS FROM A CROSS-SECTIONAL HOUSEHOLD SURVEY IN BANGLADESH, CAMBODIA, AND THAILAND

Meiwen Zhang[1], Shayla Islam[2], Aninda Sen[2], Md Akramul Islam[2], Amit Kumer Neogi[2], Rupam Tripura[1], Lek Dysoley[3], Carlo Perrone[1], Nan Shwe Nwe Htun[1], Marco Liverani[4], Richard J. Maude[1], Nicholas P.J. Day[1], Sue J. Lee[1], Yoel Lubell[1], Thomas J. Peto[1]

[1]Mahidol-Oxford Tropical Medicine Research Unit, Bangkok, Thailand, [2]Communicable Diseases Program, BRAC, Dhaka, Bangladesh, [3]Cambodian National Malaria Control Program, Phnom Penh, Cambodia, [4]Department of Global Health and Development, London School of Hygiene & Tropical Medicine, London, United Kingdom

To guide rural disease control priorities the South and Southeast Asia Community-based Trials Network conducted cross-sectional household health surveys in Bangladesh, Cambodia, and Thailand during 2022-2023. We used two-stage cluster sampling to recruit participants of all ages to assess disease prevalences and identify community-perceived concerns related to risk factors, health status, and health services. Adult participants' (≥ 15 years old) demographic and socioeconomic characteristics, concerns about health-related factors, and previously diagnosed diseases were collected through questionnaire interviews. Among adults (Bangladesh: n=1168, Cambodia: n=937, Thailand: n=1210), the majority were women (Bangladesh: 57.9%, Cambodia: 54.3%, Thailand: 59.5%), aged 15-44 years in Bangladesh (71.8%) and Cambodia (60.4%), and 45 years or older in Thailand (70.7%). Many had no formal education (Bangladesh: 33.8%, Cambodia: 21.6%, Thailand: 28.2%). The self-reported prevalence of raised cholesterol, hypertension, and diabetes was highest in Thailand (cholesterol: 17.2%, hypertension: 25.2%, diabetes: 10.2%), followed by Cambodia (cholesterol: 4.2%, hypertension: 13.1%, diabetes: 4.6%) and Bangladesh (cholesterol: 0.8%, hypertension: 10.9%, diabetes: 4.3%). Healthcare costs were the leading concern in Cambodia (82.1%) and Bangladesh (46.1%), and were most likely to be reported by Bangladeshi adults with hypertension (57.5% vs. 44.7%, p=0.006). No specific concern was most commonly reported (38.8%) in Thailand, but timeliness of health service delivery (20.9%) was more frequently mentioned by those reporting hypertension (24.9% vs. 19.3%, p=0.038) or diabetes (29.8% vs. 18.7%, p=0.008). This survey highlights the large variation in self-reported disease prevalences and community concerns, and the need for healthcare interventions tailored to local contexts. Further laboratory assays will confirm disease status and identify the extent of undiagnosed and untreated conditions, and determine associations between non-communicable and infectious diseases by sero-epidemiological assays.

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DEVELOPING THE CONCEPT AND PRACTICE OF ANTICIPATORY ACTION FOR EPIDEMICS A THE HUMANITARIAN SECTOR

Tilly Alcayna[1], Franziska Kellerhaus[2], Léo Tremblay[3], Juan Chaves[4], Rachel Goodermote[5], Mauricio Santos-Vega[6], Bhargavi Rao[1], Rachel Lowe[7]

[1]London School of Hygiene and Tropical Medicine, London, United Kingdom, [2]German Red Cross, Berlin, Germany, [3]Medecins Sans Frontieres, Toronto, ON, Canada, [4]United Nations Office for the Coordination of Humanitarian Affairs, New York City, NY, United States, [5]International Federation of the Red Cross Red Crescent, Geneva, Switzerland, [6]Universidad de los Andes, Bogota, Colombia, [7]Barcelona Supercomputing Center, Barcelona, Spain

Within the humanitarian sector, a novel anticipatory approach to climate and disaster risk management is emerging. 'Anticipatory action' makes use of climate or weather forecasts and observations as well as in-depth risk analysis to predict where and when a disaster may unfold in order to intervene in advance and prevent or reduce negative impacts. Specifically, it codifies the release of pre-emptive emergency funding to enable pre-agreed actions based on predefined triggers within a forecast of a hazard. Advances in the understanding climatic drivers of infectious disease mean that developing anticipatory action as part of epidemic prevention, preparedness, and response can help humanitarian actors and their partners increase the effective timing of outbreak interventions, reduce the delay to response, prioritise and allocate the use of limited resources, and improve coordination and clarification of roles and responsibilities amongst relevant actors. Specifically, climate-informed early warnings for climate-sensitive infectious diseases can provide information on (1) a change (earlier or later) in the onset or cessation of the transmission seasons in endemic areas, (2) geographic locations that are at higher risk, and (3) the likely magnitude of cases (including the potential surpassing of the epidemic threshold) in a forthcoming season. Anticipatory action for epidemics does not necessarily entail designing new interventions, rather it helps improve decision-making on the optimal timing of existing disease control and prevention. This paper contributes to building a common understanding on the concept of anticipatory action for epidemics for humanitarian practitioners. We detail the three main triggering methods that can be used to establish anticipatory action for epidemics and provide purposively selected case studies to illustrate the methods.

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ASSESSMENT OF COMMUNITY AWARENESS, CONDUCT AND HABITS ON YELLOW FEVER IN THE UPPER EAST REGION OF GHANA

Millicent Captain-Esoah[1], Kwadwo K. Frempong[2], Abdul Malik Hussein[1], Francis Balungnaa D. Veriegh[3], Martin N. Donkor[4], Abigail Asiedu[1], Vanessa N. Kayan[1], Emmanuel Frimpong[1], Daniel K. Aluu[1], Iddrisu Fuseini[5], Elijah D. Angyiereyiri[1], Dorothy Obuobi[6], Bernice Olivia A. Baako[7], Chrysantus Kubio[8], Victor Asoala[7], Daniel A. Boakye[2], Samuel K. Dadzie[2]

[1]Department of Applied Biology, School of Environment and Life Sciences, C. K. Tedam University of Technology and Applied Sciences, Navrongo, Ghana, [2]Department of Parasitology, Noguchi Memorial Institute for Medical Research (NMIMR), College of Health Sciences, University of Ghana, Legon, Accra, Ghana, [3]Department of Biological Science, School of Sciences, University of Energy and Natural Resources,, Sunyani, Ghana, [4]Department of Biochemistry and Forensic Sciences, School of Chemical and Biochemical Sciences, C. K. Tedam University of Technology and Applied Sciences,, Navrongo, Ghana, [5]Department of Statistics and Actuarial Science, School of Mathematical Sciences, C. K. Tedam University of Technology and Applied Sciences, Navrongo, Ghana, [6]Vestergaard NMIMR Vector Labs, Noguchi Memorial Institute for Medical Research, Legon, Accra, Ghana, [7]Department of Entomology, Navrongo Health Research Centre, Navrongo, Ghana, [8]Volta Regional Health Directorate, Ghana Health Service, Volta Region, Ho, Ghana

Recent update by WHO revealed that from January, 2021 to December, 2022, Ghana recorded 62 confirmed cases and 12 confirmed deaths as a result of Yellow Fever (YF) outbreak. Designing efficient control and preventive methods requires a thorough assessment of the community's awareness, knowledge and practices on YF. We determined community's knowledge, practices and awareness on YF using structured questionnaire with a data collection application software KOBO collect. A total of 1000 participants from two municipalities and two districts in the Upper East Region, were selected for the survey from May to July 2023. Seven hundred and ninety-two (79.2%) of the participants indicated they have heard about YF. Four hundred and fifteen participants, 415 (41.5%) had knowledge that transmission of YF is through mosquito bites. Participants from two districts; Bulsa North District, 158 (15.8%) and Kassena Nankana West District 153, (15.3%) showed very little knowledge on YF, (Mann-Whitney test =19523.000 $P = 0.567$) as well as in Bolgatanga Municipal, 295 (29.5%) and Kassena Nankana East Municipal, 186 (18.6%) (Mann-Whitney test=32300.000, P=0.000). Preventive measures used by participants to reduce spread of YF include sleeping in mosquito net, 303 (30.3%) clearing of bushes, 110 (11.0%) and vaccination, 170 (17.0%). The vaccination of respondents was not influenced by time taken to access health service

(Spearman Rho correlation coefficient=0.311). Continuous education and sensitization of inhabitants in the Upper East Region is required to create the needed awareness on YF outbreak preparedness and control.

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ECOLOGICAL STUDY OF TERRESTRIAL SMALL MAMMALS IN AN ENDEMIC PLAGUE FOCUS IN THE CENTRAL HIGHLANDS OF MADAGASCAR, IMPACT ON SURVEILLANCE STRATEGIES

Mamionah Noro Jully Parany[1], Nils Christian Stenseth[2], Fanohinjanaharinirina Rasoamalala[1], Soanandrasana Rahelinirina[1], Soloandry Rahajandraibe[1], Gauthier Dobigny[3], Eric Valade[4], Olivier Gorgé[5], Beza Ramasindrazana[1], Minoarisoa Rajerison[1]

[1]Pasteur Institute of Madagascar, Antananarivo, Madagascar, [2]Center for Pandemics and One Health Research, Sustainable Health Unit (SUSTAINIT), Faculty of Medicine; Centre for Ecological and Evolutionary Synthesis, Department of Biosciences, University of Oslo; Vanke School of Public Health, Oslo, Norway, [3]Institut de Recherche pour le Développement, UMR CBGP, Montpellier, France, [4]Institut de Recherche Biomédicale des Armées; Ecole du Val-de-Grâce, Paris, France, Paris, France, [5]Institut de Recherche Biomédicale des Armées, Paris, France

In Madagascar, human plague outbreak occurs almost every year in endemic foci and the extent of its burden may vary according to localities, case management and response. In order to investigate the implications of reservoirs in plague maintenance, we implemented a longitudinal survey. Six small mammal trapping sessions were conducted in six areas in Ankazobe during two years. Spleen from each individual of small mammals was used for Yersinia pestis detection by bacteriology and qPCR. Further, the presence of anti-F1 IgG antibodies was investigated by ELISA. A total of 2,762 small mammals were trapped and R. rattus represented 88% of all captures, with their relative abundance being significantly between trapping sessions and plague seasons. A pic of reproduction was observed during the dry and humid season. None of the tested individuals were neither PCR nor culture positive and a global seroprevalence of 0.4% was observed. Although Y. pestis appeared to circulate at low levels during the present survey, our study highlighted marked seasonal variations of R. rattus abundance and reproduction, thus allowing us to assess the most valuable periods to implement rodent reservoirs management in this recurrently active endemic focus of plague.

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RETROSPECTIVE EVALUATION OF THE DIAGNOSTIC ACCURACY OF THE RELASVPAN LASSA ANTIGEN RAPID DIAGNOSTIC TEST FOR THE DETECTION OF ACUTE LASSA VIRUS INFECTION IN NIGERIA USING REAL TIME POLYMERASE CHAIN REACTION AS REFERENCE STANDARD

Hanesh Fru chi[1], Johnson Etafo[2], Frtiz Fonkeng[1], Olufunke Ibitokun[2], Ronke Ireneh[2], Chuckwuyem Abejegah[2], Sampson Owhin[2], Aurelia Vessiere[1], Adedosu. Nelson[2], Emmanuel Agogo[1], Devy Emperador[1]

[1]FIND, Geneva, Switzerland, [2]Federal Medical Center Owo, Owo, Nigeria

Lassa virus (LASV) is a biosafety level 4, priority pathogen under the World Health Organization R&D Blueprint. LASV infects up to 300,000 persons annually across West Africa, causing a zoonotic, potentially severe viral hemorrhagic fever known as Lassa fever (LF). The LASV Rapid Diagnostic Test (RDT) landscape is currently not only limited, there is also limited independent performance data on the available LASV RDT to inform their use. This study was set up to assess the diagnostic accuracy of a LASV antigen RDT to determine its suitability for widespread use as a potential screening tool for acute LASV infection. This was an observational, retrospective, diagnostic accuracy study to determine the performance of the ReLASV™ Pan Lassa Antigen RDT (Zalgen Labs, LCC, Germantown, USA) using archived, frozen blood samples collected from individuals in Nigeria. The overall performance of this RDT was measured against the reference test, Altona RealStar LASV qRT-PCR 2.0 (Altona Diagnostics, Hamburg, Germany). Point estimates were calculated based on standardized definitions and the 95% confidence interval for each point estimate was derived based on Wilson's score method. With an observed PPA and NPA of 65% and 50.7% respectively, this test performed below the average expected performance. This test might therefore not be suitable for making critical diagnostic or treatment decisions without further validation or improvement. These findings underscore the importance of thoroughly assessing the performance characteristics of tests, to ensure their reliability and accuracy in real-world applications, especially in healthcare settings where diagnostic accuracy is critical.

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COMPARISON OF KNOWLEDGE, ATTITUDES AND PERCEPTIONS ON RESPONSE TO THE COVID-19 PANDEMIC BETWEEN RURAL AND URBAN COMMUNITIES IN DEMOCRATIC REPUBLIC OF CONGO

Mireille Amba Ngale, Samuel Mampunza Ma Miezi, Thérèse Mpiempie Ngamasata, Tarcisse Kilara Kapene, Joel Kiniati Fumwankau, Nsengi Ntamabyaliro, Gauthier Mesia Kahunu, Gaston Tona Lutete

University of Kinshasa, Kinshasa, Congo, Democratic Republic of the

SARS-COV-2 outbreak highlighted difficulties experienced by DRC population, a country of more than 90 million inhabitants located in central Africa. As soon as the first positive national case was diagnosed, the government implemented measures to protect the public. However, there are great differences between the large cities and the hinterland, which sometimes lack everything: access to water, electricity and quality health care. This project aims to compare knowledge, attitudes and practices on the government response to the COVID-19 pandemic between urban and rural communities in the DRC. This is a mixed study on knowledge, attitudes and practices conducted in two sites: Kinshasa for the urban area and at Kimpese, for the rural area. Data were collected through individual questionnaires administered to medical staff and group interviews with patients' carers. The study included 90 participants, 46 from the Kinshasa site and 44 from the Kimpese site. While 67% of Kinshasa residents surveyed trusted government reports on the spread of the epidemic and statistics on the number of cases of COVID-19 and deaths, that perception in Kimpese was lower (47%). Of the various government measures taken, the most popular were face masks (97%), lockdowns (97%) and travel restrictions (82%). Economic and social intervention policies, at 12% and 22%, were the least known. Just over six out of ten people questioned were satisfied with these measures. Proactive government management and logistical organisation prevented the spread of the C0VID-19 pandemic throughout the country. However, government management was marred by setbacks: communication crisis and financial mismanagement. Lack of contextualisation to national realities could be one of the causes of the non-appropriation of communities more concerned with their survival to the point of denying the existence of the disease. COVID-19 pandemic response in the DRC has taken into account the gap existing between urban and rural communities. An assessment of the consequences should be made. An epidemic risk management plan is needed to avoid making the same mistakes in the future.

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A MIXED-METHOD STUDY TO DETERMINE CAUSES OF DEATH USING MINIMAL INVASIVE TISSUE SAMPLING AND VERBAL AUTOPSY IN THE BONO EAST REGION, GHANA.

Grace Manu[1], Samuel Bernard Ekow Harrison[1], Dennis Adu-Gyasi[1], Mahama Abukari[1], Jones Opoku-Mensah[1], Farrid Boadu[1], Charles Zandoh[1], Abubakari Sulemana[1], Norman Goco[2], Kaali Seyram[1], Ernest Akwasi Adjei[3], Christiana Paganelli[2], Kwaku Poku Asante[1]

[1]Kintampo Health Research Centre, Kintampo, Bono East Region- Ghana, Ghana, [2]RTI International, Durham, NC, United States, [3]Komfo Anokye Teaching Hospital, Kumasi, Ashanti Region, Ghana

High-quality mortality data is needed for decision-making in health delivery. However, this is lacking in most low and middle-income countries. The Kintampo Health and Demographic Surveillance System (KHDSS) records all deaths of registered members and uses the WHO Verbal Autopsy (VA) tool to determine causes of death, but this process is limited by imprecise diagnosis and recall bias which ultimately reduces data quality and hampers evaluation of health policies. Minimal Invasive Tissue Sampling (MITS) is proven to improve the accuracy of cause-of-death (CoD) determination in low-resource settings. This study aimed to combine MITS and VA techniques to determine CoD in 300 cases involving stillbirths, children under five, and adults 60 years and above in the Bono East Region of Ghana. Initiated in May 2023, this study will explore the feasibility of integrating MITS into KHDSS to determine CoD and the acceptability of the MITS procedures. The inclusion criteria are deaths among individuals registered in the KHDSS occurring at the study hospitals or brought to study hospitals within 12 hours of death and written informed consent given by relatives. Cases of legal issues such as murder and accidents are excluded. Tissues and fluid samples are collected for molecular, microbiological, and histopathological analyses; placenta and umbilical samples are included in stillbirths and neonatal deaths. A multi-disciplinary panel of experts determines the CoD based on laboratory results, VA open narratives, and whenever available, clinical history. A total of 90 cases have been completed in the preceding 11 months since study initiation:15 stillbirths (66.7% females), 13 children under five (61.5 % males), and 62 adults (51.6% females). Thirty-one percent of these cases have been assigned causes of death (28/90) and the main CoD in stillbirth and adults are septicemia and pneumonia, respectively.

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USING MINIMALLY INVASIVE TISSUES SAMPLING TO DETERMINE CAUSES OF DEATHS IN THE MIDDLE-BELT OF GHANA: IMPLEMENTATION SUCCESSES, CHALLENGES AND OPPORTUNITIES

Mahama Abukari[1], Grace Manu[1], Samuel Bernard Ekow Harrison[1], Dennis Adu-Gyasi[1], Jones Opoku-Mensah[1], Farid Boadu[1], Sulemana Abubakari[1], Kaali Seyram[1], Ernest Akwasi Adjei[2], Norman Goco[3], Christina Paganelli[3], Kwaku Poku Asante[1]

[1]Kintampo Health Research Centre, Kintampo, Ghana, [2]Komfo Anokye, Kumasi, Ghana, [3]RTI International, Durham, NC, United States

Determination of underlying Causes of Death (CoD) and death registration remains a challengein low-resource settings. Minimal invasive tissue sampling (MITS) is an innovative interventionthat improves the accuracy of CoD information, is more culturally acceptable, and is less costlycompared to the conventional autopsy. A mixed-method study is currently being conducted inGhana to determine CoD among stillbirths, children under five, and adults 60 years and aboveusing MITS. The study team presents challenges and successes to inform future implementationof MITS in similar settings. Several challenges were identified as part of the baseline capacityassessment of health facilities and mortuaries, notable among them was the absence of apathologist in study sites. Others include 1) deficiencies in infrastructure like mortuaries, 2) lackof temperature-controlled cold boxes for transporting tissue samples, and 3) relatives' hesitancy toconsent to MITS on their deceased family members. To address the limited pathology expertise,following training, MITS sample collection was task-shifted to non-pathologists including medicalofficers, midwives, nurses, and laboratory scientists. Additionally, a virtual network of expertsincluding a pathologist provided technical support in sample analysis and interpretation. Otherstrategies implemented to address the challenges included: 1) improving the physical infrastructureby renovating mortuaries to ensure suitable environments for MITS sample collection, 2)establishing an electronic death notification system at hospitals and mortuaries, and, 3) conductingextensive community engagement involving stakeholders and developing context-specificguidelines to support the MITS consent process. Task-shifting of sample collection to non-pathologists, leveraging virtual networks to enhance capacity, optimizing existing facilities, andeffective community stakeholder engagement strategies have been key to implementing MITS inthis setting and are relevant considerations for scaling MITS within Ghana.

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COLLABORATING WITH KEY COMMUNITY ACTORS TO PREPARE FOR FUTURE OUTBREAK RESPONSES: LESSONS FROM LIBERIA

Hannah Berrian[1], Stephen B. Kennedy[1], Bartholomew Wilson[2], Mark Kieh[2], Tamba Fayiah[2], Ophelia Bongolee[2]

[1]UL-PIRE Africa Center, Monrovia, Liberia, [2]Partnership for Research on Ebola Virus in Liberia, J FK Hospital, Monrovia, Liberia

The West African sub-region was unprepared to manage the 2014-2016 Ebola outbreaks. The outbreaks exposed many systemic and structural challenges within the health sector, including limited recognition of community engagement and preparedness. In Liberia, the virus spread via person to person transmission, household clusters, and community transmissions. There were fears and misconceptions among community dwellers leading to lack of trust in the health care systems. Although Liberia and other regions are currently Ebola-free, the risk of another epidemic remains eminent thereby justifying the need for community preparedness to prevent, respond to, and contain any future outbreaks. We conducted a mixed methods quantitative and qualitative study design. This presentation focuses on the qualitative components conducted in three prevalence counties for EVD to identify the determinants of community preparedness for outbreak response, readiness for Merck ZEBOV vaccination, and the roles of informal and formal community structures. We used purposive sampling methods to conduct key informant interviews and focus group discussions with community groups, and leaders of informal and formal structures. Most of the participants described the communities as not being prepared to manage outbreak response. High death rates led communities to adhere to preventive measures although others were still engaged in traditional practices. Community leaders developed preventive measures including washing buckets at major intersections to prevent the spread of the virus. They highlighted limited trust in health authorities. Participants expressed varying opinions on Ebola vaccine, with some hesitant to take it due to lack of information, mistrust, and cultural beliefs and practices. They described community engagement and awareness as crucial for addressing mistrust and misinformation about the Ebola vaccine. There is a need for collaboration with key community leaders to prevent, detect and respond to threats from emerging and reemerging pathogens, build robust and resilient health systems, and strengthen community response.

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SEROLOGICAL SURVEY OF A COMMUNITY IN GHANA INVADED BY BLACKFLIES

Kingsley Nana Adu-Wiafe

Komfo Anokye Teaching Hospital, Ministry Of Health, Kumasi, Ghana

The Greater Accra region of Ghana is nonendemic for onchocerciasis, however, due to climate change, illegal mining activities, urban migration, and other factors, the transmission status is uncertain and confirmation may be required. The recent upsurge of blackflies in the La Nkwantanang Madina Municipality were investigated to ascertain the infectivity of

onchocerciasis among community members. The Neglected Tropical Disease program of the Ghana Health Service commenced a serological and entomological assessment in the identified communities to confirm or otherwise the onchocerciasis infectivity. This was a cross-sectional design involving interviews with a structured questionnaire. The survey purposefully sampled three firstline communities near the breeding sites of the blackflies. A total of 100 adults of 20 years and above were finger prick and dried blood spots collected for laboratory confirmation. Of the 602 samples, 24 were positive for onchocerciasis giving an overall prevalence of 3.9%. The seroprevalence of onchocerciasis in the community was 4.97%. Among the persons who tested positive for onchocerciasis, 50% were males and 21% were 60 years old and above. Two areas in the community attained the statistical threshold for mass drug administration with ivermectin for onchocerciasis.

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THREAT OF URBAN ARBOVIRAL DISEASES FROM *AEDES AEGYPTI* IN COLOMBIA

Rosa Margarita Gélvez Ramírez

Centro de Atencion y Diagnostico de Enfermedades Infecciosas-CDI, INFOVIDA, Bucaramanga, Colombia

Several arboviruses have emerged and/or reemerged in the New World in the past decades. While yellow fever and dengue are historical diseases which continue to cause deadly epidemics, chikungunya and Zika have recently invaded the South American continent, causing great concern. In Colombia, unplanned urbanization combined with growing demographics produce conditions suitable for the proliferation of *Aedes aegypti*, setting the scene for arbovirus epidemics. We collected eggs and adults of *Ae. aegypti* in Medellin, Colombia (from February to March 2020) for mosquito experimental infections with dengue (DENV), chikungunya (CHIKV), yellow fever (YFV) and Zika virus (ZIKV) and viral detection using the BioMark Dynamic arrays system. We show that *Ae. aegypti* from Medellín was more prone to become infected, to disseminate and transmit CHIKV and ZIKV than DENV and YFV. Thus, in Colombia, chikungunya is the most serious threat to public health based on our vector competence data.

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DEMONSTRATION OF RNA ACTIVATION IN TICKS

Kofi D. Kwofie[1], Emmanuel P. Hernandez[2], Anisuzzaman .[3], Hayato Kawada[1], Yuki Koike[1], Sana Sasaki[1], Takahiro Inoue[1], Fusako Mikami[1], Danielle Ladzekpo[1], Rika Shirafuji-Umemiya[4], Makoto Matsubayashi[5], Md Abdul Alim[3], Samuel K. Dadzie[6], Naotoshi Tsuji[1], Takeshi Hatta[1]

[1]Kitasato University School of Medicine, Sagamihara, Kanagawa, Japan, [2]University of the Philippines at Los Baños, laguna, Philippines, [3]Agricultural University, My-mensingh, Bangladesh, [4]Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido, Japan, [5]Osaka Metropolitan University,, Izumisano, Osaka, Japan, [6]University of Ghana, Legon, Accra, Ghana

RNA activation (RNAa) is burgeoning area of research in which small activating RNAs (saRNAs) mediate the targeted upregulation of specific genes. So far, the phenomenon has been limited to mammals, plants, bacteria, *Caenorhabditis elegans* and recently, *Aedes aegypti,* with no indication of its presence in other arthropods including ticks, despite the presence of Argonaut 2, an indispensable requirement for the formation of RNAinduced transcriptional activation complex. In this study, we demonstrated the presence of RNAa phenomenon for the first time in the Asian longhorned tick, *Haemaphysalis longicornis.* We targeted the 3'-UTR of a novel endochitinase-like gene in embryogenic eggs, for dsRNA-mediated gene activation. Our results showed an increased expression of the gene in *H. longicornis* endochitinase-dsRNA (dsHI-CHT) tick eggs at day-13 post-oviposition. Furthermore, we observed that the dsHI-CHT tick eggs exhibited relatively early egg development and hatching. Results therefore suggests that the dsRNA-mediated gene activation of HI-CHT led to the early development and hatching of dsHI-CHT tick eggs. This is therefore the first evidence of RNA activation in ticks and the outcome of this study provides new opportunities, based on the usefulness of RNAa as a tool for over expression of genes, for future research in tick biology, to reduce the global burden of ticks and tick-borne diseases.

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TICKS ON DROMEDARY CAMELS (*CAMELUS DROMEDARIUS*, LINNAEUS, 1758) FROM SOMALIA

Aamir M. Osman[1], Flávia CM Collere[1], Vanessa S. Coradi[1], Abdalla M. Ibrahim[2], Ahmed A. Hassan-Kadle[2], Mohamed A. Shair[2], Marcos R. André[3], Thiago F. Martins[4], Thállitha SWJ Vieira[5], Rosangela Z. Machado[3], **Rafael FC Vieira**[5]

[1]Universidade Federal do Paraná, Curitiba, Brazil, [2]Abrar University, Mogadishu, Somalia, [3]Universidade Estadual Paulista, Jaboticabal, Brazil, [4]Instituto Pasteur, Sao Paulo, Brazil, [5]The University of North Carolina at Charlotte, Charlotte, NC, United States

Ticks are prominent parasites and competent vectors of pathogens that may affect both humans and animals. Dromedary camels (*Camelus dromedarius*, Linnaeus, 1758) are vital livestock in Somalia but are susceptible to tick infestations, which pose health risks to both animals and humans due to pathogen transmission. Understanding tick diversity and prevalence in camel populations is crucial for effective management strategies. Therefore, this study aimed to collect and identify tick species parasitizing dromedary camels. A cross sectional study was utilized, involving the examination of 155 dromedary camels from Mogadishu and Lower Shabelle regions of Somalia. Ticks were removed from dromedaries using a commercial hook and kept in absolute ethanol labeled tubes for identification according to morphological taxonomic keys. A total of 346 (223 M, 112 F, and 11 nymphs) ticks were collected from 79/155 (50.9%; 95% CI: 42.8-59.1%) dromedary camels with a mean of 4.4 ticks per animal. Ticks were identified as *Rhipicephalus pulchellus* (174/346; 50.3%), *Hyalomma dromedarii* (103/346; 29.8%), *H. rufipes* (35/346; 10.1%), *H. marginatum* (16/346; 4.6%), *R. humeralis* (14/346; 4.0%), *Amblyomma lepidum* (2/346; 0.6%), *A. gemma* (1/346; 0.3%,), and *Ornithodoros* sp. (1/185; 0.5). The study identifies tick species infesting dromedary camels in Somalia, including the first report of *A. lepidum* and *R. humeralis* ticks in dromedary camels in the country. The economic importance of *Amblyomma* and *Rhipicephalus* ticks has long been recognized due to their ability to transmit multiple pathogens to humans and animals. Our data highlights the need for targeted control measures to mitigate health risks for animals and humans, emphasizing the importance of further research for comprehensive tick management and disease prevention in camel populations.

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UNDERSTANDING BARRIERS IN TRIATOMINE SURVEILLANCE: CHALLENGES AND COMMUNITY - DRIVING SOLUTIONS IN AREQUIPA, PERU

Laura D. Tamayo[1], Carlos E. Condori-Pino[1], Raquel Gonçalves[1], Ricardo Castillo-Neyra[2], Michael Z. Levy[2], Valerie A. Paz-Soldan[3]

[1]Zoonotic Disease Research Laboratory, One Health Unit, School of Public Health and Administration, Universidad Peruana Cayetano Heredia, Arequipa, Peru, [2]Department of Biostatistics, Epidemiology and Informatics, University of Pennsylvania, Philadelphia, PA, United States, [3]Department of Tropical Medicine and Infectious Disease, Tulane University, School of Public Health and Tropical Medicine, New Orleans, LA, United States

Vector-borne diseases remain a significant public health challenge globally, yet funding and support for vector control programs are diminishing. This study explores the systemic barriers to effective triatomine surveillance in Arequipa, Peru, by analyzing existing surveillance strategies and identifying critical inefficiencies that compromise their efficacy. Using qualitative methods, we conducted interviews and focus groups with stakeholders, applying purposive sampling to capture diverse perspectives. Our findings highlight significant systemic barriers, including inadequate community engagement, administrative inefficiencies, and resource constraints, which hinder the efficacy of both passive and active surveillance systems. We developed "AlertaChirimacha", an internet-based surveillance innovation,

to streamline the reporting process and expedite response times. "Alertachirimacha" was received positively and demonstrates the capacity of digital tools to improve the efficiency and reactivity of vector surveillance in environments with constrained resources. Our findings underscore the need for a systems thinking approach to reevaluate and enhance triatomine surveillance and control strategies. Integrating better training, improved resource allocation, and enhanced community participation are essential.

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AEDES AEGYPTI AND OTHER MOSQUITO SPECIES COHABITATING IN THE CHEKWOPUTOI CAVE, UGANDA

Austin Jose Mejia[1], Teddy Nakayiki[2], Julius J. Lutwama[2], Fred Ssenfuka[2], George Ongodia[2], Kivumbi Brian[2], Rebekah C. Kading[1]

[1]Colorado State University, Fort Collins, CO, United States, [2]Uganda Virus Research Institute, Entebbe, Uganda

Aedes aegypti is a major mosquito vector of globally significant human pathogens. *Ae. aegypti* can transmit viruses such as dengue (DENVs), Zika, chikungunya, and yellow fever. *Ae. aegypti* exhibits a complex genetic structuring among populations in Africa. Significant knowledge gaps remain pertaining to the sylvatic larval habitats of *Ae. aegypti*. We opportunistically collected mosquito larvae (n=113) from a rock pool at the entrance to Chekwoputoi cave located in the Kween District, Uganda. This cave is is the known roosting site for a large colony of the African sheath-tailed bat, *Colura afra* and is regularly utilized by domestic and other wild mammal species. Mosquitoes were reared to adults at the Uganda Virus Research Institute and morphologically identified. This collection comprised seven species: *Ae. aegypti formosus* (n=5), *Anopheles rhodesiensis*, and five additional *Culex* and *Aedes* species. Species identifications were confirmed using molecular techniques (ND4 and COI) and documented using high resolution photography. These observations represent unique ecological insight into the larval habitat and mixed-species larval community of medically-important mosquito species in Uganda. We hope to utilize this information to understand mosquito vector ecology in this poorly-studied area, and how these vectors cohabitate with each other.

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MULTIDIMENSIONAL EVALUATION OF FACTORS ASSOCIATED WITH TICK INFESTATION AMONG DOGS LIVING IN ECOTONES OF MADRE DE DIOS, PERU

Cusi Ferradas[1], Oliver A. Bocanegra[1], Veronica K. Castro[1], Carla M. Yauris[1], Winnie M. Contreras[1], Raúl Flores-Mara[1], Caroline Glidden[2], Andrés G. Lescano[1], Maureen Laroche[3]

[1]Emerge, Emerging Diseases and Climate Change Research Unit, Universidad Peruana Cayetano Heredia, Lima, Peru, [2]Department of Biology, Stanford University, Stanford, CA, United States, [3]Department of Microbiology and Immunology, School of Medicine, University of Texas Medical Branch, Galveston, TX, United States

Tick-borne pathogens circulate among humans, pets, and ectoparasites in Madre de Dios (MdD), Peru, and domestic dogs may act as a major host and reservoir. Identification of the risk factors associated with tick infestation in dogs, especially species involved in the transmission of human pathogens, is critical for a proper estimation of zoonotic transmission risk. We conducted a cross-sectional study in 120 houses in ecotones of MdD, systematically checking 170 dogs from 84 houses for ticks. We evaluated factors potentially associated with tick infestation among dogs: owners' socio-demographic information, housing characteristics (main wall and floor materials, nearby trash accumulation, etc.), dogs' signalment and acaricide use. MapBiomas Amazonia was used to determine the land coverage in a 100 meters-radius buffer around houses. Ticks were morphologically identified, and bivariate mixed effects logistic regressions were used to determine the association between these factors and tick infestation. Forty-one percent of dogs were infested with ticks (*R. sanguineus*: 90%; *A. ovale*: 4.3%, both: 4.3%). Living in houses surrounded with at least some land covered by urban infrastructure and houses close to trash accumulation were associated with higher odds of tick infestation in dogs. In contrast, dogs with owners with outdoor occupations in rural environments, dogs who live in houses surrounded with at least some land covered by a mosaic of uses (agriculture and/or pasture), and dogs who live in houses with floors built mainly with wood had lower odds of tick infestation. Our results suggest that the environmental factors and owners' outdoor habits are driving the odds of tick infestation in dogs, with no significant contribution of dog characteristics. Land coverage may be associated with microenvironmental variables or may be a proxy of other factors, like dog density. Owners who work outdoors in rural areas may be more aware of the risks associated with tick infestation. Our findings characterize the impact of individual- and community-based factors on tick infestations and highlight strategic targets for tick control interventions.

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TRACKING THE SOURCE POPULATION OF *SIMULIUM* BLACKFLY INVASION IN URBAN SETTINGS IN GHANA: A GENOMICS APPROACH

Millicent Opoku[1], Neha Sirwani[1], Emily N. Hendrickson[1], Himal Shrestha[1], Kwadwo K. Frempong[2], Sampson Otoo[2], Franklin Ayisi Ayisi[3], Millicent S. Afatodzie[2], Abena A. Nyarko[2], Sarah M. Dogbe[2], Joseph H.N. Osei[2], Sellase Pi-Bansa[2], Sindew M. Feleke[1], Warwick Grant[1], Daniel Boakye[2], Shannon Hedtke[1]

[1]La Trobe University, Melbourne, Australia, [2]Noguchi Memorial Institute for Medical Research, Accra, Ghana, [3]University of Ghana, Accra, Ghana

Simulium blackflies of the *damnosum* species complex exhibit long flight ranges of 20 to 600km when assisted by wind. They are vectors of the parasitic nematode that causes human onchocerciasis (river blindness), characterised by severe skin lesions, irreversible blindness, and epilepsy. Onchocerciasis occurs predominantly in remote rural areas in sub-Saharan Africa, Yemen, and small foci in Brazil and Venezuela. Control is by community-based mass drug administration (MDA) with ivermectin. Extensive small-scale mining in the Eastern Region have led to the pollution of fast-flowing rivers, which serve as natural breeding habitats for the *Simulium* vectors. These blackflies are likely to migrate in search of suitable breeding habitats, posing a potential risk of onchocerciasis transmission in areas previously unaffected by the disease. Reports of blackflies in parts of the capital city, Accra (an onchocerciasis naïve area) in June 2023, where blackflies had not previously been found, warranted prompt investigation of the source of the blackflies invading urban areas to assess risk of onchocerciasis transmission. We collected 270 female adult blackflies by human landing catch (HLC) from 14 communities in Ghana. Whole genome sequences were obtained from genomic DNA extracted from the blackflies. Based on principal components analysis (PCA) of 138,128 SNPs, the blackflies from two communities in the Volta region (Elavayno, n=10; Holuta, n=10) were genetically distinct from those collected from Accra (Paradise Valley, n=10; Teiman Borga Town, n=9) and the Eastern region (Asuoyaa, n=14), and elsewhere in Ghana. A PCA and k-means clustering of these distinct groups showed that the blackflies from Accra exhibited greater genetic similarity to those from the Eastern region. This implies a potential origin in southeastern Ghana. Despite the small sample size, we further identified potential migration of flies northward from the south. Further investigation into the corridors for fly movement and the implications of urban migration on the risk of onchocerciasis transmission is warranted.

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LAND REVERSION AND PALM FEATURES ARE MAJOR DRIVERS INFLUENCING THE OCCURRENCE OF A CHAGAS DISEASE VECTOR IN RURAL AREAS IN PANAMA

Juliana Hoyos[1], Vanessa Pineda[2], Kadir Gonzalez[2], Daniel Mendieta[2], Azael Saldaña[2], Jose Calzada[2], Bryna Wilson[3], Chystrie Rigg[2], Vanessa Vasquez[2], Luis F. Chaves[4], Sonia Altizer[1], Nicole Gottdenker[1]

[1]University of Georgia, Athens, GA, United States, [2]Instituto Gorgas, Panama City, Panama, [3]University of Florida, Gainesville, FL, United States, [4]Indiana University, Bloomington, IN, United States

In tropical countries, Royal Palms (*Attalea* spp.) constitute the most important element in the common arrangement defining the ecological niche of kissing bugs of the genus *Rhodnius*. The presence of palm-dwelling kissing bugs near human settlements, coupled with high infection rates with *T. cruzi* serve as indicators of the risk of transmission in rural areas. In this study, we assessed the impact of land use changes and palm characteristics on the occurrence, abundance, and infection status of *Rhodnius pallescens*, the primary vector of Chagas Disease in Panama. We sampled Kissing Bugs from Royal Palms in 12 communities distributed along a landscape gradient, with varying percentages of native forest, grassland, cropland, and early successional forest cover in Central Panama. Genomic DNA was extracted from whole bodies, and real-time PCR (RT-PCR) assays were performed using probes targeting the 28S ribosomal RNA (rRNA) genes of *Trypanosoma* parasites. We used robust design occupancy modeling to evaluate hypotheses for factors that might correlate with the occurrence of *R. pallescens* on Royal Palms, using 10 m resolution land cover data at 100 and 300 buffers, as well as specific palm traits. To account for potential spatial autocorrelation, we ran spatial occupancy model versions of the top-performing models and compared the outputs. We tested infection in populations of *R. pallescens* and found prevalence to be over 70%. We observed that elevation, amount of palm infructescence and successional forest cover have a positive effect on the probabilities of the presence of *R. pallescens* in these areas, whereas the percent of native forest showed a significant negative effect on these probabilities. Our models with quadratic effects outperformed those with linear effects for landscape metrics, indicating that predicted occupancy peaks at optimal amounts of these cover types and palm features. Our findings suggest that, in rural areas of Panama, anthropogenic landscape alterations, mainly forest regeneration, are associated with higher probabilities of palm infestation by Chagas disease vectors and with higher vector population densities.

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INSECT CELL LINES DERIVED FROM OLD AND NEW WORLD VECTORS OF TRYPANOSOMES

Lesley Bell-Sakyi, Catherine S. Hartley, Jing Jing Khoo, Alistair C. Darby, **Ben Makepeace**

University of Liverpool, Liverpool, United Kingdom

Continuous insect cell lines play important roles in research into biology and control of vectors of human and livestock pathogens. In sub-Saharan Africa, tsetse flies of the genus *Glossina* transmit the salivarian trypanosomes that cause sleeping sickness in humans and nagana in domestic animals, while in Central and South America triatomine bugs of the genera *Triatoma* and *Rhodnius* are major vectors of *Trypanosoma cruzi*, causative agent of Chagas disease in humans. Part of the remit of the Tick Cell Biobank is to generate new cell lines from neglected tropical disease vectors for distribution to the global research community as research tools. We have recently established new cell lines from three trypanosome vectors: *Glossina morsitans morsitans*, *Triatoma infestans* and *Rhodnius prolixus*. The *G. m. morsitans* cell line GMA/LULS61 is derived from tissues of adult female tsetse flies. Three *T. infestans* cell lines, TIE/LULS54, TIE/LULS65 and TIE/LULS69, and three *R. prolixus* cell lines, RPE/LULS53, RPE/LULS57 and RPE/LUCH66, were generated from embryonic tissues. With a view to their possible application in development of vector control strategies, GMA/LULS61, TIE/LULS54 and RPE/LULS53 have been tested for susceptibility to infection with *Wolbachia*. GMA/LULS61 cells supported infection and growth of 6/7 insect-derived *Wolbachia* strains, whereas neither of the triatomine bug cell lines became infected. TIE/LULS54 and RPE/LULS53 cells do not harbour Triatoma virus, and GMA/LULS61 cells do not harbour salivary gland hypertrophy virus, indicating that the cell lines could be used to propagate these previously uncultured viruses proposed as possible biological control tools. All the cell lines described here are available, subject to Material Transfer Agreements, from the Tick Cell Biobank https://www.liverpool.ac.uk/research/facilities/tick-cell-biobank/.

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DETERMINING *TRYPANOSOMA CRUZI* INFECTION PREVALENCE, BLOOD-MEAL PREFERENCE AND MICROBIOME COMPOSITION IN *TRIATOMA RUBIDA, TRIATOMA RECURVA, TRIATOMA PROTRACTA* AND *PARATRIATOMA HIRSUTA* COLLECTED BY *I*-NATURALIST CITIZEN SCIENTISTS IN THE AMERICAN SOUTHWEST

Richard M. Oxborough[1], **Zoee Sanchez**[1], Chad L. Cross[1], Evan Teal[2], Susan D. Carnahan[3], Michael Z. Levy[4], Jeff Hill[5], Michael M. Webber[6], Caryn Bern[7], Jeffrey Whitman[8], Louisa A. Messenger[1]

[1]University of Nevada, Las Vegas, Las Vegas, NV, United States, [2]University of California, San Francisco, San Francisco, CA, United States, [3]The University of Arizona, Tuscon, AZ, United States, [4]University of Pennsylvania, Pennsylvania, PA, United States, [5]United States Bureau of Reclamation, Las Vegas, NV, United States, [6]College of Southern Nevada, Las Vegas, NV, United States, [7]University of California, San Francisco, San Franscisco, CA, United States, [8]University of California, San Francisco, San Franscisco, NV, United States

Triatomine bugs are vectors of *Trypanosoma cruzi,* the etiological agent of Chagas disease. There are 11 species of triatomines reported in the US, with seven reported in the Southwest. Autochthonous *T. cruzi* transmission in the US is rare, owing to the sylvatic nature of triatomine species present and the inefficient mode of transmission. With ever-expanding anthropogenic land-use changes, the overlap between human and triatomine habitat is increasing and it is important to study triatomine bionomics and spatial distributions to update estimates of regional transmission risk. Citizen scientists who documented triatomine species observations on the i-Naturalist website from May-September 2023 were contacted with a request to safely collect, kill, and mail triatomine specimens to UNLV along with information regarding location and date of collection. Triatomine specimens were identified morphologically using standard keys before DNA was extracted from the abdomen of individual bugs and *T. cruzi* infection prevalence assessed using qPCR. A multiplex amplicon-sequencing assay has been designed to simultaneously characterize triatomine genetic diversity (CytB, ITS2), triatomine vector blood meal preferences (vertebrate 12S rRNA, CytB) and microbiome composition (16s rRNA, 18s rRNA). Overall, 449 triatomines were received, with all specimens from California being *T. protracta protracta* (n=17), while *P. hirsuta* (n=35) and *T. protracta* (n=10) were received from Nevada, and *T. rubida* (n=161) and *T. recurva* (n=206) were the predominant species from Arizona. *T. cruzi* infection prevalence by species was *T. recurva*: 94.7%, *T. rubida*: 92.9%, *T. protracta*: 72.2%, and *P. hirsuta*: 71.9%, for an overall infection prevalence of 90.7%. This is believed to be the first reported natural infection of *P. hirsuta* with *T. cruzi.* Amplicon sequencing characterization is ongoing, with results forthcoming. Additionally, entropy-based habitat models are being developed for the Southwest based on the spatial distribution of samples received, to identify putative hot spots of triatomine bug infestation for targeted surveillance efforts.

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RHIPICEPHALUS MICROPLUS SERPINS RMS-3 AND RMS-17 AND *IXODES RICINUS* SERPIN IRIPIN-3 EMPLOY DISTINCT MECHANISMS TO INHIBIT PROLIFERATION OF MOUSE T CELLS

Adela Chlastakova[1], Jindrich Chmelar[2], Anderson Sa-Nunes[3], Itabajara da Silva Vaz Jr[4], Lucas Tirloni[1]

[1]Tick-Pathogen Transmission Unit, Laboratory of Bacteriology, Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Hamilton, MT, United States, [2]Department of Medical Biology, Faculty of Science, University of South Bohemia in Ceske Budejovice, Ceske Budejovice, Czech Republic, [3]Departamento de Imunologia, Instituto de Ciencias Biomedicas, Universidade de São Paulo, São Paulo, Brazil, [4]Centro de Biotecnologia, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil

While feeding on hosts, ticks secrete saliva containing immunosuppressive molecules. These molecules enable ticks to attenuate hosts' immune responses, thereby facilitating the acquisition of a full blood meal. Proteins contained in tick saliva include serpins, which act as inhibitors of serine proteases. Two serpins from the tick *Rhipicephalus microplus* (RmS-3 and RmS-17) and one serpin from the tick *Ixodes ricinus* (Iripin-3) have been demonstrated to inhibit T cell proliferation. However, the mechanisms underlying the anti-proliferative activities of these serpins remain unknown. To elucidate the mechanism of action of the serpins, we treated mouse spleen cells with RmS-3, RmS-17, and Iripin-3. Subsequently, we stimulated T cell proliferation by adding the mitogen concanavalin A (ConA) to the cell cultures. Using flow cytometry, we assessed the expression of the T cell growth factor interleukin-2 (IL-2), the alpha-chain of the receptor for IL-2 (CD25), and the proliferation marker Ki-67 by T cells 24 h after the addition of ConA. Simultaneously, we measured the amount of secreted IL-2 using the ELISA method. Lastly, we employed flow cytometry to analyze the distribution of T cells within the G0/G1, S, and G2/M phases of the cell cycle at 48 h and 72 h after ConA addition. The experiments showed that the treatment with RmS-3 and RmS-17 led to a decrease in IL-2 production and CD25 expression by T cells. Consequently, the expression of Ki-67 was reduced in the presence of both serpins, and the cell cycle was arrested in the G0/G1 phase. In contrast, Iripin-3, while diminishing IL-2 production, did not affect the ability of T cells to express CD25. Initially, the entry of T cells into the cell cycle was hindered by Iripin-3, as indicated by decreased expression of Ki-67. However, cell cycle analysis performed at 48h and 72h time points revealed that T cells were eventually able to resume progression through the S and G2/M phases. Altogether, these results indicate that the *R. microplus* serpins RmS-3 and RmS-17 and the *I. ricinus* serpin Iripin-3 inhibit T cell proliferation through distinct mechanisms.

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EXPLORING THE TRANSCRIPTOME OF IMMATURE STAGES OF *ORNITHODOROS HERMSI*, THE SOFT-TICK VECTOR OF TICK-BORNE RELAPSING FEVER

Lucas C. Sousa-Paula[1], Markus Berger[1], Octavio A. C. Talyuli[2], Cindi L. Schwartz[1], Greg A. Saturday[1], José M.C. Ribeiro[2], Lucas Tirloni[1]

[1]National Institutes of Health, Hamilton, MT, United States, [2]National Institutes of Health, Bethesda, MD, United States

Ticks affect host's hemostasis, immunity, and tissue repair processes by injecting saliva into skin. Soft ticks exhibit a rapid-feeding strategy, completing blood meals within minutes, and possess powerful bites, causing discomfort and skin disorders. However, studies on bite effects and the genetic mechanisms underlying their feeding strategy are limited. Here, we focused on *Ornithodoros hermsi*, the vector of the tick-borne relapsing fever in the U.S. Through a combination of histopathological and whole-body transcriptomic analyses, we aimed to gain insights into *O. hermsi* feeding behavior across various feeding (unfed, 6h, 12h, 24h, and 5 days post-feeding) and developmental stages (larvae, 1st-, and 2nd-nymphs). Analysis of mouse-bitten skin showed extensive subcutaneous hemorrhages at the bite site, suggesting the presence of potent proteolytic enzymes and anti-hemostatic agents in tick saliva. By transcriptomics, we identified a diverse array of proteases, e.g., metalloproteases (M12B and M13) and serine-proteases (S01A), suggesting a potential involvement in host tissue degradation. We also identified homologs of anticoagulants described in other soft ticks, including factors Xa, thrombin, and platelet aggregation inhibitors. Clustering revealed distinct transcriptional profiles: unfed, early-fed (6h–24h), and late-fed (5d) across all developmental stages. Modulation in the expression of most annotated protein functional classes displayed a similar pattern: high expression in unfed, followed by a sharp decline in early-fed, then a subsequent increase as digestion progresses, akin to baseline expression at late-fed as seen in unfed groups. While the classical salivary genes (e.g., metalloproteases, lipocalins, proteases, and mucins), exhibited the same pattern, a gene expression switch between unfed and late-fed groups suggests a yet-to-be-elucidated strategy to alter the molecular repertoire for subsequent blood meals. Overall, our findings highlight the intense pre-feeding transcriptional activity of *O. hermsi*, providing valuable insights into its rapid-feeding strategy.

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ECTOPARASITE BURDEN OF SMALL MAMMALS LINKED TO LAND USE AND LAND COVER IN THE SOUTHEASTERN PERUVIAN AMAZON

Winnie Contreras[1], Cusi Ferradas[1], Oliver A. Bocanegra[1], Raúl Flores-Mara[1], Caroline Glidden[2], Andrés G. Lescano[1], Maureen Laroche[3]

[1]Emerging Diseases and Climate Change Research Unit, School of Public Health and Administration, Universidad Peruana Cayetano Heredia, Lima, Peru, [2]Department of Biology, Stanford University, Stanford, CA, United States, [3]Department of Microbiology and Immunology, School of Medicine, University of Texas Medical Branch, Galveston, TX, United States

Rodents are known reservoirs of zoonotic vector-borne pathogens. Understanding their ecology and infestation patterns is pivotal to accurately assess disease transmission risk. We studied the association between land use/land cover (LU/LC) types, host characteristics and ectoparasite burden in rodents through a cross-sectional study in Madre de Dios (MdD), Peru. Ectoparasites were collected from 207 rodents and taxonomically identified. LU/LC type of the capture location was obtained from MapBiomas Amazonia. Bivariate logistic and negative binomial regression models were used to characterize infestation patterns across ectoparasite groups (mites, fleas, lice, and ticks) and at the genus level. In total, 159 (76.81%) small mammals were infested with ectoparasites: 150 (72.46%) with mites, 15 (7.25%) with ticks, 10 (4.83%) with lice, and 8 (3.86%) with fleas. We found the presence of ticks, lice, and mites to be positively correlated with the presence of vegetation. Forest formation and proximity to water bodies were associated with specific patterns on infestations for various mites (Demodecidae, *Gigantolaelaps* spp., *Androlaelaps* spp., *Mysolaelaps* spp.). Looking at host characteristics, louse abundance was lower in male rodents, and mite abundance was lower in animals with higher body weight for all the species captured. While our results on factors associated with tick abundance are in accordance with published literature, they are conflicting with reports on louse abundance, which might vary according to proximity to dwellings. We confirm the role of the vegetation and water bodies in promoting rodent infestation with various ectoparasites, which serve as a strategic environmental target to reduce the risk of transmission of zoonotic rodent-borne pathogens.

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CHARACTERIZATION OF TICK-BORNE ENCEPHALITIS VIRUS SAMPLES FROM *IXODES* TICKS COLLECTED IN MONGOLIA

Bazartseren Boldbaatar[1], Noel Cote[2], Andrew G. Letizia[2], Doniddemberel Altantogtokh[3], Graham A. Matulis[4], **Nora G. Cleary**[4], Michael E. von Fricken[4]

[1]Mongolian University of Life Sciences, Ulaanbaatar, Mongolia, [2]Naval Medical Research Unit TWO (NAMRU-2), Sembawang, Singapore, [3]National Center for Zoonotic Diseases, Ulaanbaatar, Mongolia, [4]University of Florida, Gainesville, FL, United States

Tick-Borne Encephalitis Virus (TBEV) can cause neurological disease in humans with a range of clinical severity including death, depending on the subtype. Far-Eastern subtype has the highest mortality rates while Siberian is more likely to cause chronic disease. Tick-Borne Encephalitis (TBE) is endemic in Mongolia including both Siberian and Far-Eastern TBEV subtypes and has been detected since the 1980s. *Ixodes persulcatus* is the main vector of TBEV in Mongolia but this flavivirus has also been found in *Dermacentor* species. Understanding the epidemiology and evolutionary dynamics of TBEV is necessary in shaping the public health response to this deadly disease in Mongolia. Thirteen hundred *Ixodes persulcatus* ticks were collected in May 2020 from Eruu, Khuder, and Mandal in Selenge using the dragging and flagging method. Tick samples were homogenized, pooled (pool sizes ranged from 20-50), and then the supernatant was inoculated into Vero cells. Upon observing cytopathic effect (CPE), two reverse transcription polymerase chain reactions (RT-PCRs) were conducted on cell supernatant; one was to detect TBEV and the second was to subtype TBEV. Lysed cell culture supernatant was processed with Next-Generation Sequencing (NGS) using Illumina technology to obtain FASTA files for analysis. TBEV was detected from these samples and identified as the Siberian subtype using PCR. Ongoing phylogenetic analysis of NGS results will analyze genomic changes of TBEV to previously published TBEV sequences in the region. Subtype analysis of TBEV and tracking the viral evolution in ticks, specifically *Ixodes persulcatus* in Selenge, is vital to understanding the risk to the local populations. Current vaccines have been developed based on the European and Far-Eastern strains and genomic analysis of wildtype TBEV can inform vaccine strategies. Given the high concentration of this tick species and previous documentation of TBE infection in humans and ticks in this province, further characterization of TBEV in Mongolia is needed.

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GENOMIC INSIGHTS INTO THE *SPIROPLASMA SYMBIONT* OF *GLOSSINA FUSCIPES FUSCIPES*: IMPLICATIONS FOR TRYPANOSOME TRANSMISSION CONTROL

Daniel J. Bruzzese[1], Fabian Gstöttenmayer[2], Brian L. Weiss[1], Adly M.M Abd-Alla[3], Serap Aksoy[1]

[1]Yale University, New Haven, CT, United States, [2]Insect Pest Control Laboratory, Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture, Vienna, Austria, [3]nsect Pest Control Laboratory, Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture, Vienna, Austria

Tsetse flies (*Glossina* spp.) are primary vectors of parasitic trypanosomes responsible for human and animal African trypanosomiasis in sub-Saharan Africa. In addition to trypanosomes, tsetse flies harbor both obligate and facultative symbionts, which play crucial roles in host physiology and vector competence. *Spiroplasma*, a bacterium found in the tsetse fly species *Glossina fuscipes fuscipes* (*Gff*), has emerged as a potential candidate for reducing trypanosome transmission in its tsetse host. Previous research demonstrates a negative correlation between *Spiroplasma* presence and trypanosome infection in *Gff* flies and has shown *Spiroplasma* to be an apt manipulator of *Gff* physiology. However, the mechanisms behind the putative *Spiroplasma*-induced trypanosome resistance remain unknown. Here, to better understand the *Spiroplasma* strain that infects *Gff* flies, we conducted comparative genomics of *Spiroplasma* collected from the colony located at the FAO/IAEA Insect Pest Control Laboratory (IPCL) in Seibersdorf Austria and from a population located at Toloyang village in Northwestern Uganda. Leveraging Oxford Nanopore (ONT) sequencing technology, we generated closed *Spiroplasma* genomes from individual *Gff* flies. Both the colony and field assemblies had a high degree of similarity in gene content and structure, suggesting they belong to the same strain, denoted as *s*Gff. Phylogenomic analyses placed *s*Gff within the *Spiroplasma poulsonii* clade, which is a clade predominantly comprised of other Dipteran-infecting strains. Within the *s*Gff genome, we found genes involved in nutrient transport showing that *s*Gff relies on its host for many essential metabolites. We also identified numerous mobile genetic elements and putative defensive genes, including prophages, plasmids, and toxin genes, that could be responsible for the *s*Gff-induced resistance to trypanosomes. This study enhances our understanding of the *s*Gff strain and its potential role in modulating trypanosome transmission in its *Gff* host and highlights the efficacy of ONT sequencing to rapidly unravel the biology of symbionts.

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MICROBIAL DIVERSITY OF *CULICOIDES REEVESI* FROM CHIHUAHUA, MEXICO: A METAGENOMIC ANALYSIS OF RRNA 16S

Rodolfo Gonzalez Peña[1], David Orlando Hidalgo Martínez[2], Herón Huerta[3], Erick de Jesús De Luna Santillana[4], Jaime Raúl Adame Gallegos[5], Carlos Arturo Rodríguez Alarcón[2], Stephanie Viridiana Laredo Tiscareño[2], Ezequiel Rubio Tabarez[2], Julián Everardo García Rejón[1], Luis M. Hernández Triana[6], Javier Alfonso Garza Hernandez[2]

[1]Universidad Autónoma de Yucatán, Merida, Mexico, [2]Universidad Autónoma de Ciudad Juárez, Ciudad Juárez, Mexico, [3]Instituto de Diagnóstico y Referencia Epidemiológicos, Ciudad de México, Mexico, [4]Laboratorio Medicina de la Conservación, Centro de Biotecnología Genómica del Instituto Politécnico Nacional, Reynosa, Mexico, [5]Universidad Autónoma de Chihuahua, Chihuahua, Mexico, [6]Animal and Plant Health Agency, Virology Department, Rabies and Wildlife Zoonoses Research Group, Addlestone, United Kingdom

This study aimed to investigate the microbial diversity of bacteria in the composite microbial community associated with *Culicoides reevesi* biting midges from Buenaventura municipality in the state of Chihuahua, Mexico, using a Sanger sequencing 16s rRNA metagenomics approach. Adult females of *Culicoides reevesi* were collected by human landing catches in the rainy season of 2023 and morphologically identified. They were grouped into pools of 25 individuals from which genomic DNA (gDNA) was extracted. Sanger sequencing of 16S rRNA was performed for a total of 4 pools, and the amplicon sequencing of the V3-V4 hypervariable region was done on Illumina Mseq platform to detect bacterial communities. The bioinformatic analysis included quality assessment, taxonomic classification, and visualization. The evaluation of the microbial community involved assessing taxa abundance and diversity using Mothur and QIIME2 software included in Galaxy Tool Shed (https://usegalaxy.eu/). Our study presents, for the first time in México and worldwide, an in-depth analysis of the bacteriome composition in *C. reevesi*, utilizing a 16S rRNA metagenomic approach. We emphasize the prevalence of dominant bacterial phyla, particularly Proteobacteria, alongside varying abundances of Actinobacteria, Firmicutes, Acidobacteria, and Bacteroidota, with a notable occurrence of Tenericutes. We identified intriguing species of both human and animal pathogenic bacteria. Moreover, we observed the absence of unidentified bacterial sequences, alongside the presence of other bacterial groups associated with the environment or plants. This has implications for both healthcare and ecological management, potentially simplifying control measures but also posing risks if the dominant species are harmful. This research enhances our understanding of the microbiome associated with *Culicoides* species, such as *Culicoides reevesi*, underscoring the need for further investigation to fully grasp their ecological importance and impact on public health.

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ASSESSING FINE-SCALE ENVIRONMENTAL INFLUENCE ON COMMUNITIES OF CUTANEOUS LEISHMANIASIS VECTORS IN SOUTHERN IN PERU

Sergio Méndez-Cardona[1], Alejandro Lopera-Toro[2], Juliana A. Morales-Monje[2], Adrian Adrian Forsyth[2], Amely Bauer[1], Alexandra Bauer[1], Olivia Magaletta[1], Panpim Thongsripong[1], Olga L. Cabrera-Quintero[3]

[1]Florida Medical Entomology Laboratory, University of Florida, Vero Beach, FL, United States, [2]Andes Amazon Fund, Washington, DC, United States, [3]Instituto Nacional de Salud de Colombia, Bogota, Colombia

The departments of Madre de Dios and Cusco have the highest incidence of cutaneous leishmaniasis (CL) in Peru, accounting for about 30% of cases annually. Despite the significant public health impact of CL in this region, knowledge on sand fly ecology and distribution is still lacking. To better understand the transmission risks, it is crucial to examine the fine-scale environmental factors that favor the abundance of the potential vectors of CL. Therefore, our goal was to use a novel light trap to investigate the structure of sand fly communities across habitats with differing levels of anthropogenic impact at Manu Biological Station, located within the Manu Biosphere Reserve in Southern Peru. We hypothesized that the proportion of potential vector species would be greater in habitats with less canopy cover and associated with human activity. The low-cost light trap (Katchy UV light trap) was validated by comparing the abundance and species richness of phlebotomine sand flies to collections with standard CDC light traps using a Latin square design. The Katchy trap was then used to sample five habitat classes: secondary forest, bamboo dominated forest, riparian forest, abandoned fruit crops and peridomicile. For each trapping location, temperature, relative humidity, foliage cover and basal area were recorded. A total of 1184 sand flies were collected during the trap comparison and our results suggest that the Katchy light trap offers a viable low-cost alternative for phlebotomine sand fly sampling. Across the five habitat types, a total of 3047 sand flies belonging to 31 species were collected. *Nyssomyia shawi* was the most abundant species across all sites, representing 36.9% of all sand flies collected. Preliminary results indicate variation in community composition and abundances across different habitat types. Quantifying the influence of fine-scale environmental factors on phlebotomine sand fly communities across anthropogenically impacted habitats can provide insights relevant to understanding potential transmission risks and improve prevention and management of CL in Peru's endemic sylvatic regions.

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FIRST REPORT OF NATURAL INFECTION OF *ANOPHELES GAMBIAE S.S.* AND *ANOPHELES COLUZZII* BY WOLBACHIA AND MICROSPORIDIA IN BENIN: A CROSS-SECTIONAL STUDY

Wilfrid Sewade[1], Minassou Juvénal Ahouandjinou[1], Arthur Sovi[1], Aboubakar Sidick[1], Come Koukpo[1], Saïd Chitou[1], Linda Towakinou[1], Bruno Adjottin[1], Steve Hougbe[1], Filémon Tokponnon[1], Germain Gil Padonou[1], Martin Akogbéto[1], Louisa A. Messenger[2], Razaki Ossè[1]

[1]CREC, Cotonou, Benin, [2]School of Public Health, University of Nevada, Reno, NV, United States

Recently, bacterial endosymbiont, including *Wolbachia* and Microsporidia were found to limit the infection of *Anopheles* mosquitoes with *Plasmodium falciparum*. This study aimed to investigate the natural presence of key transmission-blocking endosymbionts in *Anopheles gambiae* and *Anopheles coluzzii* in Southern Benin. The present study was conducted in seven communes of Southern Benin. Anopheles were collected using indoor/outdoor Human Landing Catches (HLCs) and Pyrethrum Spray Catches (PSCs). Following morphological identification, PCR was used to identify *An. gambiae* sensu lato (*s.l.*) to species level and to screen for the presence of both *Wolbachia* and Microsporidia. *Plasmodium falciparum* sporozoite infection was also assessed using ELISA. Results: Overall, species composition in *An. gambiae s.l.* was 53.7% *An. coluzzii*, while the remainder was *An. gambiae* sensu stricto (*s.s.*). Combined data of the two sampling techniques revealed a mean infection prevalence with *Wolbachia* of 5.1% (95% CI 0.90–18.6) and 1.3% (95% CI 0.07–7.8) in *An. gambiae s.s.* and *An. coluzzii*, respectively. The mean infection prevalence with Microsporidia was 41.0% (95% CI 25.9–57.8) for *An. gambiae s.s.* and 57.0% (95% CI 45.4–67.9) for *An. coluzzii*. *Wolbachia* was only observed in Ifangni, Pobè, and Cotonou, while Microsporidia was detected in all study communes. Aggregated data for HLCs and PSCs showed a sporozoite rate (SR) of 0.80% (95% CI 0.09–2.87) and 0.69% (95% CI 0.09–2.87) for *An. gambiae* and *An. coluzzii*, respectively, with a mean of 0.74% (95% CI 0.20–1.90). Of the four individual mosquitoes which harbored *P. falciparum*, none were also infected with *Wolbachia* and one contained Microsporidia. This study is the first report of natural infections of field-collected *An. gambiae s.l.* populations from Benin with *Wolbachia* and Microsporidia. Sustained efforts should be made to widen the spectrum of bacteria identified in mosquitoes, with the potential to develop endosymbiont-based control tools; such interventions could be the game-changer in the control of malaria and arboviral disease transmission

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INTRA-POPULATION DIFFERENCES IN CTMAX AND EGG SURVIVAL IN USA POPULATIONS OF THE TIGER MOSQUITO, *AEDES ALBOPICTUS*: IMPLICATIONS FOR CLIMATE ADAPTATION?

Katie Westby, Ben Orlinick, Angela Smith, Kim Medley

Washington University in St. Louis, Eureka, MO, United States

In recent decades, we seen increases in the number of reported cases of vector-borne disease as well as vector range expansions in the USA. These increases have, in part, been attributed to climate change. Extreme weather events, such as heat waves and droughts, are also becoming more common and have the potential to negatively impact vectors if temperatures regularly go above, or humidity goes below, the thresholds to which ectothermic vectors can withstand. Very little is known about what these thresholds are for different vector species, and less is known about population differences within species. To address these gaps in knowledge, we established eight populations of the tiger mosquito, *Aedes albopictus,* from urban locations across its range in the eastern United States spanning four climate zones. Using a series of common garden experiments, we experimentally determined CTmax for adults and larvae, and survival rates of eggs exposed to different temperatures and relative humidities. We found significant population differences in CTmax for both adult males and females that were not correlated with latitude or longitude. Larvae had, on average, significantly higher CTmax (mean 44.8C) than adults (37.7C) and population differences were less pronounced for larvae. We interpret these results as larvae living below their thermal maximum and being less locally adapted than adults. We also found significant differences by population in egg survival but only when eggs were exposed to a higher temperature and lower humidity treatment (31C and 65% RH). Interestingly, the adult populations with the highest CTmax were not the same populations with the highest egg survival indicating that different selection pressures may be acting on the different life stages potentially because adults are mobile and eggs are sessile. These results present evidence of genetic based differences in heat tolerance at broad spatial scales for an important species with a near global distribution. As genetic variation is critical for thermal adaptation, this work implies that *Ae. albopictus* may be able to adapt to higher temperatures than they currently experience.

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PYRUVATE KINASE AND SIRTUIN 2 PROTEIN INTERACTION TIGHTLY REGULATES CARBON AND NITROGEN METABOLISM IN *AEDES AEGYPTI* MOSQUITOES

Patricia Y. Scaraffia[1], Natthida Petchampai[1], Jun Isoe[2]
[1]Tulane Unversity, New Orleans, LA, United States, [2]The University of Arizona, Tucson, AZ, United States

Aedes aegypti females are vectors of pathogens that cause serious human diseases. The implementation of better mosquito mitigation strategies requires a better understanding of mosquito metabolism. Previously, the application of positional 13C-stable isotope tracer analysis allowed us to provide evidence that *Ae. aegypti* mosquitoes use the carbon skeleton of glucose for ammonia detoxification and uric acid synthesis *via* multiple metabolic pathways, including glycolysis. Later, the kinetic characterization of the recombinant pyruvate kinase 1 from *Ae. aegypti* (AaPK1), the enzyme that catalyzes the last step of the glycolytic pathway, showed that AaPK1 is allosterically regulated by specific amino acids and phosphorylated sugars. Mass spectrometry-based target metabolomics, stable-label isotope tracing coupled with reverse genetics provided evidence that AaPK regulates both carbon and nitrogen metabolism. Recently, we discovered that AaPK is a lysine-acetylated protein post-translationally regulated by sirtuin 2 (AaSirt2), an NAD^+-dependent deacetylase that catalyzes the removal of acetyl groups from acetylated lysine residues. Western blotting of immunoprecipitated proteins showed that AaPK binds with AaSirt2 in the cytosolic fractions of tissues dissected from non-starved and starved females. In addition, knockdown of AaSirt2 by RNA interference significantly decreased AaPK protein abundance in fat body of starved females indicating that both AaPK and AaSirt2 tightly modulate how mosquitoes respond to starvation. We also found that AaSirt2 localized in both cytosolic and mitochondrial cellular compartments of mosquito tissues. To identify potential additional targets of AaSirt2, we took a proteomics-based approach to analyze immunoprecipitated lysine-acetylated proteins from cytosolic and mitochondrial fractions isolated from non-starved and starved mosquitoes. Our acetylomics data indicate that AaPK and other lysine-acetylated proteins significantly change their relative abundance in females deprived of sugar to cope with starvation.

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EXPLORING THE IMPLICATIONS OF TRAIT VARIATION AND LIFE HISTORY TRADE-OFFS FOR VECTOR-BORNE DISEASE TRANSMISSION

Brit Johnson, Greg Jacobs, Courtney Murdock
Cornell University, Ithaca, NY, United States

Vector-borne diseases pose significant challenges to public health worldwide and our current understanding of mosquito-borne disease transmission relies heavily on mechanistic mathematical models that can vary in complexity. One limitation to these models is a lack of incorporation of trait variation associated with dynamic environments more characteristic of field conditions. Additionally, it is assumed that these traits are often varying independently of each other. Yet, life history theory and evidence from a diversity of vertebrate and invertebrate systems suggest organisms experience life history trade-offs resulting in negative correlations across life history traits. To interrogate these assumptions, we explored the integration of life history theory into transmission models to investigate how accounting for life history trade-offs affects our understanding of vector-borne disease transmission. We utilized the basic reproductive number of a mosquito-borne pathogen (R_0) as a simple heuristic model to examine the effects of life history trade-offs that have been well established in other systems, specifically trade-offs between mosquito current reproduction (e.g., lifetime egg production) and immune defense (e.g., with consequences for vector competence and the extrinsic incubation period) as well as current reproduction and future survival. We found that incorporating correlations across traits, such as reproduction and immunity, leads to a substantial change in vectorial capacity and subsequently the predicted relative R_0 compared to a model that does not account for these trait correlations. This study underscores the importance of considering ecological and evolutionary factors in disease transmission dynamics and highlights the potential of integrating life history theory into epidemiological research for more robust disease control strategies.

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EXPLORING HOW LARVAL DIET AND REARING WATER INFLUENCE *AEDES AEGYPTI* FITNESS, MICROBIOTA AND VECTOR COMPETENCE

Elodie Calvez[1], Isaure Quetel[1], Caitlin Gaete[1], Ludmina Saint-Alban[1], Gladys Gutierrez-Bugallo[2], Christelle Dollin[1], Cédric Ramdini[3], Anubis Vega-Rua[1]
[1]Institut Pasteur de la Guadeloupe, Les Abymes, Guadeloupe, [2]Institute of Tropical Medicine Pedro Kourí, Havana, Cuba, [3]Regional Health Agency, Les Abymes, Guadeloupe

Mosquito rearing optimization in laboratory conditions is crucial for both vector research and control. Although the addition of nutriments is important for *Aedes aegypti* development from immature stages to adult mosquitoes, little is known about the nutriment composition of commercial diets used for mosquito rearing and their influence on *Ae. aegypti* life traits and ability to transmit pathogens. Here, we evaluated the influence of diets commonly used in laboratory rearing on *Ae. aegypti* fitness, lifespan, microbiota and vector competence. First, we characterized the effect of four diets and two different rearing waters (laboratory *versus* field-collected waters) on *Ae. aegypti* development, lifespan and microbiota. Our investigations demonstrated that nutriment composition (protein, lipid, carbohydrate) of the diets tested influenced *Ae. aegypti* development (time to pupation and emergence), size and survival. Metagenomic analysis revealed specific modulations of adult microbiota composition according to both diet and rearing water. Indeed, in laboratory water, if new emerged females demonstrated a high proportion of *Chryseobacterium* after independent rearing with three different diets, mosquitoes reared with yeast contained a more diverse microbiota composition. For field collected water, the diversity of the microbiota composition was high for three diets. However, for TetraMin condition, female microbiota was mainly composed by *Shingobacterium*. Then, we investigated the influence of the larval diet on *Ae. aegypti* vector competence for dengue virus (DENV). Our results highlight differences on vector infection and virus dissemination according to the diet used. Mosquitoes reared at larval stage with diets containing a higher concentration of protein seemed to be more susceptible to DENV infection and dissemination. Taken together, these results emphasize the importance of the standardization of arbovirus transmission estimation protocols to be as close as possible to field conditions and obtain accurate transmission risk estimations.

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HARNESSING MOSQUITO SYMBIONTS FOR MALARIA TRANSMISSION BLOCKING

Jeremy K. Herren, Thomas Onchuru, Joseph Gichuhi, Oscar Mbare
international centre of insect physiology and ecology, Kenya, Nairobi, Kenya

A possible malaria control approach involves the dissemination in mosquitoes of inherited symbiotic microbes to block *Plasmodium* transmission. However, in the *Anopheles gambiae* complex, the primary African vectors of malaria, there are limited reports of inherited symbionts that impair transmission. We have established the SYMBIOVECTOR project to investigate the prospect of deploying a recently discovered Anopheles symbiont, *Microsporidia MB*, as a *Plasmodium* transmission blocking tool. The ability of *Microsporidia MB* to block *Plasmodium* transmission together with vertical transmission and avirulence makes it an excellent candidate for symbiont-based transmission blocking. We show that a vertically transmitted microsporidian symbiont (*Microsporidia MB*) in the *An. gambiae* complex can impair *Plasmodium* transmission. *Microsporidia MB* is present at moderate prevalence in geographically dispersed populations of *An. arabiensis* in Kenya, localized to the mosquito midgut and ovaries, and is

not associated with significant reductions in adult host fecundity or survival. We investigated the mechanistic basis and efficiencies of *Microsporidia MB* transmission between *Anopheles arabiensis* mosquitoes. We show that *Microsporidia* can be transmitted both vertically (mother to offspring) and sexually between adult mosquitoes. The dynamics of spread and optimal dissemination strategies have been investigated under semi-field conditions and used to determine the likely outcomes of releasing *Microsporidia MB* in the field.

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CULEX MODESTUS CAN TRANSMIT USUTU VIRUS AND CAN BE COLONIZED IN A LAB SETTING

Alina Soto, Lotte Wauters, Leen Delang

Rega Institute for Medical Research, KU Leuven, Leuven, Belgium

Usutu virus is an emerging pathogen transmitted primarily by *Culex* mosquitoes. Recent preliminary data suggested that *Culex modestus* from Belgium may be strong Usutu virus vectors, potentially more efficient than the primary vector *Culex pipiens*. Yet, *Culex modestus* is a poorly understood species, despite their potential role in pathogen transmission and widespread establishment across Europe, Asia, and Northern Africa. Here we captured *Culex modestus* from Belgium to investigate their vector competence for Usutu virus and to establish a lab colony. Larvae and pupae were collected from a reedbed pond and brought to the lab to rear to adulthood. Adult *Culex modestus* were placed in cages and allowed to mate, lay autogenous egg rafts, and produce new generations. Testing different rearing conditions revealed that this species has specific requirements for larval diet, breeding water, and blood-feeding hosts. For example, despite readily feeding on chicken and rabbit blood, only females that fed on live mice effectively produced offspring. Through the establishment of the colony we experimentally designed a rearing protocol and gained insights into novel aspects of their biology, including their mating in confined spaces, small body size, and active and aggressive behavior. Meanwhile, female *Culex modestus* from the third generation were experimentally infected with a Belgian strain of Usutu virus via a blood meal. After 14 days of incubation at 25°C, the Usutu virus transmission capacity was evaluated by measuring infectious titers in a plaque assay and RNA copies by qRT-PCR. Most *Culex modestus* had an Usutu virus infection in their abdomen and heads (61%; n=17/28) with relatively high median titers (5560 PFU/abdomen and 778 PFU/heads). We observed a transmission efficiency of 54% (n=15/28) based on the presence of infectious Usutu virus in the mosquito saliva. This research offers compelling evidence that Belgian *Culex modestus* are potent vectors for Usutu virus, highlighting their potential role in Usutu virus circulation. In addition, we hope this study aids future researchers who wish to establish lab colonies of these mosquitoes.

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TIME TO LOSS OF PHYSICAL INTEGRITYOF ATTRACTIVE TARGETED SUGAR BAIT STATIONS IN WESTERN PROVINCE, ZAMBIA: A SURVIVAL ANALYSIS

Refilwe Y. Karabo[1], Mundia Masuzyo[2], Mwansa Mwenya[2], Kochelani Saili[3], Erica Orange[4], Zhiyuan Mao[1], John Miller[2], Kafula Silumbe[2], Javan Chanda[5], Busiku Hamainza[6], Joe Wagman[7], Irene Kyomohangi[1], Angela Harris[7], Megan Littrell[7], Thomas P. Eisele[1], Ruth A. Ashton[1], Joshua Yukich[1]

[1]Tulane University, New Orleans, LA, United States, [2]PATH, Zambia, Lusaka, Zambia, [3]Macha Research Trust, Choma, Zambia, [4]PATH, Seattle USA, Seattle, WA, United States, [5]Path, Zambia, Lusaka, Zambia, [6]National Malaria Elimination Center, Lusaka, Zambia, [7]PATH, Washington DC, Washington DC, DC, United States

The Westham Attractive Targeted Sugar Bait (ATSB) stations are a potential addition to the integrated vector management strategy against malaria. Each station, measuring approximately the size of A4 paper, consists of 16 cells filled with a bait sugar paste and ingestion toxin (dinotefuran), contained between a white plastic backing and a black perforated membrane to allow mosquito feeding. This study measured the length of time ATSB stations maintained physical integrity when hung on external walls of residential structures in a field setting in Western Province, Zambia as part of a Phase III cluster-randomized trial of ATSB efficacy. Loss of physical integrity was defined according to pre-defined criteria for bait station replacement due to damages which included tears, mold, leakage, and depletion of bait. A total of 5696 visits were made to 1107 ATSB stations, that were placed on 304 eligible structures to assess for their physical presence and condition using pre-defined criteria. Kaplan-Meier curves and Cox-Proportion Hazard models were used to assess survival probabilities, including risk factors associated for increased physical deterioration. The overall median ATSB station survival time was 149 days, or equivalent to 5 months. It was evident that the most documented damage on bait stations were holes/tears, and mold. Longevity of ATSB station survival was extended on those that were hung on structures that had “excellent protection” (Hazard Ratio 0.36, 95% CI {0.25-0.49}, p<0.001) compared to bait stations that had “no protection”. Thatched roof also extended ATSB station survival, and resulted in median survival time of 218 days (HR 0.37, 95% CI {0.26, 0.47}, p<0.001), or equivalent to 7 months, when compared to roofing made of iron sheets. Results suggest that ATSB stations may remain intact over the malaria transmission season in this setting in rural Zambia, and that longevity increases when housing characteristics provide sufficient protection from the weather, such as when placed under wide thatch roofs. Further research is needed to understand the relationship between physical damage of the bait station and efficacy.

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IDENTIFICATION OF NOVEL *WOLBACHIA* INFECTIONS IN FLORIDA MOSQUITOES

Leena Salama, Daniel W. Pérez-Ramos, Ian J. Sandum, Alex Bauer, Alex Urlaub, Abdullah Alomar, Lawrence E. Reeves, Eric P. Caragata

Florida Medical Entomology Laboratory, Department of Entomology & Nematology, Institute of Food & Agricultural Sciences, University of Florida, Vero Beach, FL, United States

Mosquito-transmitted diseases, such as malaria, dengue fever, and mosquito-borne encephalitis, cause thousands of deaths annually. Scientists have been developing novel approaches to reduce the incidence of these diseases and several rely on artificial transinfections with *Wolbachia*, an endosymbiotic bacterium. *Wolbachia* can spread rapidly through mosquito populations and block infection and transmission of important pathogens like dengue virus. Critically, it is unclear which mosquito species harbor native *Wolbachia* infection and how the prevalence of such infections varies across mosquito populations. This is important because laboratory studies show that native *Wolbachia* can have variable impacts on pathogen infection. For instance, some native *Wolbachia* infections decrease mosquito susceptibility to arbovirus and reduce the rate of transmission, while others have no effects. To better understand mosquito-*Wolbachia*-pathogen dynamics in nature, it is first necessary to establish which mosquitoes naturally harbor *Wolbachia*. Florida is a prominent site for arboviral disease in the United States. It is also home to approximately 90 different mosquito species. We have collected and screened approximately 35 of those species for the presence of *Wolbachia*, sampling from conservation land in eastern central Florida. This list includes mosquito species that are implicated in pathogen transmission as well as highly abundant nuisance biters. We used a custom qPCR assay to assess *Wolbachia* infection frequencies and titers and Sanger sequencing to assess *Wolbachia* diversity. Our results will facilitate the examination of the role of native *Wolbachia* in pathogen transmission for those species.

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VERTICAL AND HORIZONTAL TRANSMISSION OF *MICROSPORIDIA MB* IN *ANOPHELES ARABIENSIS* OCCURS THROUGH GERMLINE INFECTIONS

Thomas O. Onchuru, Edward Makhulu, Joseph Gichuhi, Jeremy Herren

International Centre of Insect Physiology and Ecology, Nairobi, Kenya

Microsporidia MB is a promising candidate for developing a symbiont-based strategy for malaria control because it disrupts *An. arabiensis*' ability to transmit the *Plasmodium* parasite. The symbiont is predominantly localized in the host's reproductive organs as it is vertically transmitted from mother to offspring and horizontally (sexually) transmitted during mating. Due to efficient transmission by both routes, *Microsporidia MB* has the potential to invade and spread in target vector populations to establish high prevalence rates. The stability and efficiency of *Microsporidia MB* transmission in *An. arabiensis* is important for its sustainable use for malaria control. In this study, we investigated the mechanistic basis of vertical and horizontal transmission of *Microsporidia MB* in *An. arabiensis* by establishing the localization patterns of this symbiont in the reproductive organs. We found that the germline stem cell niche, the primary and secondary follicles of newly emerged female *An. arabiensis* mosquitoes were infected with different stages of *Microsporidia MB* in high intensities. These infections were consistent across the pre and vitellogenic stages of egg development. Furthermore, *Microsporidia MB* replicated and increased intensities in the oocyte of developing eggs when mosquitoes were given a blood meal. Additionally, we investigated the *Microsporidia MB* infection rates of developing eggs and adult F1 offspring of infected female mothers. The adult-to-adult vertical transmission rate of *Microsporidia MB* was lower than the primary follicle infection rate indicating a significant impact of symbiont clearing during mosquito development. In males, *Microsporidia MB* also was localized in the stem cell niche. The symbiont replicated in infected cells and formed cyst-like structures within the testis and migrated into the ejaculatory duct with the sperms for transfer to females during mating. The ability of *Microsporidia MB* to consistently maintain infections in the germline stem cell niche and developing eggs in *An. arabiensis* provide evidence of an intimate association of this symbiont and its mosquito host.

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ZOONOTIC AND HUMAN MALARIA TRANSMISSION BY VECTOR SPECIES AND LANDSCAPES IN INDONESIA

Thomas R. Burkot[1], Boni Sebayang[1], Bram van der Straat[1], Ahadi Kurniawan[2], Inke Lubis[3], Matt Grigg[4], Triwibowo Ambargarjito[5], Tanya L. Russell[1]

[1]Australian Institute of Tropical Health and Medicine, Cairns, Australia, [2]Public Health Laboratory of Medan, Medan, Indonesia, [3]Universitas Sumatera Utara, Medan, Indonesia, [4]Menzies School of Health Research, Darwin, Australia, [5]National Research and Innovation Agency, Salitiga, Indonesia

Zoonotic malaria infections in humans, particularly *Plasmodium knowlesi*, are increasing across Southeast Asia, notably in rural areas of Malaysia and Indonesia, and have been associated with changes in land use patterns. Nine species of the *Anopheles leucosphyrus* and *Anopheles dirus* species complexes are vectors of zoonotic malarias to humans in Southeast Asia but, little is known about their bionomics. The bionomics of zoonotic (*An leucosphyrus* group) and malaria (*An maculatus* group) vectors were studied in three land-use types: an oil palm plantation, a residential area, and a mixed-crop agriculture area in northern Sumatra, Indonesia. All night human landing collections characterised vector distributions, abundance (biting rates), endophily, seasonality and sporozoite rates and behaviours. Larval surveys characterised the anopheline immature habitats. The members of the *An leucosphyrus* group mosquitoes collected in Sumatra belonged to the *An dirus* complex. Distributions of anophelines varied significantly by land use within a limited geographic area with larvae of both *An dirus* and *An maculatus* complex species being found in both natural and man-made habitats. Biting rates of the *An dirus* complex was highest in a mixed-crop agriculture area while *An maculatus* group biting rates were highest in the oil palm plantation. Albeit significantly less in the village, *Ans dirus* complex biting rates in the village were greater than *An. maculatus.* The *An dirus* complex bit throughout the night and was highly exophagic in Sumatra. PCR analyses detected *Plasmodium vivax, P. inui, P. fieldi* and *P. coatneyi* in areas where *P. knowlesi* infections in humans were detected. Indoor interventions such as insecticide treated nets and indoor residual spraying are likely not as effective against the *An dirus* complex species in northern Sumatra given their exophagic habit and their lesser abundance in residential areas. The risk of both being bitten by *An dirus* complex mosquitoes and transmission of zoonotic malarias to humans will depend on human movement patterns among the different landscapes present in Sumatra.

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ADVANCING MOSQUITO SURVEILLANCE USING MALDI-TOF-MS

Mercy Tuwei[1], Jonathan Karisa[1], Caroline Wanjiku[1], Caroline Kiuru[2], Edith Ramaita[3], Martin Rono[1], Joseph Mwangangi[1], Derek Charlwood[4], Charles Mbogo[5], Marta Maia[1]

[1]KEMRI-Wellcome Trust Research Proramme, Kilifi, Kenya, [2]Barcelona Institute for Global Health, Barcelona, Spain, Barcelona, Spain, [3]Ministry of Health Kenya, Nairobi, Kenya, [4]Instituto de Higiene e Medicine Tropical, Lisbon, Portugal, Lisbon, Portugal, [5]Kenya Medical Research Institute, Nairobi, Kenya

Vector surveillance is pillar of malaria control however it is hindered by the cost and time needed to process the numerous mosquitoes collected during surveys. Current methods require multiple laboratory assays to gather species, bloodmeal source, and infection data. This project investigates a single spectroscopic approach using MALDI-TOF MS (matrix-assisted laser desorption/ionization time-of-flight) reducing costs and processing time, maximizing information from entomological collections. Sampling of malaria vectors was done in different sites across Mozambique and Kenya using CDC light traps. The collected samples were morphologically identified to species, preserved in silica gel, and transported to the lab for further analysis. The cephalothorax mosquitoes of were aseptically dissected into two halves - one half for gold standard molecular assays and the other half for MALDI-TOF MS spectra acquisition. The malaria vectors were processed using PCR and bidirectional Sanger sequencing for species. A subset (a minimum of 10 per species) of the samples with quality spectra were selected were for unsupervised clustering using dendrograms and database build-up and the rest for validation. MALDI-TOF MS was able to identify primary and secondary malaria vectors including members of the An. gambiae s.s, An. arabiensis, An. merus, An. quadriannulatus, An. funestus s.s, An. rivulorum, An. leesoni, An. parensis, An. rivulorum, An. coustani, An. cf. coustani, An. cf. rivulorum, An. rufipes and An. pretoriensis. Additional species are continuously being added to the database. The development of a comprehensive database is anticipated, providing valuable insights into lesser-known vectors, and potentially aiding our understanding of the role of different primary and secondary vector species in transmission leading to improved surveillance and control. The ease of performance, the rapid turn-around time to results, and the minimal cost per sample make it a novel methodology that could bring about a paradigm shift in routine entomological surveillance.

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FACTORS AND EXTENT OF DISCORDANCE BETWEEN HOUSEHOLD DECLARATIONS OF INSECTICIDE TREATED BED NET USE AND CONFIRMATORY DIRECT OBSERVATIONS OF NETS HANGING ON OR LYING NEAR THE BED

Martin C. Akogbéto[1], Gil G. Padonou[1], kefilath Badirou[1], Bruno Akinro[1], Saïd Chitou[1], Moustapha Idrissou Souler[1], Salako S. Albert[1], Rock Aïkpon[2], Cyriaque Affoukou[2], Virgile Gnanguenon[3], Raoul Oloukoi[3], Daniel Impoinvil[4]

[1]CREC, Cotonou, Benin, [2]MNCP, Cotonou, Benin, [3]USAID, Cotonou, Benin, [4]CDC, Atlanta, GA, United States

Five triennial mass distribution campaigns and annual ancillary continuous distribution of insecticide treated-bed nets (ITNs) occurred in Benin from 2011 to 2023 with malaria incidence (cases/1000 population) ranging between about 294 in 2011 to 270 in 2022 (peak: 340 in 2016); previous studies report ITN use rates after net distribution based on household-declaration of net use, but there is uncertainty on the reliability of these self-reports. This study compares household declaration of ITN use with confirmatory direct ITN observation in houses to determine the factors and the extent of discordance between declared and observed use rates. The study took place 4-months after the 2023 ITN distribution campaign. To capture the variation in cultural and socio-economic characteristics of the country, in this study, 1567 households were visited in 24 randomly selected villages along a south-north Benin transect. ITN use rates were calculated by 1) obtaining a declaration of having slept under a net last night by the head of the household and 2) confirmatory direct observations of nets in the household. Any net found hanging on a bed was considered in use; nets not hanging but found over the bed or laid on or near the bed were also considered in use when the heads of households confirmed it. Survey data was recorded using the Open Data Kit (ODK) software loaded on tablets. Most ITNs directly observed were campaign nets 77% (3,223/4,210) and for campaign nets and other nets found in households, the coverage rate was 46% [44% - 48%] (1 ITN for every 2 people). Of 1567 households visited, 1492 (95%) declared sleeping under a net the night before; however, only 82% (3434/4210) of all nets, and 82% (2653/3223) of the 2023 campaign nets were observed hanging over the bed or lying on or near the bed where use was confirmed by the heads of the households. One of the reasons for the absence of hanging nets is the lack of space; some rooms serve as a place for sleeping and resting. While the declared ITN use rate seemed high (~95%), confirmatory direct observation suggested rates may be lower.

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COMPREHENSIVE ASSESSMENT OF SOCIODEMOGRAPHIC PROFILE, MALARIA EPIDEMIOLOGY AND VECTOR BIONOMICS IN NORTHEASTERN TANZANIA: A PRE-INTERVENTION BASELINE SURVEY FOR A PROSPECTIVE CLUSTER RANDOMIZED CONTROLLED TRIAL ASSESSING THE EFFICACY OF A NOVEL 3D-WINDOW DOUBLE SCREENS (3D-WDS) FOR SUSTAINABLE MALARIA VECTOR CONTROL

Subam Kathet[1], Venneranda Mbwana[2], Mwantumu A. Fereji[2], Jane B. Mhando[2], Gladness Kiyaya[2], Patrick Tungu[2], Victor S. Mwingira[2], Frank S. Magogo[2], William N. Kisinza[2], Seppo Meri[1], Ayman Khattab[1]

[1]University of Helsinki, Helsinki, Finland, [2]National Institute for Medical Research, Amani Medical Research Centre, Muheza, Tanzania, United Republic of

The emergence of insecticide-resistant malaria vectors has necessitated the development of alternative methods that are non-reliant on these chemicals. In response, a novel window screen, the 3D-Screen, has been developed in Finland. Prior to implementing a cluster-randomized controlled trial (cRCT) to assess its efficacy in reducing malaria at the community level, a cross-sectional survey was conducted to establish the sociodemographic profile, malaria prevalence, and vector bionomics in 20 villages across the Muheza district in northeastern Tanzania. Blood samples were collected from 778 children aged 6 months to 14 years to detect *Plasmodium* parasite using malaria RDTs and to measure hemoglobin concentration for anemia. Adult mosquitoes were captured indoors using CDC light traps followed by morphological identification and molecular analyses to establish sibling species, *Plasmodium* infection, and blood meal sources. Insecticide resistance to common pyrethroids was evaluated using WHO cylinder test, recording knockdown time and mortality rate, followed by the genotyping of L1014F kdrE mutation using TaqMan assay. A total of 1203 households (HH) from 20 villages were enumerated during the study. The average LLINs per HH was 1.7 with universal coverage in 54.53% HH. The average malaria prevalence in the study area was 40.23%. *An. gambiae* and *An. funestus* were the two major malaria vectors at an overall proportion of 29.78% and 70.21%, respectively. The average bites per person per night were 5.219, and the overall *Plasmodium* infection rate in *Anopheles* was 2.15%. High levels of resistance to pyrethroids were observed with mortality of 56.50% and 52.77% against permethrin and deltamethrin, respectively, and the average occurrence of kdrE resistant and susceptible alleles in *An. gambiae* s.s. across the study area was 0.49 and 0.41, respectively. The study reported high prevalence of malaria and widespread resistance to common pyrethroids, underscoring the necessity for a non-insecticidal approach in the study area. These findings also guided the selection of study clusters for the cRCT of the 3D-WDS conducted from 2019 and 2021.

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THERMAL ADAPTATION IN *AEDES ALBOPICTUS*

Ayda Khorramnejad[1], Stefano Quaranta[1], Hugo Perdomo[1], Romina Bahrami[1], Chloé Lahondère[2], Mariangela Bonizzoni[1]

[1]University of Pavia, Pavia, Italy, [2]Virginia Polytechnic Institute and State University, Blacksburg, VA, United States

Ectotherms depend on environmental temperature to support their physiology and use several physiological (e.g., synthesis of heat shock proteins) and behavioral (e.g., shift in microhabitat selection) strategies to avoid the risk of thermal stress when exposed to rapid changes in temperature. Long-term exposure to a thermal challenge can have profound and lasting physiological changes. In *Drosophila* these changes include both plastic responses and genetic adaptations. How arboviral vectors respond to a long-term thermal challenge like that imposed by global warming is still not fully understood. To address this knowledge gap, we used an experimental evolution approach on *Aedes albopictus*, the primary vector of arboviruses in temperate areas of the world. We have been rearing mosquitoes through 13 generations under tropical (32°C for 14 h and 26°C for 10 h) and control (28°C for 14 h and 26°C for 10 h) conditions. We compared mosquito fitness at G_1, G_5 and G_{10} with respect to G_0 and observed significant fitness differences between G_0 and G_1 mosquitoes, indicating acclimation, as well as among G_0 and $G_{5\text{-}10}$, suggesting adaptation. Importantly, we observed 14.93% mortality one day after emergence in G_1 females, which reached 31.21% in G_5 and 27.54% in G_{10} for the mosquitoes under tropical conditions. This result suggests that this condition represents a strong selective force. We further took a batch of G_{13} eggs and reared them under control condition (28°C). We observed that the fitness of these mosquitoes is different from the fitness of mosquitoes at G_0, $G_{1\text{-}5\text{-}10}$. Overall, these result support thermal adaptation includes more than plasticity in *Ae. albopictus*.

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THE EFFECT OF VARIATION IN MICROCLIMATE AND LAND USE ON THE DISTRIBUTION OF THE *AEDES ALBOPICTUS* AT THE INVASION EDGE

Anna V. Shattuck[1], Brandon D. Hollingsworth[1], Scott Campbell[2], Christopher L. Romano[2], Courtney C. Murdock[1]

[1]Cornell University, Ithaca, NY, United States, [2]The Arthropod-Borne Disease Laboratory, Suffolk County, Yaphank, NY, United States

In recent decades, there has been a significant rise in vector-borne disease incidence, posing a significant public health threat. The increase in vector-borne disease risk can partially be attributed to the increased distribution of several vector species. Furthermore, with invasion often occurring in environments with high densities of susceptible people (many urban centers), another principal concern is being able to use vector distribution information to identify populations most at risk. Mosquito surveillance data is often lacking for recently invading species, so it is important to understand the extent at which surveillance data can be extrapolated into new, often heterogenous, landscapes to predict species distribution. In this study, we use long-term (2004 to 2023) mosquito surveillance data collected in Suffolk County, Long Island, NY, to explore the environmental drivers of spatial variation in *Aedes albopictus* populations, a recently arrived invasive species, across a heterogenous landscape using a variety of Species Distribution Modeling methods. We use cross-validation and field collections to validate model results and to compare accuracy between methods. Additionally, we sample outside the Suffolk County to evaluate each model's ability to spatially extrapolate mosquito distributions. Finally, we combine predicted species distributions with human census data to identify populations of people most likely to experience greater mosquito population burdens. We specifically explore correlation of income status and access to medical care with predicted mosquito distributions. Ultimately, this study will help improve the deployment of targeted vector control efforts for invasive vector species in new environments.

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PREDICTION MODELING OF THE GEOGRAPHICAL DISTRIBUTION OF *AEDES ALBOPICTUS* IN TUNISIA

Hala S. Thabet[1], Walid Barhoumi[2], Ifhem Chelbi[2], Nada Toumi[2], Reham TagEldin[1], Oumaima Jlassi[2], Kais Ben-Ahmed[2], Mbarek Nasri[2], Matthew Montgomery[1], James Harwood[1], Elyes Zhioua[2]

[1]US Naval Medical Research Unit-EURAFCENT, Cairo detachment, Egypt, [2]Unit of Vector Ecology, Pasteur Institute of Tunis, Tunis, Tunisia

The Asian tiger mosquito, *Aedes (Stegomyia) albopictus* (Skuse), was first reported in Tunisia in 2018, at an archeological site, located in the northern part of the country. The Tunisian strain of *A. albopictus* has been shown experimentally to be competent for transmission of Chikungunya, Dengue, and Zika viruses; thus, the country is facing increased risk of Asian tiger mosquito-borne viruses—serious threats to public health. Currently there is limited information concerning the geographical distribution of *A. albopictus* in Tunisia, and the absence of comprehensive data and accurate modeling to predict geographical distribution hinders vector control efforts. To address this gap, we collected larval and adult mosquitoes from nine Tunisian governorates between October 2022 and January 2024, gathering a total of 52 positive occurrence points. We developed a Maximum Entropy (MaxEnt) model incorporating one vegetation, two topographic, and 19 bioclimatic variables as potential predictors for the habitat distribution of *A. albopictus*. These variables were selected based on their biological relevance to the target species distribution. The model predicts that the most suitable areas for *A. albopictus* are located in northern and northeastern Tunisia. Key factors contributing to the distribution of *A. albopictus* in Tunisia include elevation, temperature seasonality, precipitation during the warmest quarter, annual precipitation, and mean temperature during the wettest quarter. The phenology of *A. albopictus* showed a major peak during the month of October. Since 2018, and driven by climate changes and urbanization, *A. albopictus* has extended its reach in the country. Our findings offer crucial insights for monitoring the spread of this invasive mosquito species and provide guidance for decision-makers aiming to implement comprehensive surveillance and control programs in Tunisia.

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FIRST RECORD OF *AEDES ALBOPICTUS* IN YEMEN

Alia Zayed[1], Yasser Baheshm[2], Matthew J. Montgomery[3]

[1]Naval Medical Research Unit EURAFCENT, Cairo, Egypt, [2]National Malaria Control Program, Aden, Yemen, [3]Naval Medical Research Unit EURAFCENT, Sigonella, Italy

Aedes albopictus was recorded for the first time in Yemen in September 2023, during routine surveillance in Al Mahara Governorate. This invasive mosquito species is considered a public health threat due to its ability to transmit viral diseases such as dengue and chikungunya, its wide range of hosts and its ecological plasticity. Five villages, namely Rahn, Al Fatk, Damqout, Jadheb and Houf, were screened for the presence of mosquitoes. We surveyed water bodies and containers, and deployed Communicable Disease Control traps (CDC), Biogents Sentinel (BG) traps, spray catches and aspiration for adult collection. Adults and immature stages of *Ae. albopictus* were identified in Houf, a city 17 m above sea level in the easternmost part of the Governorate of Al Mahara, which is located less than 8 km from the border with Oman. Our findings show that *Ae. albopictus* was coexisting with both *Ae. aegypti* and *Anopheles stephensi* and co-inhabiting the same container with *Ae. aegypti* with a ratio of 1:2. The role of continuous human movement and transportation in facilitating cross-border dispersal of *Ae. albopictus* between Al Mahara and Oman, will be addressed. Intensive efforts should be undertaken to monitor and manage *Ae. albopictus* spread in the country The presence of both Aedes vectors together highlights the need for surveillance for associated diseases and consideration of countermeasures.

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THE USE OF EDNA AS A METHOD TO DETECT PRESENCE OF *AEDES AEGYPTI* AND *AEDES ALBOPICTUS* IN INTERSPECIFIC LARVAL HABITAT

Stephen Opoku Afriyie, Corey Brelsfoard, Matthew A. Barnes, Joseph McMillan

Texas Tech University, Lubbock, TX, United States

Environmental DNA (eDNA) analysis is an emerging technology that could be utilized as a novel tool to test for the presence of *Ae. aegypti* and *Ae. albopictus* in areas suspected of recent invasions. To investigate the utility of eDNA for detection of *Ae. aegypti* and *Ae. albopictus,* we first validated detection of these species from single-species larval rearing containers using species-specific qPCR assays targeting the ribosomal internal transcribed spacer 1 region. We then designed a series of experiments to: (i) test the sensitivity of real-time qPCR to detect eDNA of *Ae. aegypti* and *Ae. albopictus* in mixed species containers at different ratios, and (ii) detect eDNA as a proxy for the presence of *Ae. aegypti* and *Ae. albopictus* in containers treated with varying concentrations of larvicides. Experiments were performed in three replicates under controlled laboratory conditions using sterilized containers, distilled water, and nutrient sources including appropriate controls. Larvae rearing water in each container was vacuum-filtered using 1.2 µm membrane filters before eDNA extraction using the CTAB-chloroform method. After extraction, quantification of *Ae. aegypti* and *Ae. albopictus* eDNA was performed using qPCR. Our preliminary results suggest that, *Ae. aegypti* and *Ae. albopictus* can be accurately detected in mixed species larval habitats. Experiments are currently ongoing to test possible detection of *Ae. aegypti* and *Ae. albopictus* eDNA in containers treated with larvicides. Our findings will provide important preliminary data to assess the potential of using eDNA approaches to enhance surveillance of *Ae. aegypti* and *Ae. albopictus* in the High Plains and Mountain West regions of the US, where invasion of these medically important species is suspected.

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IMPACT OF SUGARCANE IRRIGATION SCHEME ON ANOPHELINE MOSQUITO ECOLOGY, BEHAVIOR, MALARIA TRANSMISSION RISK AND INSECTICIDE RESISTANCE IN SOUTHWESTERN ETHIOPIA

Assalif Demissew[1], Abebe Animut[2], Solomon Kibret Birhanie[3], Dawit Hawaria[4], Arega Tsegaye[5], Teshome Degefa[5], Hallelujah Getachew[6], Ming-Chieh Lee[7], Guiyun Yan[7], Delenasaw Yewhalaw[5]

[1]Ambo University, Ambo, Ethiopia, [2]Addis Ababa University, Aklilu Lemma Institute of Pathobiology, Ethiopia, [3]West Valley Mosquito and Vector Control District, Southern California, California, CA, United States, [4]Hawassa University, Hawassa, Ethiopia, [5]Jimma University, Jimma, Ethiopia, [6]Arba Minch College of Health Sciences, Arba Minch, Ethiopia, [7]University of Californiia, Irvine, California, CA, United States

Ethiopia is expanding extensive irrigation developments to meet food demands and alleviate poverty in the country. However, the effect of such water development projects on malaria transmission risk is not well investigated. Moreover, agrochemicals used in irrigation activities are blamed to drive resistance selection in malaria vectors. Studies evaluating the impact of these agrochemicals on malaria vector's resistance are lacking. This study investigated impact of sugarcane irrigation on vector dynamics, behavior, transmission risk and insecticide resistance of malaria vectors in Southwestern Ethiopia. Adult *Anopheles* mosquitoes were collected using CDC light traps and human landing catches from irrigated and non-irrigated clusters of Arjo-Didessa sugarcane irrigation scheme in wet and dry seasons, between 2018 to 2021. Mosquito species composition, abundance, seasonality, behavior (biting & blood feeding) and *Plasmodium* infection rates were compared. Mosquitoes were identified to species morphologically and using molecular techniques. Mosquito host blood meal sources were determined by polymerase chain reaction (PCR). *Plasmodium* sporozoite infections were analyzed using CSP ELISA. Adult *Anopheles gambiae* s.l. were tested for their susceptibility to insecticides using WHO tube test. Among 6,058 female *Anopheles* mosquitoes collected, 72.3% (n= 4379) were from irrigated and 27.7% (n= 1679) from non-irrigated clusters. Mosquito composition, abundance and density was significantly higher in the irrigated than non-irrigated clusters during the wet and dry seasons. Anophelines in the irrigated clusters were more anthropophagic and showed overnight as well as outdoor biting activity. A 2-fold higher *Plasmodium* infection rates were recorded in the irrigated than non-irrigated areas. *Anopheles gambiae* s.l. was resistant to deltamethrin and alphacypermethrin insecticides. Thus, malaria vector interventions should be strengthened in Arjo-Didessa sugarcane irrigation scheme to reduce malaria transmission risk during wet and dry seasons. Integrated resistance management strategies should also be implemented.

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CONTRIBUTION OF *ANOPHELES FUNESTUS* IN MALARIA ENDEMIC TRANSMISSION ON THE EAST COAST OF MADAGASCAR

Alice Zilera Suzanantsoa[1], Tauni Razafimanantsoa[1], Iavonirina Randriananjantenaina[1], Irma Ivonintsoa Vavifara Zilera[2], Omega Raobela[1]

[1]National Malaria Control Program, ANTANANARIVO, Madagascar, [2]University of Antananarivo Madagascar, ANTANANARIVO, Madagascar

An. funestus group is an important malaria vector in Madagascar. This species thrives in endemic areas and caused increase malaria cases during dry season. *n*evertheless A*n. funestus* has been understudied. To assess entomological indicators of *Anopheles funestus* in malaria endemic villages, Vangaindrano district, Madagascar, from August to November 2023. So Human Landing Catch (HLC), Outdoor Resting Catch (ODC) and Indoor Resting Catch (IDC) were used to collect mosquitoes. All the anopheline collected were assessed for species identify . The entomological indicators such as density, Human Bating rating (HBR), the entomological inoculation rate (EIR), human blood index (HBI) and vectorial (Cv) was used to obtain intensity of malaria transmission.A total of 453 females were identify as *An. funestus,* it was confirmed as *An. funestus s.s*. The highest densities were observed in August (42.6%). Interestingly, 75.5% of the collected *An. funestus s.s.* were captured from HLC, with biting behavior exophagic (mean exophagic rate 62.6%). The peak biting activity was between 12:00 - 03:00 hours in outdoor and indoor during the survey. Detection sporozoites showed *P. falciparum* and *P. vivax* positif on this species. According to the mean HBR and the mean sporozoite index, EIR of *An. funestus* s.s. was 0.13 to 0.22 and 0.44 infectious bites/person/night (ib/p/N) for *P. falciparum* and *P. vivax* respectively. The longest longevity recorded for *An. funestus s.s.* was six days from August. The IDC proved that *An. funestus s.s* is exophilic (mean endophilic rate under 50%), high HBI values (0.83 to 0.50), indicating that these vectors are anthropophilic .The recorded blood meals were mainly from humans (50%). The Cv of *An. funestus s.s*. for P. *falciparum, P. vivax was* decreasing to the beginning of rainy season. The ODC showed this species has a strong preference resting on cattle dwellings. The results showed that *An. funestus s.s.* maintaining the transmission of malaria in dry season in this area, the trophic and resting behavior suggests that using long-lasting insecticidal nets alone is insufficient as a vector control strategy in this area.

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COST COMPARISON ANALYSIS OF DIFFERENT WORKFLOWS FOR ENTOMOLOGICAL SURVEILLANCE USING A DECISION-TREE APPROACH

Jonathan Karisa[1], Rist Cassidy[2], Mercy Tuwei[1], Kelly Ominde[1], Brian Bartilol[1], Zedekiah Ondieki[1], Caroline Wanjiku[1], Joseph Mwangangi[1], Charles Mbogo[3], Martin Rono[1], Philip Bejon[4], Marta Maia[1]

[1]KEMRI-Wellcome Trust Research Programme, Kilifi, Kenya, [2]Virginia-Maryland College of Veterinary Medicine at Virginia Tech 205 Duck Pond Drive Blacksburg, VA 24061, Virginia, VA, United States, [3]Pan-African Mosquito Control Association, Nairobi, Kenya, [4]University of Oxford, Centre for Global Health and Tropical Medicine, Oxford, United Kingdom

MALDI-TOF MS is increasingly applied for entomological surveillance. However, the potential for cost savings using MALDI-TOF MS in routine entomological surveillance has never been evaluated. This study compares the costs of current diagnostic methods used in entomological malaria surveillance in Kenya to the expected costs when using MALDI-TOF MS. To perform a cost comparison analysis of the conventional methods currently used for entomological malaria surveillance in Kenya to the use of MALDI-TOF MS, a decision tree analytic model to provide a systematic process for calculating the costs associated with materials, labour and direct costs, and time-to-results for the two workflows was developed. The analysis compared the costs of the current methods used in entomological malaria surveillance by the NMCP in Kenya to the expenses expected if MALDI-TOF MS was used instead, assuming a sample size of 15,000 mosquitoes and accounting for time-to-results and direct costs (materials and labour). Using MALDI-TOF MS for mosquito surveillance would result in a total direct cost savings of 83% (6 times cheaper) compared to the current workflow. It would also result into a 94.31% net time savings (17.6 times faster) compared to the current workflow. MALDI-TOF MS represents a platform that significantly reduces costs for the laboratory's sample processing, materials, and labour. Despite the initial high capital cost of the instrument, the ease of performance, the rapid turn-around time to results, and the modest cost of testing for each sample make this novel methodology a paradigm shift for entomological surveillance.

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DEVELOPMENT OF A SYSTEM TO SUPPORT COMMUNITY-BASED SURVEILLANCE OF DISEASE-TRANSMITTING MOSQUITOES IN RESOURCE-CONSTRAINED SETTINGS

Dickson Samwel Msaky, Naomi Urio, Halfan Ngowo, Emmanuel Kaindoa, Fredros Okumu

Ifakara Health Institute, Dar es salaam, Tanzania, United Republic of

The identification and tracking of disease-transmitting mosquito populations are crucial for understanding their geographical distribution, behavior,

and species composition. Furthermore, understanding the dynamics of these mosquito populations is essential for guiding vector control interventions to improve their effectiveness and identify areas that require attention. Given the high burden of mosquito-borne diseases, particularly in resource-limited settings, and the limitations of traditional surveillance methods, there is a need for an innovative solution. To address this, we have developed a system to facilitate community-based surveillance of disease-transmitting mosquitoes. The system architecture comprises three main components: the user interface layer, the data processing layer, and the feedback and reporting layer. The user interface layer includes a mobile and web application that serves as the primary point of contact for users, allowing community members to submit mosquito data. The data processing layer manages the secure transmission, storage, validation, and trust assessment of the data, ensuring its integrity and reliability. The feedback and reporting layer provides necessary feedback to community members, generates comprehensive reports based on validated data, and manages compensation, thereby fostering wider community engagement. This system allows for the timely and efficient tracking of mosquito populations, significantly improving public health response capabilities across diverse geographic settings. By streamlining data collection and making it accessible to a wider audience, the system enhances the quality of surveillance data and enables targeted public health interventions. This system supports the tracking of disease-transmitting mosquito populations while simultaneously enhancing healthcare quality over the long term by informing public health practices. .

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INTEGRATION OF VECTOR AND HUMAN BEHAVIOR IN RESIDUAL MALARIA IN RURAL COMMUNITIES IN THE PERUVIAN AMAZON

Jan E. Conn[1], Carlos Acosta[2], Joaquin Gomez[2], Diana Cubas[2], Pamela Rodriguez[2], Mitchel Guzman-Guzman[2], Marlon P. Saavedra[2], Joseph M. Vinetz[3], Dionicia Gamboa[2]

[1]Wadsworth Center, New York State Dept of Health, Albany, NY, United States, [2]Universidad Peruana Cayetano Heredia, Lima, Peru, [3]Yale School of Medicine, New Haven, CT, United States

Malaria remains a significant public health concern in Peru where 22,349 cases were reported in 2023, mainly in Loreto in the Peruvian Amazonian Region. The national malaria control plans (NMCP) rely primarily on Long-lasting insecticidal nets (LLIN) and Indoor Residual Spraying (IRS) for vector intervention. This study investigates residual malaria exposure among LLIN users in Loreto, in three riverine communities from the Mazan district: Gamitanacocha (GAM) and Libertad (LIB) in the Mazán river basin; and Urcomiraño (UM) in the Napo basin. We assessed exposure levels across dry and wet seasons to identify gaps in protection to help guide Peruvian health authorities in developing NMCP. We integrated entomological and epidemiological data to adjust for mosquito exposure by human behavioral data. Our analysis reveals inter-seasonal variation in *Ny. darlingi*, with a peak of Human Biting Rates (HBR) during the wet season predominantly outdoors, ~18:00, diminishing overnight, and resurging in the morning. Previous studies in LIB and UM showed peak activity ~21:00. Additionally, Mazan watershed communities exhibited higher HBR than Napo across all seasons. Behavior-corrected exposure indices allowed us to determine that most of the main sources for remaining exposure are indoors awake and unmitigated bites, despite LLIN usage across seasons; and higher levels of outdoor remaining exposure in the wet season especially during early evening hours. We examined the malaria diagnosis associated with various potential risk factors, including socio-demographic characteristics and human behavior (age, gender, primary job), entomological indices and indoor/outdoor exposure during the early evening. Applying logistic regression with mixed effects showed that there is slight statistical evidence (p=0.123) for individuals who work outside to have a greater chance of contracting malaria. We suggest enhancing indoor protection measures for effective malaria control, particularly during early evening hours. Additionally, extra precautions should be taken with individuals outdoors in the early morning, particularly during the wet season.

6192

EFFECT OF *MICROSPORIDIA MB* INFECTION ON THE DEVELOPMENT AND FITNESS OF *ANOPHELES ARABIENSIS* UNDER DIFFERENT DIET REGIMES

Godfred Yaw Boanyah[1], Lizette K. Koekemoer[2], Tullu Bukhari[3], Jeremy Herren[3]

[1]International Centre of Insect Physiology and Ecology, Kenya, Mbita, Kenya, [2]Wits Research Institute of Malaria, University of Witwatersrand, Johannesburg, South Africa, [3]International Centre of Insect Physiology and Ecology, Kenya, Nairobi, Kenya

The symbiont *Microsporidia MB (MB)* found in *Anopheles arabiensis* tested in Kenya has shown malaria blocking potency against the transmission of the *Plasmodium* parasite. *MB* density is high in mosquito gonads, which is linked to horizontal (sexual) and vertically (transovarial) transmission from one mosquito to another. We have investigated how environmental factors such as diet affect the *MB An. arabiensis* symbiosis phenotype. F1 larvae of G_0 females confirmed to be *An. arabiensis* and infected with *MB* were either combined (Isogroup lines (IGLs)) or reared separately (Isofemale lines (IMLs)) depending on the experiments. Four diet regimes, Tetramin 0.07, Tetramin 0.3, Gocat 0.3 and Cerelac 0.3 mg/larva were tested on F1 IGLs for larva diet. IGLs reared on Tetramin 0.3 mg/larva were fed on either 1% or 6% glucose diet to determine adult survival. Larva of IMLs were fed on Tetramin 0.07mg and Tetramin 0.3mg for larva experiment. The adult experiment on IMLs were reared on 1% and 6% respectively. We found that amongst the four larval diet regimes tested on *An. arabiensis* development in the presence of *MB,* Tetramin 0.3 mg/larva gave the fastest larva development, highest adult emergence, largest body size mosquitoes, greatest prevalence, and density of *MB*. Also, adult *MB* mosquitoes fed on 6% glucose survived longer than negatives whilst with 1% glucose diet there was no significant difference between *MB*+ & -. However, development time and wing size, and the survival of adult were not significantly different between *MB* infected and uninfected *An. arabiensis* under the Tetramin 0.07 and 1% glucose diet respectively, suggesting that the *MB* conferred fitness advantage was diet dependent. *Microsporidia MB* does not adversely impact the development of *An. arabiensis*, even under limited dietary conditions. Optimal larval and adult diet regimes have been determined for mass rearing of *An. arabiensis* mosquitoes infected with *MB* for future trial releases for Malaria control and elimination. Knowledge on the effect of diet is important for understanding *MB* spread in *An. arabiensis* in the field.

6193

CLIMATE-SENSITIVE VECTOR-BORNE DISEASES: INTEGRATION OF TEMPERATURE, PRECIPITATION AND RELATIVE HUMIDITY IN A DYNAMIC PROCESS-BASED MODELLING APPROACH FOR IMPROVED SURVEILLANCE AND OUTBREAK PREPAREDNESS

Oliver Mbaoma

University of Bayreuth, Bayreuth, Germany

The global burden of climate-sensitive vector-borne diseases to public health system has increased significantly. Changing climatic conditions tend to support the environmental suitability for vector population establishment, pathogen replication and transmission at known or previously unknown locations. Public health agencies rely on surveillance and early warning systems for information on spatial and temporal patterns of vector population and pathogen transmission. Accurate and timely information are important for effective interventions and control programs. Laboratory experiments has shown that vector population density and pathogen transmission suitability are driven by temperature. However, in the natural environment, complex interactions exist where several factors play significant roles in the overall processes. We developed a series of dynamic process-based models that represent a simplified replication of the complex interactions that exists in nature. The models were forced with precipitation and relative humidity in addition to frequently used temperature variables. They can serve as the backend of an early warning system. The

epidemiological model simulates *Aedes aegypti* population, dengue and chikungunya outbreaks in different spatial and temporal scales. We applied the model to different geographical locations (Mexico, Germany, Kenya). In addition, a population model was developed to simulate population of *Culex torretium* mosquito population in Germany, Sweden and UK. Results were validated with vector occurrences and cases of outbreaks with high accuracy levels. Our research was able to demonstrate that a model forced with temperature, precipitation and relative humidity can replicate vector population and pathogen transmission in several geographical location with accurate seasonal variation better than classical models forced with temperature alone. We conclude that dynamic process-based models which can be automated, scaled and transferred between geographical areas serve as important tools for early warning systems of climate-sensitive vector-borne diseases.

6194

MODELLING WOLBACHIA REPLACEMENT FOR DENGUE CONTROL: A SCOPING REVIEW

Katie Tiley, Laith Yakob, Oliver Brady
LSHTM, London, United Kingdom

Aedes aegypti mosquitoes, the primary vector for dengue, Chikungunya, and Zika virus, have been shown to have reduced vector competence when deliberately infected by strains of the bacterium *Wolbachia*[1]. Utilising this novel technology for dengue control, the release of *Wolbachia*-infected *Ae. aegypti* mosquitoes into wild populations, where they naturally proliferate, is known as *Wolbachia* replacement. *Wolbachia* replacement has the potential to be an effective tool for dengue control, demonstrated by a successful randomised controlled field trial in 2021 which reduced dengue incidence by 77% in intervention areas[2]. While a 2018 review of *Wolbachia* replacement considered takeaways from modelling[3], computational literature which explores this technology is rapidly growing, thus, the need for a review specifically addressing modelling of *Wolbachia* replacement is pressing. This scoping review will examine novel findings in the field of modelling *Wolbachia* replacement since 2018, as well as evaluating how recent developments could be incorporated into and improve future models.

6195

FIGHTING MALARIA WITH THE MOSQUITO SYMBIONT BACTERIA SECRETED BIOACTIVE CELL-FREE SUPERNATANT

Jacques E. Gnambani, Etienne M. Bilgo, François De Sales D. Hien, Djènebou Millogo, Abdoulaye Diabate
IRSS/DRO, Bobo-Dioulasso, Burkina Faso

Malaria control is critical in reducing the disease burden caused by mosquitoes, and insecticides are an effective tool to control vector. Resistance to common insecticides is now widespread, and novel classes of insecticides or tools are needed. In previous work, we described the mosquitocidal activities of *Chromobacterium anophelis sp.nov.*, a bacterium found in association with wild mosquitoes. In this current work, we further explored the effects of bacterium cell free supernatant on *An. cuoluzzii* mosquito fitness, mosquito physiology and *P. falciparum* infection development. We found that cell free supernatant from *C. anophelis sp.nov.,* has mosquitocidal activity against a broad range of malaria mosquito. Mosquitocidal activity of *C. anophelis sp.nov*., was retained after removal of live cells from M9 medium, suggesting the bacteria secrete mosquitocidal compound(s) into the M9. 100% of mosquito fed to chromobacterium cell-free supernatant (80-100 %) die less than 5 days and 10 days post exposition respectively in lab and semi field condition. Mosquito exposure to *C. anophelis sp.nov*., cell free supernatant reduces significantly its susceptibility to *Plasmodium falciparum* infection, thereby compromising the mosquito's vector competence. Parasite inhibition rate was 72.06% when the mosquitoes took solution (20 %) through a cotton ball before taking *P. falciparum*-infected blood, and 40.24% when solution was mixed with gametocytes. Our findings suggest that *C. anophelis sp.nov.,* cell free supernatant has factor(s) with strong effects on mosquito longevity, fitness and decrease significantly *P. falciparum* infection, which may be of interest for mosquitocidal, anti-Plasmodial tools development and is promissing for malaria elimination.

6196

ASSESSMENT OF THE EFFICACY OF FLUDORA FUSION ON SPRAYED SURFACES IN THE GAMBIA

Aminata Bah
Ministry of Health, Banjul, Gambia

The use of insecticide for indoor residual spraying has significantly reduced malaria cases in The Gambia. However, this efficacy is jeopardized by a variety of factors such as mode of application method, insecticide type, and spray surface nature. In this study, we examined how different wall surfaces found in Gambia affect insecticide efficacy. The efficacy of the spraying is evaluated after a month. Different sprayed walls were randomly chosen from Gambisara village in the Upper River Region. Trained regional Vector control officers and field biologists collected mosquito larvae from various natural breeding sites. The collected larvae and pupae were separated. The anopheles pupae are reared into adults, Female anopheles mosquitos aged 2-5 days were exposed to sprayed walls for 30 minutes and the mortality rate is monitored after 24 hours. A total of 2160 female anopheles mosquitoes were exposed to different levels on sprayed walls. Dead after 24 hours exposure were, 331 (92%) mud wall, 324 (90%) mud wall plastered, 321 (89%) mud wall plastered and painted, 320 (89%) cement wall, 290 (81%) cement wall plastered 275 (76%) cement wall plastered and painted. Overall 1861 dead mosquitoes were recorded which result to 86% mortality rate. While in the control, 120 mosquitoes were exposed, 20 mosquitoes for each wall type. At the end of the test, 10 dead mosquitoes were recorded in the control representing 3% mortality rate. This short survey indicated that insecticide efficacy last longer on mud walls compared to other types. However, since factors like mode of spraying, volume of water and sprayed surfaces affect insecticide efficacy on sprayed wall make it difficult to conclude. All the walls tested were sprayed by different people who may have different mode of application speed, make it difficult to draw line on these findings

6197

SPATIOTEMPORAL CO-DISTRIBUTION AND TIME LAGGED CROSS CORRELATION OF MALARIA AND DENGUE IN LORETO, PERU

Paloma M. Carcamo[1], Gabriel Carrasco-Escobar[1], Samantha R. Kaplan[2], Jesus M. Quispe[1], Gordon C. McCord[3], Tarik Benmarhnia[3]
[1]Universidad Peruana Cayetano Heredia, Lima, Peru, [2]University of Colorado, Aurora, CO, United States, [3]University of California San Diego, La Jolla, CA, United States

Malaria and dengue account for most vector-borne disease-related cases and deaths worldwide, disproportionately affecting tropical regions such as Peru. Previously identified social, environmental, and climate determinants for both diseases are similar despite differences in vector ecologies. Control strategies for both rely on interventions such as removal of breeding sites or insecticide-based strategies, which could be integrated. We assessed synchrony (temporal correlations, temporal order, lagged relationships) and spatial correlations between malaria and dengue in the Loreto region of Peru. We conducted a time-lagged cross-correlation (TLCC) analysis between district-level dengue and malaria time series in Loreto between 2000-2021. We identified temporal patterns of dengue that could precede malaria patterns or vice versa. We categorized districts based on dengue/malaria spatio-temporal patterns and conducted Moran's tests for spatial autocorrelation of maximum TLCC coefficients and optimal lag times. The number of districts reporting both diseases has increased. Maximum TLCC coefficients varied in magnitude and direction between districts, as did corresponding lag times. In the Northwest, increases in malaria often preceded increases in dengue, while in the Northeast increases in malaria preceded decreases in dengue cases. We found spatial correlation

between coefficients in some regions in the Northwest, suggesting that characteristics of a geographic area may influence the observed associations. The identification of districts with strong associations between dengue and malaria incidence can inform implementation of targeted integrated interventions, while identification of distinct patterns of association can inform future studies assessing drivers of both diseases in different settings.

6198

DIET AFFECTS THE LONGEVITY AND THE RESPONSE TO INSECTICIDE OF *ANOPHELES GAMBIAE*

Khadidiatou Cissé[1], Jacob Koella[2]

[1]Centre Suisse de Recherche Scientifique en Cote d'Ivoire, Abidjan/Cocody, Côte D'Ivoire, [2]University of Neuchatel, Neuchatel, Switzerland

The longevity of mosquitoes is a key parameter underlying the epidemiology of malaria, and it is the main target of insecticides in malaria control. The evolution of resistance is therefore threatening the success of control. While several genes underlie a mosquito's longevity and resistance, it is often overlooked that both can be influenced by the mosquito's environment, and in particular its diet, i.e. the combination of sugar obtained from plants and the blood obtained from its hosts. Within this context we aimed to determine with a series of experiments (i) how nectar influences a mosquito's longevity, (ii) how the type of sugar obtained in nectar affects a mosquito's response to insecticide, and (iii) how sugar and blood meal interact to influence resistance. First, we fed mosquitoes for five days with four types of sugar diluted to either 1.97 or 19.7 kcal per 100 ml of water and then measured their resistance with a WHO bioassay test. While the mosquitoes fed on the lower concentration were 2 times more likely to die within 24 hours of being exposed to the insecticide than those fed on the higher concentration, the type of sugar did not influence mortality. Second, we let mosquitoes feed throughout their lives on one of five species of local plants - *Thevetia nerifolia*, *Mandalium coromandelianum*, *Ixora coccinea*, *Tabernanthe iboga* or *Carica papaya* - and linked their longevity to the types and concentrations of sugar in the plants' nectar. The mean longevity ranged from 9 days with *C. papaya* to 22 days with *T. nerifolia*, but was not linked to the concentration of any of the sugars in the nectar. Third, we fed mosquitoes on one of three plant species for four days, then gave half of them a blood meal and measured the resistance of all of the mosquitoes. The blood-fed mosquitoes were less likely to be killed by the insecticide than those that had fed only on plants - Plant species not influence their response to insecticide. Our studies reveal that other compounds of nectar have an important impact on mosquito - there is a close link between blood meal and the detoxification of insecticide. These concepts will lead to improve attractive toxic sugar baits.

6199

ABUNDANCE & CHARACTERIZATION OF MALARIA VECTORS IN SAKASSOU, CENTRAL IVORY COAST

Louise Golou Bellai[1], Constant A. Edi[2], Amanda Ross[1], Benjamin G. Koudou[2], Jürg Utzinger[1], Pie Müller[1]

[1]Swiss Tropical and Public Health Institute, Basel, Switzerland, [2]Centre suisse de recherches scientifiques en Côte d'Ivoire, Abidjan, Côte D'Ivoire

In preparation of an indoor residual spraying programme to control malaria in Sakassou, Central Côte d'Ivoire, we assessed whether insecticide-treated nets and indoor residual spraying are potentially suitable vector control strategies. From November 2018 to July 2020, we collected mosquitoes using human landing catches, pyrethrum spray catches and CDC light traps. We identified all mosquitoes morphologically and further determined members of the *Anopheles gambiae* species complex using molecular PCR diagnostics. In addition, we estimated sporozoite rates using enzyme-linked immunosorbent assays. We collected 98,314 mosquitoes with higher numbers in months with increased rainfall*. Anopheles coluzzii* was the most prevalent species (90%), showing a *Plasmodium* infection rate of 0.017 and biting throughout the night with a peak around 1.00 am. In the houses selected for human landing catches, an individual was exposed to 516 infective bites per year. As *An. coluzzii* is resting indoors and is the main vector biting indoors at night, both insecticide-treated nets and indoor residual spraying are potentially effective malaria control interventions in Sakassou. However, as biting was also observed earlier in the night and outdoors, additional interventions should be considered.

6200

OPTIMIZING AND VALIDATING THE HOST-FREE TUNNEL TEST: A MORE AFFORDABLE, PRACTICAL, AND ETHICAL TOOL FOR THE EVALUATION OF INSECTICIDE-TREATED NETS

Kath Gleave, Rosemary Lees, Jessica Williams

Liverpool School of Tropical Medicine, Liverpool, United Kingdom

The tunnel test is recommended by the World Health Organization (WHO) to investigate the biological activity of an insecticide-treated net (ITN) surface by observing relevant effects on mosquitoes subjected to exposure that is more representative of interaction while host seeking. Data generated using the tunnel test is routinely used by ITN manufacturers as part of the dossier generation for applications for listings by the WHO Prequalification (PQ) team to demonstrate efficacy and/or quality, and by implementers to monitor chemical durability and ongoing efficacy post-deployment. The tunnel test involves a series of chambers where mosquitoes must pass through a holed ITN sample to reach a live animal bait to blood feed. It is necessary to have a bait in the test to provide cues that stimulate mosquitoes to be attracted towards, and through, the holed ITN sample. Mosquitoes are scored for their passage through the net as well as blood feeding and mortality. Currently, the WHO tunnel test is the best available method for testing the bioefficacy of chlorfenapyr nets at the laboratory scale. However, the reliance on live animal hosts raises ethical concerns and logistical challenges if suitable hosts aren't available. To address this iiDiagnostics, with support from IVCC and Syngenta, developed an alternative method that eliminates the need for live hosts. Preliminary data shows that there is evidence that this method is appropriate with mosquito feeding rate over 50% in control arms and mortality above 80% for treatment arms. Innovation to Impact (I2) are utilising a method validation framework to establish an optimised, validated, and accessible alternative to the standard tunnel test. It is imperative that a robust and validated bioassay is available for conducting durability monitoring for those dual-AI ITNs already on the market and those in development. Following engagement with the WHO PQ/VCT team our aim is for a version of the final Standard Operating Procedure (SOP) for the host-free tunnel test to be incorporated as an Implementation Document supporting the WHO Guideline for Prequalification Assessment of ITNs.

6201

RESIDUAL EFFICACY OF WALL CONTACT BIOASSAYS AND FUMIGANTS EFFECTS INDUCED BY ACTELLIC®300CS AND FLUDORA®FUSION WP-SB 56.25 INSECTICIDES USED FOR INDOOR RESIDUAL SPRAYING AGAINST SUSCEPTIBLE *ANOPHELES GAMBIAE* S.S.

Beatus Cyubahiro

Rwanda Biomedical Centre - Malaria and Other Parasitic Diseases Division, Kigali, Rwanda

From 2020 to 2022, the study area of Nyagatare District located in Eastern Province of Rwanda rotated two insecticides for Indoor residual spraying (IRS), Fludora® Fusion WP-SB 56.25 and Actellic® 300CS**.** This assessed the residual efficacy of wall contact and non-contact (fumigant effect) bioassays induced by the two new insecticides sprayed during the IRS. The residual efficacy tests used WHO standard protocol and was performed in two sites. The surveys were conducted September 2020 to July 2021 for Fludora® Fusion WP-SB 56.25 and August 2021 to June 2022 for Actellic®CS300. Six houses per month were tested for fumigant effect while the direct wall contact bioassays were carried out in 12 houses. At 10-months, Fludora® Fusion WP-SB 56.25 provided a residual efficacy of five months for direct mortality (24h) and 6 months to 10 months for

the delayed mortality (96 hours) while Actellic® 300CS provided a residual efficacy for more than 10 months period with mortality only counted at 24 hours. The fumigant effect was four months and five months of delayed mosquito mortality for Fludora® Fusion WP-SB 56.25 and Actellic® 300CS respectively. This study showed the efficacy of 10 months for both insecticides using wall cone bioassay tests and complemented by a fumigant effect of four months for Fludora® Fusion WP-SB 56.25 and five months for Actellic® 300CS. These results show that the application of one IRS round per year is sufficient to ensure adequate annual prevention against malaria (month to month) in Rwanda. More research is needed to evaluate the effects of fumigant effects on mosquito control for reductions in malaria cases and anything about sustaining this intervention.

6202

IMPACT OF FOCAL MALARIA CONTROL USING TARGETED INDOOR RESIDUAL SPRAYING (IRS), 4 YEARS RESULTS FROM RUSIZI DISTRICT, WESTERN PROVINCE OF RWANDA

Dunia Munyakanage[1], Beatus Cyubahiro[1], Elias Niyituma[1], Xavier Misago[1], Jules Nahimana[2], Phocas Mazimpaka[1], Michee Kabera[1], Naomi W. Lucchi[3], Kaendi Munguti[4], Aimable Mbituyumuremyi[1], Emmanuel Hakizimana[1]

[1]Rwanda Biomedical Centre, Kigali, Rwanda, [2]PMI EVOLVE Project, Abt Associates, Inc., Kigali, Rwanda, [3]The Centers for Disease Control and Prevention, Division of Global Health Protection, Nairobi, Kenya, [4]US President's Malaria Initiative, USAID, Kigali, Rwanda

After facing its highest malaria incidence rate of 409 per 1000 population in 2016, Rwanda developed a malaria contingency plan (MCP) that focused on high malaria burden districts. In Rusizi, a district with heterogeneous malaria transmission, the MCP recommended focal indoor residual spraying (IRS), targeting 9 out of 18 sectors within the district. The goal of focal IRS is to cover epidemiological hotspots in geographic areas that experience regular seasonal increases in confirmed malaria cases with high transmission activity in comparison to surrounding areas. This assessment evaluated the impact of IRS focal spraying in Rusizi district comparing 2019 and 2023, after four years (2020-2023) with focal IRS. Monthly uncomplicated and severe malaria cases and deaths were retrospectively extracted from the Health Management Information System (HMIS) for 2019-2023. Monthly entomological data were collected using Human Landing Catches from the same period in three villages of Mashesha entomological sentinel site in Rusizi. The sporozoite rate (SR) derived from *Plasmodium* sporozoite positive ELISAs by the total vector mosquitoes tested. The entomological inoculation rate (EIR) was calculated as the product of the human biting rate (HBR) and the SR.A substantial decrease of 95.7% ($W = 305$, $p < 0.001$), in uncomplicated cases was observed, from 256,271 in 2019 to 10,918 in 2023. Severe malaria cases and deaths were reduced by 92.3% (from 689 to 53 cases), and 100% (from 15 to 0 cases) for the same period. An 86% reduction of HBR for *Anopheles gambiae s.l,* the main malaria vector, (from 69.95 to 9.63 bites/person/night) for the same period. The EIR decreased by 93%, from 97.93 to 6.74 infectious bites per person per year. These findings from Rusizi District demonstrate the sustained success of a focal IRS intervention for controlling hot spots of malaria transmission, ultimately advancing progress towards sustainable elimination objectives while optimizing resource allocation for IRS.

6203

BUILDING AN EVIDENCE BASE TO SUPPORT INSECTICIDE-TREATED NET DISTRIBUTION IN TWO HIGH-BURDEN TO HIGH-IMPACT COUNTRIES

Angharad Steele[1], Nnamdi Dum-Buo[2], Jackline Martin[3], Erica Vigano[1], Alphaxard Manjurano[3], Siân Clarke[4], Jo Lines[4], Katherine Theiss-Nyland[1]

[1]Malaria Consortium, London, United Kingdom, [2]The Centre for Research in Infectious Diseases, Yaoundé, Cameroon, [3]National Institute for Medical Research (NIMR), Mwanza, Tanzania, United Republic of, [4]London School of Hygiene and Tropical Medicine (LSHTM), London, United Kingdom

Insecticide-treated nets (ITNs) are a key tool for preventing malaria. The World Health Organization (WHO) recommends delivering ITNs through mass campaigns and continuous distribution channels, such as antenatal clinics and the expanded programme on immunisation. These mechanisms, and how they are operationalised vary between countries and at present the information that underpins decision-making at different administrative levels for distribution is not well documented. Building an evidence base of the factors that influence decisions on ITN distribution is critical to optimise this intervention. This qualitative study investigates planning and delivery experiences with ITN distribution at national and subnational levels in two high-burden countries - Cameroon and Tanzania. Participants are selected through purposive sampling, in consultation with the National Malaria Control Programme (NMCP). At national level, we conduct semi-structured interviews with the NMCP, other relevant government departments and external partners. Focus group discussions are also carried out at two sub-national sites and four delivery-points in each country with operational-level NMP staff, community focal points and individuals overseeing net distribution. Topic guides are informed by the WHO Health Systems Building Blocks. Thematic data analysis is used to understand the planning and delivery experiences of individuals involved in ITN distribution at different administrative levels and across countries. We present our results under the key themes of leadership and governance, financing, access to essential commodities, service delivery, health workforce, and information, learning and accountability. Findings across administrative levels allow for comparison of influencing factors within countries as well as across different national contexts. Our results highlight key commonalities, differences, barriers, and enablers associated with ITN distribution mechanisms used in these contexts.

6204

COMBINING HOUSE-SCREENING AND ODOUR BAITED MOSQUITO TRAPS FOR SUSTAINABLE CONTROL OF MALARIA TRANSMISSION IN LOW INCOME COMMUNITIES DOMINATED BY *ANOPHELES FUNESTUS*

Doreen Josen Siria[1], Halfan Said Ngowo[1], Anold Mmbando[1], Emmanuel Kaindoa[1], Frances Hawkes[2], Heather Ferguson[3], Fredros Okumu[1]

[1]Ifakara Health Institute, Morogoro, Tanzania, United Republic of, [2]University or Greenwich, Greenwich, Greenland, [3]University of Glasgow, United Kingdom, United Kingdom

Tanzania is making tremendous progress against malaria by scaling up long-lasting insecticide-treated nets (LLINs), indoor residual spraying (IRS), and effective treatments. There's a greater need for new complimentary tools to monitor the persistent malaria transmission. Two simple interventions that fit this target profile are house screening and odor-baited mosquito traps which have not previously been tested jointly. This current study, therefore, investigated the impact of combining house-screening and odor-baited traps in reducing malaria vector density by; a)identifying an effective trap for *Anopheles arabiensis*, b)measuring the impact of house-screening with and without an odor-baited trap, c)testing the personal/household and community level of protection of users (either or both interventions) and non-users(control), and d)conducting a small-scale field experiment on testing the combination of house-screening (eaves and windows) and outdoor baited traps in rural setting in Tanzania. This

study aimed to assess the impact of house-screening and odour-baited traps on reducing mosquito vector density and biting risks. Semi-field and field experiments were conducted in rural Tanzania, focusing on *Anopheles arabiensis* mosquitoes, a dominant malaria vector. In semi-field experiments, the Suna trap exhibited higher mosquito recapture rates than the BGM trap in both non-competitive and competitive evaluations of odor-baited traps revealing it as an effective outdoor trap for mosquito collection. House screening, whether alone or combined with traps, demonstrated substantial reductions in indoor biting risk, offering protection efficacies of 80% and 93%, respectively. Also, in the field experiments, a total of 38,281 mosquitoes were collected both by CDC light trap and Suna trap. House screening whether alone or combined with traps provided more than 80% protection indoors whereas there was a clear diversion and recapture of mosquitoes from the odor-baited Suna traps outdoors. It is evident that house screening can potentially reduce indoor mosquito-biting risk.

6205

THE EFFECT OF REPEATED WASHING OF THE ROYAL GUARD, INTERCEPTOR G1 AND G2 NETS ON BLOOD FEEDING BEHAVIOR AND SURVIVAL OF *ANOPHELES* MOSQUITOES

Judith Sinkanako Banda[1], Owen Ndalama[1], Shupikayi Kambiring'oma[1], Raphael Linno[2], Hilary Ranson[3], Themba Mzilahowa[1], Elizabeth Bandason[4]

[1]Kamuzu University of Health sciences, Blantyre, Malawi, [2]Lilongwe University of Agriculture and Natural resources, Lilongwe, Malawi, [3]London school of tropical medicine, London, United Kingdom, [4]Kamuzu University of Health Sciences, Blantyre, Malawi

Long-lasting insecticidal nets offer longer time of protection because they are wash resistant. Whether the new LLINs (Interceptor IG2 and Royal guard) are wash resistant compared to the conventional nets are unknown. This study assessed the wash resistance of two nets, Interceptor G2 (IG2) and Royal guard (RG) compared to the mono-treated version of InterceptorG1 and a negative control (untreated net). Blood feeding behavior and mortality were measured against Anopheles mosquitoes. WHO cone bioassays were conducted using 2-3 days *An. gambiae* Kisumu and *An.gambiae s.l*. A total of 1,400 mosquitoes were exposed to untreated net and 0-20th washed IG1,IG2 and RG nets for 3minutes. Each test had 10 replicates and 50 mosquitoes per replicate. Knockdown was observed from 5 minutes to 60 minutes after which mosquitoes were provided with blood to check the feeding behavior after exposure. Final mortality was observed from 24hrsto 72 hours for each net. High mortality (>80%) of mosquitoes and high blood feeding inhibition (100%) was observed across the washes for all the three treated nets and test mosquitoes. Time to knock down increased with number of washes for IG1. There was a significant difference in knock down mortality for *An. gambiae s.l* between untreated vs RG (95% CI -98.26 to -92.54, p=<0.001). The difference was not significant in IG1 and IG2 (95% CI -5.737 to 2.137, p=0.6317). No difference was observed in blood feeding inhibition and mortality due to repeated washing of the two new nets, Interceptor IG2 and Royal guard nets which shows that the distribution of these nets in Malawi may have a positive impact in reducing malaria vectors.

6206

DISTRIBUTION OF ANOPHELES VECTORS AND THEIR ROLE IN MALARIA TRANSMISSION ACROSS HIGH MALARIA BURDEN AREAS IN MALAWI INCLUDING CHIKWAWA, KARONGA AND NKHATA BAY DISTRICTS

Nellie Chikondi Kaunde[1], Leonard Dandalo[1], Yemane Yihdego[2], Abdoulaye Bangoura[1], Miriam Williams[2], Austin Gumbo[3], Lumbani Munthali[3], Liliah Gerberg[4], Pius Masache[5], Themba Mzilahowa[6]

[1]U.S President's Malaria Initiative, Malawi Evolve project, Abt Associates, Lilongwe, Malawi, [2]U.S President's Malaria Initiative, Evolve Project, Abt Associates, Rockville, MD, United States, [3]National Malaria Control Program, Lilongwe, Malawi, [4]U.S President's Malaria Initiative, USAID, Washington DC, CO, United States, [5]U.S President's Malaria Initiative, USAID, Lilongwe, Malawi, [6]Malaria Alert Centre, Kamuzu University of Health Sciences, Blantyre, Malawi

A comprehensive understanding of vector distribution and malaria transmission dynamics at a local scale is essential for implementing and evaluating effectiveness of vector control strategies. In Malawi, *Anopheles funestus* s.l. and *An. gambiae* s.l. are the main malaria vectors. To assess the intersection between vector distribution and malaria transmission dynamics, we collected vector bionomics and malaria case data from July 2022 to June 2023 in three high malaria burden districts: Chikwawa, Karonga and Nkhata Bay. Monthly malaria case data were obtained from the National Malaria Control Program database from health facilities affiliated with the entomological sentinel sites. Monthly mosquito collections were carried out using pyrethrum spray catches (PSCs) and Center for Disease Control light traps (CDC-LTs). Sporozoite rate (SR) and entomological inoculation rates (EIR) were calculated from mosquitoes collected using CDC-LTs. The total number of *An. funestus* s.l. and An. gambiae s.l. mosquitoes collected were 1,745 and 8,979, respectively. *An. funestus* s.l. was predominant in Nkhata Bay and the densities were high throughout the year with a peak in May. An. gambiae s.l. were more abundant in Karonga and densities were highest between August and September. In Chikwawa both Anopheles species were almost evenly distributed. The SRs were higher for *An. funestus* s.l. ranging from 1.4 to 8.3% than for An. gambiae s.l. with a range from 0 to 0.8%, among the three areas. The EIR of *An. funestus* s.l. was higher in Nkhata Bay [26.0 infective bites/person/month (ib/p/m)], followed by Chikwawa (7.0 ib/p/m). *An. gambiae* s.l. infective bite rate was highest in Karonga (6.5 ib/p/m. Malaria cases were high in all three districts during and immediately after the rainy season from January 2023 to June 2023 ranging from 160 to 370 malaria cases per 1,000 population per month with highest cases reported from Nkhata Bay. EIR and malaria burden were highest in Nkhata Bay where *An. funestus* s.l. is dominant. Species composition, spatial and temporal distribution of vectors should be considered when planning targeted vector control interventions.

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EFFECTS OF SAMPLE PRESERVATION METHODS AND DURATION OF STORAGE ON THE PERFORMANCE OF MID-INFRARED SPECTROSCOPY FOR PREDICTING THE AGE OF MALARIA VECTORS

Jacqueline Nicholaus Mgaya[1], Doreen J. Siria[1], Faraja E. Makala[1], Joseph P. Mgando[1], John-Mary Vianney[2], Emmanuel Peter Mwanga[1], Fredros O. Okumu[3]

[1]Ifakara Health Institute, Morogoro, Tanzania, United Republic of, [2]The Nelson Mandela African Institution of Science and Technology, Arusha, Tanzania, United Republic of, [3]University of Glasgow, Glasgow, Scotland, United Kingdom

Monitoring the biological attributes of mosquitoes is critical for understanding pathogen transmission and estimating the impacts of vector control interventions on the survival of vector species. Infrared spectroscopy and machine learning techniques are increasingly being tested for this purpose and have been proven to accurately predict the age, species, blood-meal sources, and pathogen infections in *Anopheles* and *Aedes* mosquitoes. However, as these techniques are still in early-stage implementation, there are no standardized procedures for handling samples prior to the infrared scanning. This study investigated the effects of different preservation methods and storage duration on the performance of mid-infrared spectroscopy for age-grading female *Anopheles arabiensis.* Laboratory-reared *An. arabiensis* (N=3,681) were collected at 5 and 17 days post-emergence, killed with ethanol, and preserved using either silica desiccant at 5°C, freezing at - 20°C, or absolute ethanol at room temperature. For each preservation method, the mosquitoes were divided into three groups and stored for 1, 4 or 8 weeks, then scanned using a mid-infrared spectrometer. The best performing classifier for age-grading mosquitoes was the support vector machine (SVM). The classification of mosquito ages (as 5 or 17-day-olds) was most accurate when the samples used to train the SVM model (training samples) and samples being tested (test samples) were preserved the same way or stored for equal durations.

However, when the test and training samples were handled differently, the classification accuracies declined significantly. When using mid-infrared spectroscopy and supervised machine learning to age-grade mosquitoes, the highest accuracies were achieved when the training and test samples are preserved in the same way and stored for similar durations. This underscores the critical need for standardized sample-handling protocols for infrared-based entomological studies. These protocols not only enhance accuracy but also holds significant implications for advancing malaria vector control strategies.

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EXPLORING THE INFLUENCE OF MOSQUITO FEEDING BEHAVIOR AND EXISTING VECTOR CONTROL INTERVENTIONS ON THE IMPACT OF ENDECTOCIDES FOR MALARIA CONTROL

Nilani Chandradeva[1], Joseph D. Challenger[1], Ellie Sherrard-Smith[1], Hannah C. Slater[2], Thomas S. Churcher[1]

[1]Imperial College London, London, United Kingdom, [2]PATH, Seattle, WA, United States

Endectocides are mosquito-killing drugs; upon taking a bloodmeal on a treated host, the mosquito ingests the drug and is killed. If approved for malaria control, endectocides will be distributed in areas with different mosquito species, feeding behaviours and on top of existing vector control interventions, predominantly LLINs. The aim of this study focuses on using a malaria transmission model to predict the impact of an ivermectin-like endectocide (3x300 µg/kg, 80% coverage) in settings differing in LLIN usage, mosquito species, pyrethroid resistance and LLIN age. In one model version, endectocide uptake was determined by the LLIN-mediated human biting rate and endectocide coverage, whereas in another, uptake was independent of the impact of LLINs. Model-specific intervention dynamics and efficacy were explored by comparing prevalence in under 5-year-olds and the annual entomological inoculation rate (EIR), across a range of entomological contexts, between settings with LLINs or LLINs and endectocide. Model-derived trends in mosquito life expectancy and time between human bloodmeals, across LLIN usage and resistance profiles, were explored. Both models predicted a reduction in EIR and slide prevalence when endectocides were added to settings with historic LLIN usage, compared to when only LLINs were used, across a range of insecticide resistance profiles. Both models predicted similar dynamics and reductions in the EIR and slide prevalence in under 5-year-olds, indicating a marginal impact of LLINs on endectocide uptake. Endectocides in humans were predicted least impactful in settings with *Anopheles stephensi*-like zoophilic vectors. Across a range of settings, the differences in the average time between human bloodmeals were relatively small compared to the differences in the average mosquito life expectancy. This work suggests that as the LLIN-induced delays in human bloodmeals are relatively small compared to respective reductions in mosquito life expectancy (as assumed by the model) across these LLIN usage and entomological settings, LLINs have a negligible impact on endectocide uptake.

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EVALUATION OF THE CHAIN OF CUSTODY OF THE RESIDUAL INSECTICIDE USED IN MALARIA VECTOR CONTROL IN THE AMAZON REGION OF BRAZIL

Marcela Lima Dourado[1], Marcia Caldas de Castro[2], Pablo Secato Fontoura[3], Daniele Pereira de Castro[4], Jose Bento Pereira Lima[4]

[1]Brazilian Ministry of Health, Brasília, Brazil, [2]Harvard University, Boston, Boston, MA, United States, [3]Ministry of Health, Brazil, Brasília, Brazil, [4]Instituto Oswaldo Cruz - Fundação Oswaldo Cruz, Rio de Janeiro, Brazil

In Brazil, malaria remains a significant public health issue, particularly in the Amazon region where 99% of malaria cases are concentrated, primarily affecting the most vulnerable populations. The primary method for controlling the main malaria vector, the *Anopheles* darlingi mosquito, continues to be the use of various residual chemical insecticides. However, there is no complete overview of the chain of custody of these products in Brazil. The objective of this research is to evaluate the cost-effectiveness of the insecticide supply chain utilized for malaria control in three municipalities with a high disease incidence in the Brazilian Amazon region from 2017 to 2023. The study involved a statistical analysis that included proportions, frequencies, and Pearson correlations. Data on costs from the National Program for the Prevention and Control of Malaria (PNCM) over the past six years, details on the supply chain process, and epidemiological information from São Gabriel da Cachoeira, Tefé, and Barcelos in Amazonas state were examined. Preliminary results indicate that the total expenditure on national purchases of Vectron and Lankron insecticides over the last six years amounted to US$ 6,690,358.16. Comparing the period from 2017 to 2023, there was a 39% decrease in purchases of these products by the municipalities under review. However, these inputs still represent a considerable cost for the PNCM. During the same timeframe, there were variation in reported malaria incidence, with a 6% increase in São Gabriel da Cachoeira, 43% reduction in Tefé and 24% decrease in Barcelos. Although there was a decline in the distribution of insecticides in the municipalities, the expenses still total US$ 514,430.33. The analyses indicate a variation in the quantity of insecticides procured alongside variation in the incidence of malaria cases in the studied areas. This study aims to contribute to enhancing the cost-effectiveness assessments of chemical insecticides employed by the PNCM for malaria control in Brazil.

6210

THE INTERSPECIFIC COMPETITION BETWEEN LARVAE OF *AEDES AEGYPTI* AND MAJOR AFRICAN MALARIA VECTORS IN A SEMI-FIELD SYSTEM IN TANZANIA

Sperancia Coelestine Lushasi

Ifakara Health Institute, Morogoro, Tanzania, United Republic of

The interspecific competition between larvae of *Ae. aegypti* and *Anopheles* species may influence adult life history traits such as body size, fecundity, pathogen susceptibility, longevity, vector competence, flight capacity and overall vectorial capacity of both species and affect their ability to transmit diseases. We examined the effects of intra and interspecific competition on individual fitness between *Ae. aegypti* and *An. arabiensis, Ae. aegypti* and *An. gambiae,* as well as *Ae. aegypti* and *An. funestus* at the larvae stage in a semi field system. We designed the experiment with intra and interspecific competition under three species combinations, with and without food; 100:100, 200:0, 0:200. Two habitat sizes were utilized, small (0.5 liter of water) and medium (1 liter of water). Tetramin fish food (0.02g) was provided on daily basis to the food assigned group. Interspecific competition had significant effects on developmental time, larval survival to adulthood, and adult body size via wing size. However, these effects were more prominent in *Anopheles* species in interspecific than *Ae. aegypti*. Cannibalism and predation were observed in both experiments, with and without food, for both species. In the absence of food, *Ae. aegypti* exhibited prolonged survival compared to *Anopheles* species, although no larvae survived to adulthood for the two species. Our results suggest that the interspecific competition significantly impacted malaria vectors than *Ae. aegypti*. Owing to the epidemiological importance of the two species in diseases transmission, it is crucial to understand the outcomes of competition between these two species on species distribution and individual fitness and performance for effective vector control strategies especially in urban and suburban settings.

6211

ESTIMATING SPATIAL DISTRIBUTIONS OF *AEDES AEGYPTI*, *AEDES ALBOPICTUS* AND *CULEX QUINQUEFASCIATUS* IN HAITI

Ian A. Pshea-Smith[1], Bernard Okech[2], Ian Sutherland[3], James Dunford[3], John So[3], Jeffrey Koehler[4], Jacques Boncy[5], Graham A. Matulis[6], Jason K. Blackburn[1], Michael E. von Fricken[6]

[1]Department of Geography, University of Florida, Gainesville, FL, United States, [2]Department of Preventative Medicine and Biostatistics, Uniformed Services University, Bethesda, MD, United States, [3]Navy Entomology

Center of Excellence, Naval Air Station, Jacksonville, FL, United States, [4]United States Army Medical Research Institute Diagnostic Systems Division, Frederick, MD, United States, [5]National Public Health Laboratory, Port-au-Prince, Haiti, [6]Department of Environmental and Global Health, University of Florida, Gainesville, FL, United States

Here we modelled the geographic distributions of three arbovirus vectors, *Aedes aegypti*, *Ae. albopictus* and *Culex quinquefasciatus* across Haiti. All three of these mosquito species are known to occur across Haiti; however, determining their most likely distributions is crucial for tailoring vector-borne disease control strategies. Idiosyncratic presence points for all three species were obtained from the VectorMap and Global Biodiversity Information Facility databases and were supplemented by a recent mosquito collection project within Haiti. An ecological niche model experiment using maximum entropy (MaxEnt) was used to estimate the probability of presence across the landscape for each of the three target species. The model incorporated several predictor variables: human population, nighttime lights, distance to roadways, distance from inland waterways, distance from the coast, elevation, mean annual temperature, maximum temperature, minimum temperature, annual precipitation, wind speed, normalized difference vegetation index and built surface extents. All covariates were obtained in raster format from publicly available databases. The MaxEnt-based prediction elucidated several key patterns for the three mosquito species, with the highest probability of presence for all species being found at and around urban and highly populated areas, especially around the capital of Port-au-Prince and other cities such as Léogâne, Jacmel, Gonaïves and Cap-Haitien. This trend persists for all three mosquito species, with significant overlap between the predicted distributions for *Ae. aegypti* and *Ae. albopictus*, with *C. quinquefasciatus* having a wider distribution than the others. These models corroborate existing research that depicts the frequent co-existence of these three species and their synanthropy. While additional count modelling approaches may elucidate additional species-specific preferences, these presence/absence models provide first pass, actionable spatial data for public health policy and tailored vector management strategies in Haiti.

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ALARMINGLY EXPANDING GEOGRAPHIC DISTRIBUTION OF *ANOPHELES STEPHENSI* IN ETHIOPIA

Dawit Hawaria Logita[1], Solomon Kibret[2], Daibin Zhong[3], Ming-Chieh Lee[3], Delenesaw Yewhalaw[4], Guiyun Yan[3]

[1]Hawassa University, Hawassa, Ethiopia, [2]West Valley Mosquito and Vector Control District, Ontaria, ON, United States, [3]University of California, Irvine, CA, United States, [4]Jimma University, Jimma, Ethiopia

Anopheles stephensi is a primary vector of urban malaria in India and the Middle East. It has been introduced to Africa in the last decade. Its expansion into new geographical regions has posed a threat to malaria control and elimination efforts in the region. Strengthening surveillance and targeted vector control have been advocated to limit its spread. This study sought to determine the geographic distribution of *An. stephensi* in Ethiopia. A targeted entomological survey, both larvae and adult, was conducted in three major cities in southern Ethiopia; Hawassa, Dilla, and Arba Minch, between 2023 and 2024. *Anopheles* larvae collected from the field were reared to adults for species identification. Adult *Anopheles* mosquitoes were also collected using BG pro traps and Prokopack aspirators. Species identification was made using morphological keys. Mosquito blood meal sources was examined using qPCR. A high larval positivity rate was recorded for *An. stephensi* in Hawassa (16.6%), Arba Minch (14.1%) and Dilla (9.0%). Out of 1284 adult female *Anopheles* mosquitoes reared from larvae, 514 (40.0%) were identified as *An*. *stephensi*. This is the highest proportion of *An. stephenis* ever documented in Ethiopia or any Eastern African countries. Blood meal analysis indicated that a zoophagic tendency of *An. stephensi*. The study, for the first time, confirmed the wide range spread of *An. stephensi* with high larval positivity rates in Ethiopia. The presence of both larval and adult stages of *An. stephensi* proves that the species has established in southern Ethiopia. The findings suggest the need for further investigations into the ecology, behavior, population genetics, and the role of *An. stephensi* malaria transmission in Ethiopia.

6213

A SYSTEMATIC REVIEW OF ENTOMOLOGICAL INDICATORS AND SAMPLING APPROACHES USED IN THE EVALUATION OF CLUSTER RANDOMIZED TRIALS FOR MALARIA VECTOR CONTROL PRODUCTS

Victoria James Githu[1], Alex MacConnachie[1], Samson Kiware[2], Joseph Biggs[3], Jackie Cook[3], Paul Johnson[1], Heather Ferguson[1]

[1]University of Glasgow, Glasgow, United Kingdom, [2]Ifakara Health Institute, Dar es salaam, Tanzania, United Republic of, [3]London School of Hygiene and Tropical Medicine, London, United Kingdom

Vector control plays a crucial role in the fight against malaria and other vector-borne diseases. Recommendations for designing vector control trials in assessing vector control products emphasize the importance of well-designed epidemiological trials, particularly cluster Randomized Controlled Trials (cRCT), to demonstrate public health value. Entomological indicators as secondary outcomes complement epidemiological outcomes by providing valuable insights into the effectiveness of vector control interventions and aiding in the interpretation of epidemiological trial outcomes. There remains ambiguity regarding the selection of these parameters, their relationship with epidemiological indicators and the approach taken to collect entomological data in trials is not standardized. This study aims to assess the methodological variability and constraints in cRCTs evaluating vector control interventions by analysing how entomological outcomes are incorporated into epidemiological trials, study designs for entomological monitoring, and the value of resultant data for interpreting epidemiological impacts. Through a systematic review of existing methodologies focusing on malaria, we will determine the frequency with which entomological outcomes are measured, assess variability in the types of entomological indicators collected and study designs used to measure them. This study underscores the importance of robust trial methodologies in vector control research and highlights the need for standardized approaches to the selection of entomological indicators and associated study designs. By clarifying the variability in trial methodologies and its impact on trial outcomes, this research aims to inform the development of guidelines for conducting high-quality vector control trials, ultimately enhancing the effectiveness of vector control interventions, and reducing the burden of malaria and other vector-borne diseases worldwide.

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MALARIA VECTOR CONTROL IN SUB-SAHARAN AFRICA; COMPLEX TRADE-OFFS TO COMBAT THE GROWING THREAT OF INSECTICIDE RESISTANCE

Richard M. Oxborough[1], Karen C. Figueroa[1], Tatchémè F. Tokponnon[2], Louisa A. Messenger[1]

[1]University of Las Vegas, Nevada, Las Vegas, NV, United States, [2]Centre de Recherche Entomologique de Cotonou (CREC), Cotonou, Benin

Mass distribution of insecticide-treated nets (ITNs) has been a key factor in reducing malaria cases and deaths in sub-Saharan Africa. A shortcoming has been overreliance on pyrethroid (PY) insecticides, with more than 2.1 billion PY ITNs distributed in the past two decades, leading to widespread PY resistance. Progressive changes are occurring, with increased deployment of more effective PY-chlorfenapyr or PY-piperonyl butoxide (PBO) ITNs in areas of PY resistance. We performed a critical review of contemporaneous trends in the malaria vector control landscape in sub-Saharan Africa and cost implications associated with use of new chemical classes for improved malaria vector control and resistance management. In 2023, PY-PBO ITNs accounted for 58% of all ITNs shipped to sub-Saharan Africa. Pyrethroid-PBO and PY-chlorfenapyr ITNs are 30-37% more expensive than standard PY ITNs, equating to an additional $132-159 million required per year in sub-Saharan Africa to fund the shift to more effective ITNs. Several countries are withdrawing or scaling back indoor residual spraying (IRS) programs to fund the shortfall. This is reflected by the number of structures sprayed by the US-President's Malaria Initiative decreasing by 30% from 5.67 million (2021) to 3.96 million (2023). Benin is a prime example of a country which ceased IRS in 2021 after fourteen

years of annual spraying. Our economic evaluation indicates that IRS in Benin cost $3.50 per person protected per year, around five times more per person protected per year compared to PY-PBO ($0.73) or PY-chlorfenapyr ITNs ($0.76). While relatively costly to implement, a major advantage of IRS is the portfolio of at least three chemical classes for prospective resistance management. With loss of synergy to PBO developing rapidly, there is a grave danger of overreliance on PY-chlorfenapyr ITNs. Based on current projections, the WHO estimates that key 2030 malaria incidence milestones will be missed by a staggering 89%; in order to enhance the prospects for malaria control and elimination in sub-Saharan Africa, it is imperative to urgently develop a diverse range of insecticide classes for ITNs.

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IVERMECTIN AND *ANOPHELES* GLUTAMATE-GATED CHLORIDE ION CHANNEL INTERACTIONS

Kevin C. Kobylinski[1], Minh N. Nguyen[2], David Hotwagner[3], Andrew K. Jones[3], Joel Tarning[1]

*[1]Mahidol Oxford Tropical Medicine Research Unit, Bangkok, Thailand, [2]BioInformatics Institute, Agency for Science, Technology and Research (A*STAR), Singapore, Singapore, [3]Department of Biological and Medical Sciences, Oxford Brookes University, Oxford, United Kingdom*

Ivermectin is a novel vector control tool for malaria as ivermectin-treated humans or animals are lethal to blood-feeding *Anopheles* mosquitoes, the vectors of malaria. In fruit flies and nematodes, the target for ivermectin is the Glutamate-Gated Chloride (GluCl) ion channel. However, *Anopheles* GluCl channel and ivermectin interactions have not been well characterized. We have been working with the primary Southeast Asian malaria vectors, *Anopheles dirus* and *Anopheles minimus*, which are the most ivermectin-tolerant and -susceptible *Anopheles* found worldwide. Interestingly, compared to ivermectin parent compound, ivermectin monosaccharide (missing second sugar ring) and ivermectin aglycone (missing both sugar rings) structures impart only partial and no mosquito-lethal effect, respectively. This suggests that there are important binding interactions with the second ivermectin sugar ring and *Anopheles* GluCl. Genomic and cDNA sequencing were used to determine the GluCl sequence and primary splice isoforms present in *An. dirus* and *An. minimus*. New AI techniques utilizing AlphaFold 2.0 software, Schrodinger software, and the A*Star CLICK method were applied to build 3-D *in silico* docking models of *Anopheles* GluCl-ivermectin, -monosaccharide, -aglycone interactions to characterize how ivermectin binds to the GluCl channel of *An. dirus* and *An. minimus*. Indeed, the *in silico* docking models indicate novel *Anopheles* GluCl-ivermectin binding interactions not observed previously during protein crystallography investigation of nematode GluCl-ivermectin interactions. This work will benefit the research community working to advance ivermectin use for malaria control as it will improve our understanding of: 1) the binding and mode of action of ivermectin and *Anopheles* GluCl, and 2) potential mechanisms for ivermectin resistance development in *Anopheles*.

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EFFECTIVENESS OF ECO BIOTRAPS - AN INNOVATIVE LARVAL SOURCE MANAGEMENT VECTOR CONTROL TOOL IN DHARAVI, MUMBAI, INDIA

Dr Susanta Kumar K. Ghosh[1], Prasad Phadke[2], Nitin Khope[2], Daksha Shah[3], Sashibala D. Shukla[3], Satish Revatkar[3], Chetan Choubal[3]

[1]Former ICMR-National Institute of Malaria Research, Bangalore Urban, India, [2]Ecobio Consulting Private Limited, Pune, India, [3]Brihanmumbai Municipal Corporation, Mumbai, India

In recent years mosquito-borne diseases especially arboviral diseases such as dengue, chikungunya, Japanese Encephalitis, and Zika virus disease are surging leading to an escalating mortality rate. Many efforts are being made to vector control. Innovations in vector control is utmost necessary. The present study presented an eighteen-month of longitudinal investigation of the effectiveness of an eco-friendly EcoBio Trap for vector control tool in two urban slum areas Kumbharwada and Rajiv Gandhi Nagar covering approximately 500,000 population in 8,000 households of Asia's largest slum Dharavi, Mumbai, India. Dharavi is endemic for dengue for several years. A set of one trial EcoBio Trap (with mosquito attractant and anti-larval IGR compound (Pyriproxyfen) and control (without attractant and IGR) was placed 6 to 8 meter apart in the study sites following the World Health Organization (WHO) guideline for mosquito larvicides. The field team comprising of two trained health staff conducted the study with a weekly follow-up period on day 7, day 14, day 21 and day 28 intervals, respectively. One senior staff supervised for quality assurance. Breading instances and larval density (hatching) data was recorded in Microsoft Excel Worksheet and frequency with proportion was tabulated for each follow-up week. The periodic change in breeding and hatching proportions in EcoBio Trap and control has been recorded and compared. Significant higher proportion of breeding instances (OR: 2.0; 95% CI: 1.7 - 2.4; $p<0.0001$) and lower rate of hatching (OR: 0.04; 95% CI: 0.01 - 0.06; $p<0.0001$) in the EcoBio Trap was reported compared with the control arm. The result of survey to assess the people perception on acceptability of EcoBio Trap as a vector control method had showed a positive responses in reduction of mosquitoes biting experience in the community. Alongside, a WHO-recommended silicone-based monomolecular film-based larvicide plus attractant showed comparable results with Pyriproxyfen. A large-scale trial in six locations in Mumbai city including the existing Dharavi sites is underway.

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ANOPHELES ARABIENSIS, A POTENTIAL THREAT TO MALARIA ELIMINATION IN HWEDZA DISTRICT, ZIMBABWE 2023

Trust Nyakunu[1], Elias Nyamande[2], Charmaine C. Matimba[1], Tanatswa X. Gara[1], Brenda Makonyere[1], Tariro P. Chikava[1], Agatha Nduna[1], Prosper Nyamutsamba[2], Brighton Sibanda[2], Aramu Makuwaza[1], Nobert Mudare[1], Busisani Dube[3], Jessica Kafuko[3], Regis Magauzi[3], Sungano I. Mharakurwa[1]

[1]Africa University, Mutare, Zimbabwe, [2]Mashonaland East Provincial Medical Directorate, Ministry of Health and Child Care, Marondera, Zimbabwe, [3]U.S. Presidential's Malaria Initiative, US Centres for Disease Control and Prevention, Mutare, Zimbabwe

In Zimbabwe most malaria cases are reported in rural areas, mainly transmitted by *Anopheles arabiensis, An. gambiae s.s.* and *An. funestus s.s.* In 2018, Hwedza transitioned to malaria elimination phase and 36,044 Deltamethrin-treated nets were distributed to 4,658 households in 5 targeted wards to achieve 100% coverage. Subsequently, Hwedza recorded 737 malaria cases in 2019, 118 in 2021, 105 in 2022, and 64 in 2023. To conduct surveillance against the residual transmission, from August – November 2023, 102 larval and 268 adult mosquitoes were collected around Garaba, Chikurumadziva, Makarara, and Zvidhuri health centers in Hwedza District. Of the 370 mosquitoes morphologically identified and confirmed by PCR, 22.4% were *An. gambiae s.l.,* 19.4% *An. rufipes,* 18% *An. coustani*, 13.5% *An. pretoriensis,* 8.9% *An. demeilloni*, 6.8% *An. funestus s.l.,* 5.7% *An. marshallii,* 1.3% *An. maculipalpis*, 0.003% *An. squamosus,* and 4% remained unidentified. Two *An. gambiae* complex species were identified by PCR, namely *An. arabiensis* (7.8% of the total samples) and *An. quadriannulatus* (9.5%). 5.1% of the *An. gambiae* complex did not amplify using the PCR protocol, requiring sequencing for identification. Pf-ELISA showed that none of the adult-collected mosquitoes were infected. Insecticide resistance tests against Deltamethrin were done on the 64 larval-collected adult specimens, among which 18 (28%) were *An. arabiensis.* These 18 were tested for Kdr and ACE-1 resistance PCR assays and showed no markers of resistance. This study showed a 7.8% relative abundance of *An. arabiensis* after a period of scarcity (median 1.2%) from 2019 – 2022. Although none of *An. arabiensis* caught as adults (11), among which 5 were caught outdoors, were found Pf-infected. As an efficient malaria vector in Zimbabwe, *An. arabiensis* needs monitoring because its escalating abundance amid residual cases may threaten the malaria elimination status of Hwedza. Its exophagic trait can pose challenges for indoor-based vector control interventions. Supplementary vector control tools could potentially complement indoor-based interventions to maintain the malaria elimination status of Hwedza.

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TAILORING MALARIA CONTROL IN ETHIOPIA: HUMAN AND VECTOR BEHAVIOR CREATE DIFFERENT EXPOSURE PROFILES IN HIGHLANDS AND LOWLANDS

Endashaw Esayas[1], Adam Bennett[2], Edward Thomsen[3], Muluken Assefa[1], Elodie Vajda[3], Steven Gowelo[3], Asefaw Getachew[4], Henry Ntuku[5], Lemu Golassa[6], Endalamaw Gadisa[1], Neil F. Lobo[7]

[1]Armauer Hansen Research Institute, Addis Ababa, Ethiopia, [2]PATH, Seattle, WA, United States, [3]University of California, San Francisco, San Francisco, CA, United States, [4]PATH, Addis Ababa, Ethiopia, [5]PATH, Geneva, Switzerland, [6]Addis Ababa University, Addis Ababa, Ethiopia, [7]University of Notre Dame, South Bend, IN, United States

Malaria elimination in Ethiopia is challenged by seasonal worker movement between highlands and lowlands, transmitting parasites and requiring targeted interventions. To understand the effectiveness and limitations of vector interventions, this study evaluated mosquito and human behavior in migrant workers, residents, and highland populations during peak and minor malaria seasons. Hourly CDC light trap collections coupled with human behavior observations were conducted in four highland and four lowland villages (eight households/farm structures per village). Sampling/observations occurred between 18:00 and 06:00 hrs. Exposure was estimated by multiplying mosquito catches inside/outside by the proportion of individuals exhibiting different behaviors during each hour. Adult mosquitoes were morphologically identified (subset confirmed by DNA sequencing). In the highlands, 4,697 *Anopheles* (13 species) were identified. *Anopheles gambiae* s.l. (41.9%), *An. demeilloni* (24.1%) and *An. cinereus* (11.0%) were the dominant species. While lowlands had less mosquito catches (3,220 *Anopheles*) but higher diversity (20 species groups). *Anopheles gambiae* s.l. (36.9%), *An. pretoriensis* (27.9%) and *An. demeilloni* (17.5%) were predominant. Indoor biting rate was highest in highlands (2.5x outdoor), while outdoor biting dominated lowlands. Human behavior data suggested the peak biting risk in early evening (18.00-20.00 hrs.) for both settings. In highlands, 87.3% of exposure occurred indoors in individuals not using bed nets. In lowlands, exposure mostly occurred outdoors, among migrants, outdoor exposure accounted for 74.5% of total exposure, while residents had 65.5% outdoor exposure. High diversity among mosquito vectors, coupled with the variability of human and mosquito behaviors, presents a significant challenge for malaria control. These factors create gaps in preventative measures, allowing malaria to persist. Thus, to achieve optimal control, the limitations of one-size-fits-all strategies must be recognized, and interventions need to be tailored to the diverse spatiotemporal behaviors of mosquito and human.

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COMPARATIVE SUSCEPTIBILITY OF WILD-DERIVED AND LABORATORY-REARED *AEDES* AND *ANOPHELES* LARVAE TO IVERMECTIN: A PRELIMINARY STUDY TOWARD EXPERIMENTAL SELECTION OF LARVAL IVERMECTIN RESISTANCE MECHANISMS

Cheick Oumar Wendpagnandé Ouedraogo[1], Emmanuel Daouda Sougue[1], André B. Sagna[2], Olive Traore[3], Dieudonné Diloma Soma[3], Sié Hermann Pooda[4], Lamidi Zela[5], Mady Ndiaye[6], El Hadji Mamadou Niang[6], Sophie Le Lamer Déchamps[7], Karine Mouline[8], Fabrice A. Some[1], Roch K. Dabire[1]

[1]Institut de Recherche en Sciences de la Santé/Direction Régionale de l'Ouest, Bobo-Dioulasso, Burkina Faso, [2]Institut de Recherche pour le Développement, Bobo-Dioulasso, Burkina Faso, [3]Université Nazi Boni, Bobo-Dioulasso, Burkina Faso, [4]Université Daniel Ouezzin Coulibaly, Dédougou, Burkina Faso, [5]Centre international de recherche-développement sur l'élevage en zone subhumide, Bobo-Dioulasso, Burkina Faso, [6]Université Cheikh Anta Diop de Dakar, Dakar, Senegal, [7]Medincell, Montpellier, France, [8]Institut de Recherche pour le Développement, Montpellier, France

Administering ivermectin to humans and livestock renders their blood toxic for mosquitoes like *Anopheles* and *Aedes*, offering a promising approach for controlling these vectors. However, the impact of such treatment on larval stages exposed to the drug through contaminated breeding sites is not fully understood. This study looked at how ivermectin affects the development of *Aedes* and *Anopheles* larvae. We exposed laboratory-reared (*An. gambiae* Kisumu and *Ae. aegypti* Bora Bora) and wild-derived (*An. coluzzii* VK5 and *Ae. aegypti* Bobo) larvae to ivermectin concentrations ranging from 1 to 100 ng/ml for 24h, and transferred surviving larvae into free-ivermectin medium to monitor development until adult stage. Parameters measured were: survival, pupation dynamics, emergence rates, and fecundity of the adult females. Four independent replicates were performed. Ivermectin effects were characterized by comparison with larvae raised in control medium. Results indicated that highest ivermectin concentrations (100, 75, and 50 ng/ml) reduced larval survival by over 50% within 24 to 48 hours post-exposure, with varying effects across different strains. Wild-derived larvae showed lower susceptibility to ivermectin compared to laboratory larvae for both *Anopheles* and *Aedes* species. The concentrations leading to 50% larval mortality (4-day-LC50) were 3.65 and 1.86 ng/ml for *Anopheles* VK5 and Kisumu strains, and 15.60 and 2.56 ng/ml for *Aedes* Bobo and Bora Bora strains, respectively. The transition from larval to adult stage was significantly affected, particularly in the Kisumu strain ($p = 0.001$). No significant effects on the number of laid eggs were observed across different strains. Overall, these data showed how lab-raised and wild-derived *Anopheles* and *Aedes* larvae and females are affected differently by ivermectin, highlighting potential implications for vector control strategies. Further investigations are planned to understand potential existing mechanisms allowing wild-derived larvae to better survive than laboratory ones despite the presence of ivermectin in their breeding environment.

6220

UPDATES ON COMMUNITY-BASED BIOLARVICIDING FOR MALARIA CONTROL IN TANGA REGION, TANZANIA

Denis Richard Kailembo[1], Tegemeo Gavana[2], Jubilate Bernard Minja[3], Elizabeth Kasagama[1], Fabrizio Molteni[1], Noela Kisoka[1], Best Yoram[4], Stella Kajange[4], Samwel Lazaro[3], Charles Dismas[3], Propser Chaki[2], Christian Lengeler[5]

[1]Swiss TPH, Dar es Salaam, Tanzania, United Republic of, [2]IHI, Dar es Salaam, Tanzania, United Republic of, [3]NMCP, Dodoma, Tanzania, United Republic of, [4]PORALG, Dodoma, Tanzania, United Republic of, [5]Swiss TPH, Basel, Switzerland

Malaria remains a disease of great public health importance. Globally in 2022, there were an estimated 249 million cases and 608,000 deaths. Tanzania accounted for approximately 4% of all malaria deaths globally. In addition to mainstream vector control interventions, the country has deployed recently community-based biolarviciding to enhance its efforts toward malaria control and elimination. Biolarviciding is implemented routinely in three councils in Tanga Region: Handeni DC, Tanga CC, and Lushoto DC, representing 'high', 'moderate', and 'low' malaria risk strata/councils respectively, as well as both rural and urban settings. Implementation started in June 2022 following a community-based approach using trained community-owned resource persons (CORPs) to monitor breeding habitats and apply biolarvicide. CORPs are supervised using existing local government structures. Two biolarvicide products produced in-country are used: *Bacillus thuringiensis var. israelensis* (Bti) and *Bacillus sphaericus* (Bs). Application of biolarvicide follows a discontinuous temporal pattern based on rainfall, with three rounds conducted per year. Each round comprises eight weeks of larvae monitoring and biolarvicide application. All three councils have now completed six rounds of biolarvicide application. Programmatic monitoring shows that biolarviciding reduces significantly mosquito larvae abundance and larvae occupancy. Larvae occupancy within breeding habitats across eight weeks of implementation decreased by 66% in round two, 93% in round three, 95% in round four, and 90% in round five. The analysis of the extensive entomological, epidemiological, and costing evaluation to determine the impact and cost-effectiveness of the intervention is ongoing, and will be reported at the conference. These results from an extensive real-world program will inform the decision for future scale-up of larviciding to other councils across the country, and represent an important piece of global evidence, especially with regard to larviciding in endemic rural areas.

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INVESTIGATING THE MOSQUITO MYCOBIOTA: FROM BASIC KNOWLEDGE TO POTENTIAL APPLICATIONS FOR MOSQUITO CONTROL

Irene Ricci, Alessia Cappelli, Mohammad Sameer Zubair, Valentina Cecarini, Claudia Damiani, Guido Favia
University of Camerino, Camerino, Italy

Mosquito control is a crucial aspect of public health, especially in regions where mosquitoes pose a threat for disease transmission. Traditional vector control methods such as insecticides have proven effective but are often associated with environmental concerns and the development of insecticide resistance. In recent years, there has been growing interest in alternative approaches. Understanding composition and function of mosquito microbial communities could have profound implications for new vector control strategies. While the bacterial community has been deeply studied, the fungal component is still little appreciated. It is reported that budding yeasts associated with larvae and/or adult mosquitoes are involved in symbiotic associations, but extensive investigations of the fungal community in mosquito breeding sites is still lacking. The present work represents an in-depth characterization of the larval mycobiota in wild mosquitoes. NGS analysis has unveiled a diverse fungal community including Ascomycota (budding yeasts) and Basidiomycota in vector mosquitoes including *Aedes albopictus*, *Culex pipiens* and *Aedes koreicus*. Fungi such as *Wickerhamomyces anomalus*, *Metschnikowia pulcherrima*, and *Candida parapsilosis* were detected across all analysed species, whereas *Hyaloraphidium* and *Microidium* were associated with *Cx. pipiens* and *Ae. koreicus*, respectively. Metagenomic outcomes were confirmed using culture-dependent methods and the isolated fungal strains were processed by headspace solid-phase microextraction combined with gas chromatography-mass spectrometry to extract and analyse the yeast volatile organic compounds (VOCs). Results provide the base for next functional tests of selected fungi, that are aimed at the evaluation of attractant properties towards gravid mosquitoes or entomopathogenic activity against larvae. Fungal blends might be used for the implementation of 'lure and kill' formulations to be released in artificial or natural breeding sites of mosquitoes. Such innovative fungal-based products might contribute to mosquito control through a sustainable 'ready to use' technology.

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BACTERIAL SYMBIONTS IMPACTING THE BIOLOGY AND VECTORIAL COMPETENCE OF MOSQUITOES

Guido Favia, Claudia Damiani, Alessia Cappelli, Monica Falcinelli, Silvia Sorana, Paolo Luigi Catapano, Paolo Rossi, Irene Ricci
University of Camerino, Camerino, Italy

The role of symbiotic microbes of mosquito vectors in the processes of environmental adaptation and resistance to insecticides has been proposed, however the mechanisms by which these processes occur are still unclear. Our group at the University of Camerino, in recent years has focused predominantly on the characterization of the contribution that symbionts offer to thermal adaptation and insecticide resistance to the host mosquito, thus identifying symbiotic bacteria of different mosquito species which seem to reveal a role in both mechanisms. In fact, the exposure of mosquitoes of the *Aedes* (*Ae. albopictus, Ae. koreicus, Ae. japonicus*), Culex (*Cx. pipiens*) and Anopheles (*An. stephensi*) genera to different temperatures has made it possible to identify some bacterial species that seem to allow tolerance to low or high temperatures. Similarly, the analysis of mosquitoes resistant or sensitive to pyrethroids has allowed us to identify potential bacteria that play a role in resistance mechanisms. We have identified a pyrethroid hydrolase potentially underlying these mechanisms. The relevant research in progress will be presented in detail.

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MICROBIAL COMPETITION IN MOSQUITO: POSSIBLE APPLICATION IN MONITORING AND CONTROL

Claudia Damiani[1], Alessia Cappelli[1], Marie Paul Audrey Mayi[1], Paolo Luigi Catapano[1], Lodovico Sterzi[2], Irene Ricci[1], Francesco Commandatore[2], Guido Favia[1]
[1]University of Camerino, Camerino, Italy, [2]University of Milan, Milan, Italy

Wolbachia is an obligate intracellular bacterium naturally found in 60% of all arthropod's species. There are discrepancies in Wolbachia detection, with some studies revealing it in 14% of species while others report negative results, indicating regional variability in infection rates and potential limitations in detection methods. Wolbachia can influence host fitness and vector competence thus a series of application has been developed to decline viral transmission within mosquito host. Recently we have identified Wolbachia in the sylvatic African vector Aedes africanus, a mosquito vector widely distributed throughout sub-Saharan Africa, except Madagascar, where it acts as one of the major vectors of yellow fever arboviruses. We have characterised Wolbachia of Ae. africanus and its relationships with members of the mosquito microbiota, highlighting competition dynamics between Wolbachia and Pantoea.Wolbachia was found in nearly all the specimens of Ae. africanus examined, displaying varying quantities. The phylogenetic analysis via multi-locus sequence typing revealed that this Wolbachia strain belonged to Supergroup B yet exhibited closer resemblance to Wolbachia strains observed in Lepidoptera and Hymenoptera rather than mosquitoes. Fluorescence in situ hybridization analysis revealed Wolbachia localization in both male and female reproductive organs. Moreover, microbiota analysis suggested a potential competition among highlighting competition dynamics between Wolbachia and Pantoea.Whole genome sequencing of the two bacteria is ongoing to better define the dynamics of this competition.

6224

BIOMARKERS FOR MOSQUITO AGE GRADING AND PARITY STATUS OF *ANOPHELES DIRUS*

Sirasate Bantuchai
Mahidol University, Ratchathewi, Thailand

Malaria transmission predominantly occurs through the bites of infected *Anopheles* mosquitoes. Female mosquitoes necessitate blood for egg development to initiate oogenesis and vitellogenesis. Recent research has focused on mosquito population age structure, given the consideration of microbial agents targeting mosquito lifespan in disease mitigation strategies. Mosquito age is linked to blood feeding behavior, significantly influences malaria transmission dynamics. Age grading methods rely on changes in insect morphology by the ovary tracheation method, differentiating nulliparous and parous mosquitoes based on the presence or absence of tracheole skeins. Beyond the limitation of conventional morphological techniques, transcriptome profiling of blood-fed and non-blood-fed female mosquitoes offers insights into molecular processes triggered by blood meals, aiding in characterizing mosquito aging and feeding behavior. In this study, lab reared *Anopheles dirus* was utilized for transcriptomic analysis in which several genes have been found to be regulated differently by age and parity status as candidate biomarkers. The candidate genes were validated by RT-qPCR with the controlled age mosquito under laboratory settings. Analyzing age-dependent gene expression in the changes of mosquito biological structure, including the process of completing the gonotrophic cycle, as biomarkers to be implemented for the field validation using wild-caught mosquitoes, eventually serving as indicators for effective vector control and intervention.

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MEMBRIN GENE IDENTIFICATION AND JUVENILE HORMONE PRODUCTION AND MATING EFFICIENCY IN ADULT MALE *CULEX PIPIENS* MOSQUITOES

Tatyana Martynova, Cheolho Sim
Baylor University, Waco, TX, United States

In order to successfully implement various vector control programs, such as the sterile insect technique (SIT), on *Culex* mosquito disease vectors, it is crucial to identify the specific genes that can be targeted for male sterilization. Additionally, it is important to determine the locations where these reproductively relevant genes are most concentrated in males. In our previous study, we conducted an RNA-seq and qRT-PCR analysis on the complete genome of the male accessory gland (MAG) of *Cx. pipiens*. This gland is widely recognized as a significant, albeit understudied, reproductive organ. The objective of our investigation was to identify genes that may be elevated and potentially beneficial for adult male sterilization. Subsequently, we selected the gene membrin (CPIJ006096) that is linked to increased expression in young and, consequently, reproductively viable MAGs, for a subsequent functional examination. The initial step involves conducting RNA interference (RNAi) knockdown of membrin (CPIJ006096) to assess the phenotype of *Cx. pipiens* MAG tissue between the wild type and the knockdown. To evaluate the reproductive capacity of male *Cx. pipiens* individuals with knockdown compared to those with wild type, a small cage fertility assay is conducted after analyzing variations in knockdown tissue using a light microscopy. In the test, wild type and knockdown males are segregated in distinct enclosures and given a period of 2-3 days to engage in mating. Subsequently, females are gathered and dissected to measure the rate of sperm insemination in their spermatheca. The spermatheca is monitored to determine the presence or lack of sperm. Ultimately, the MAG tissue of both wild type and knockdown males is analyzed using mass spectroscopy to ascertain the presence or absence of the potentially crucial JH hormone. This analysis aims to establish a connection between membrin activity and the sufficient production of JH hormone in the MAG.

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A RAPID, COST-EFFECTIVE, COLORIMETRIC LAMP ASSAY (CLASS) FOR DETECTING INVASIVE MALARIA VECTOR, *ANOPHELES STEPHENSI*

Cristina S. Rafferty[1], Gloria Raise[1], JeNiyah Scaife[1], Bernard Abongo[2], Seline Omondi[2], Sylvia Milanoi[2], Margaret Muchoki[2], Brenda Muchoki[2], Eric Ochomo[2], Sarah Zohdy[1]
[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]KEMRI, Kisumu, Kenya

Anopheles stephensi, an invasive malaria vector in Africa, threatens to put an additional 126 million people per year at risk of malaria. To accelerate the early detection and rapid response to *An. stephens*i, it is critical to confirm its presence and geographic extent. However, morphological identification may be easily misinterpreted, and existing molecular species assays require specialized laboratory equipment and training to interpret, requiring sequencing confirmation. A colorimetric rapid loop-mediated isothermal amplification (LAMP) assay for molecular *An. stephens*i species identification was developed and optimized. The CLASS assay requires only a heat source and reagents and can be used with or without DNA extraction resulting in positive color change in 30-35 minutes. To determine analytical sensitivity, a 1:10 dilution series of the DNA extract was conducted showing 100% assay sensitivity down to 0.0003 nanograms. To determine specificity, 3 different *An. stephens*i laboratory strains (STE2, SDA 500, UCI), 8 other *Anopheles* mosquito species, and *Aedes aegypti* were compared, and the results indicated 100% specificity across these species. To determine use without the need for DNA extraction, samples included a single mosquito leg, whole adult or larval mosquitoes, and pooled DNA extract from several mosquito species. A total of 1687 individual reactions were tested, and all LAMP assay results were compared against the conventional PCR assay and confirmed through Sanger sequencing. To validate the assay on wild caught specimens, DNA extracted from 12 wild caught, sequence-confirmed *An. stephensi* from Marsabit, Kenya, were tested and the assay was accurate in identifying all the specimens as *An. stephensi.* The assay presents an opportunity to accelerate *An. stephensi* molecular identification and offers a simple, rapid, alternative to existing PCR -based *An. stephensi* species identification strategies. The CLASS assay provides, an opportunity to better understand the spread of the species in Africa and other recently invaded areas, thus accelerating a response to mitigate its impacts on malaria on the continent.

6227

EFFECTS OF TEMPERATURE ON *AEDES* HEAT SHOCK PROTEIN EXPRESSION

Rachel Fay[1], Lisa Couper[2], Erin Mordecai[1]
[1]Stanford University, Stanford, CA, United States, [2]University of California Berkeley, Berkeley, CA, United States

In the last 50 years, increased urbanization, and climate change are believed to have contributed significantly to the increased prevalence of vector-borne diseases such as West Nile, Zika, and dengue. As global temperatures rise, vector-borne diseases are uniquely responsive as insects are ectothermic organisms, meaning that the surrounding environmental temperature modulates the host's body temperature. As mosquitoes adapt to a warming climate, it is paramount to understand what genetic determinants play a role in mosquito thermal tolerance. Thermal stress can initiate a suite of responses that mitigate the effects of extreme heat on mosquito life history traits. One such response, the heat shock response, is facilitated by a family of proteins, known as heat shock proteins, that are well conserved across *Aedes* species. Those genes associated with thermal stress include *hsp70*, *hsp26* and *hsp83*. Understanding how heat shock protein expression varies within and among populations from different thermal ranges is important in assessing the future effects of climate change on mosquito-borne disease transmission. Here, we investigated the effect of temperature on heat shock protein expression by utilizing *Aedes sierrensis*, the western tree hole mosquito, which is the primary vector of dog heartworm. *Aedes sierrensis,* a close relative of the major vector *Aedes aegypti*, is found across a wide swath of the West Coast of North America spanning different thermal regimes. We utilized samples from a common garden experiment where *Aedes sierrensis* mosquitoes collected from across a temperature gradient spanning over 1200 km were reared at various temperatures, RNA was extracted from whole mosquitoes, and heat shock protein expression was quantified via qRT-PCR. The results of this work found variability in heat shock protein expression and determined the role of source thermal environment and rearing temperature on thermal tolerance. Elucidating species-wide targets for future vector control measures is pertinent as public health officials prepare to limit vector-borne disease risk in a warming climate.

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CHARACTERIZATION OF *AEDES AEGYPTI* INFECTION WITH THE NATURALLY ATTENUATED DENV-2D30-7169 VIRUS

Sreynik Nhek[1], Sreyneang Phoch[1], Mengheng Oum[1], Somnang Man[1], Sophana Chea[1], Lyhourng Long[1], Sreyngim Lay[1], Piseth Ly[1], Octavio AC Talyuli[2], Chanthap Lon[1], Sokchea Lay[3], William Stone[2], Isabelle Conclois[4], Dorothée Misse[4], Stephen Whitehead[2], Jessica E. Manning[1], Tineke Cantaert[3], Christina Yek[2], Fabiano Oliveira[2]
[1]International Center of Excellence in Research Cambodia, Phnom Penh, Cambodia, [2]National Institute of Allergy and Infectious Diseases, Rockville, MD, United States, [3]Institut Pasteur Cambodge, Phnom Penh, Cambodia, [4]Universite Montpellier, Montpellier, France

Several dengue virus (DENV) challenge human infection models (CHIMs) have been used to assess the safety and efficacy of dengue vaccines and therapies. However, these have used needle inoculation of the virus, bypassing the natural mosquito vector. Prior research has shown that mosquito saliva can potentiate viral infections and may promote distinct DENV infection outcomes in mouse models. Here, we propose a "natural CHIM" where DENV infected *Aedes aegypti* will be used for human challenge. For safety and feasibility, we chose a cGMP-grade, naturally

attenuated DENV-2Δ30-7169 virus, that was tested in prior FDA-approved CHIMs. DENV-2Δ30-7169 infection was shown to cause mild symptoms (rash) and viremia, but no severe dengue outcomes in humans. An additional benefit of using DENV-2Δ30-7169 is its poor transmissibility back into *Aedes aegypti* by blood feeding. To overcome this paradox for our "natural CHIM", we injected *Aedes aegypti* intrathoracically with 100 DENV-2Δ30-7169 plaque forming units and evaluated viral dissemination by PCR of dissected mosquito salivary glands, heads, abdomen, legs, Malpighian tubules and midgut at days 1, 3, 5, 7, 10, 14, 18, 21, 25, and 30 post-infections. We found that all tissues had detectable virus at day 3 post-infection. Viral titers increased over time, leveling off at day 18 post-infection. Salivary glands accumulated significantly higher viral titers versus midguts and Malpighian tubules from day 7 post-infection onwards. Finally, we detected virus in mosquito saliva by PCR and confirmed its infectivity by salivary gland titration in Vero cell. In summary, we show that DENV-2Δ30-7169 can infect and disseminate within *Aedes aegypti* mosquitoes following intrathoracic inoculation, making this a suitable model to develop a "natural CHIM" using dengue infected mosquitoes.

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OPEN QUESTIONS IN *ANOPHELES* DOSAGE COMPENSATION

Niklas Klauss, **Michael B. Wells**
Idaho College of Osteopathic Medicine, Meridian, ID, United States

Two recent studies (PMIDs: 37769784, 37774706) provide insights into the process of X chromosome dosage compensation in *Anopheles* mosquitoes. These results confirm that dosage compensation is a process present across multiple Dipteran species and that the mechanism used involves a variation of the evolutionarily conserved male X chromosome upregulation approach. Important findings and limitations of this study, along with deeper analysis of the data and comparisons to dosage compensation in other species, suggest multiple lines of inquiry for future studies. In this work, we interrogate the amino acid structure of SOA/007 for clues to its molecular function, provide a more complete understanding of why binding and gene expression regulation were observed at many sites not located on the male X chromosome, highlight an updated view of the relationship between dosage compensation and sex determination in *Anopheles*, and describe follow-up experiments important for considerations in the creation of vector control strategies that rely upon male mosquitoes. Our aim is to inform future studies that intersect with *Anopheles* dosage compensation.

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NATURALLY OCCURRING RECESSIVE LETHAL ALLELES (RLAS) AND SEX-RATIO DISTORTION IN THE YELLOW FEVER MOSQUITO, *AEDES AEGYPTI*

Abdulhadi Kobiowu, Atashi Sharma, Melanie Hempel, Zhijian Tu
Virginia Tech, Blacksburg, VA, United States

Aedes aegypti female mosquitoes are critical public health issues on a global scale, as they are vectors of many arboviral diseases like Zika, yellow fever, dengue, and chikungunya viruses. Currently, prevention relies on effective vector control, which is hindered by rising insecticide resistance. There have been several research efforts, such as using genetic tools for population suppression and population modification—by reducing the female population or making the females resistant to virus transmission, respectively. Studies have reported the presence of recessive lethal alleles (RLAs) on the Y-like chromosome of *A. aegypti*, resulting in sex ratio distortion. Here, we report the discovery of naturally-occurring RLAs on the X-like chromosome. To map these novel RLAs, we generated several genetic strains that will enable rapid identification of the RLA loci through marker-assisted mapping. Upon successful completion, this study will provide insights into the evolutionary forces that led to the occurrence and persistence of sex-linked RLAs. It could also help remove the biting females so only males are released in the previously described genetic control programs.

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REDUCED GENETIC DIVERSITY OF KEY FERTILITY AND VECTOR COMPETENCY RELATED GENES IN *ANOPHELES GAMBIAE* S.L. ACROSS SUB SAHARAN AFRICA (SSA)

Fatoumata Seck[1], Mouhamadou Fadel Diop[1], Karim Mané[1], Amadou Diallo[2], Idrissa Dieng[2], Moussa Namountougou[3], Abdoulaye Diabaté[4], Umberto D'Alessandro[1], Alfred Amambua-Ngwa[1], Alistair Miles[5], Ibrahima Dia[2], Benoit Sessinou Assogba[1]
[1]Medical Research Council Unit The Gambia at the London School of Hygiene & Tropical Medicine, Banjul, Gambia, [2]Institut Pasteur de Dakar, Dakar, Senegal, [3]Université Nazi Boni, Bobo-Dioulasso, Burkina Faso, Bobodioulasso, Burkina Faso, [4]Institut de Recherche en Sciences de la Santé, Bobodioulasso, Burkina Faso, [5]Wellcome Sanger Institute, Hinxton, United Kingdom

Targeting crucial fertility and susceptibility to *Plasmodium* genes of malaria mosquito with small molecule inhibitors is a promising alternative approach to curb the vector population and transmission down. We identified 4 key potential genes associated both in *Anopheles* reproductive success and ability to transmit *Plasmodium*: *MISO*, *HPX15*, *VG* and *LP*. However, the successful application of this approach would require a comprehensive knowledge of the genes' diversity in natural vector populations to ensure its large implementation. Using whole-genomic SNPs data of the Ag1000G project, we extracted each gene from 2784 wild-caught *Anopheles gambiae* s.l across 19 SSA countries. We performed a population structure-based analysis and estimated the differentiation level and genetic diversity. We also computed neutrality tests and evaluated nsSNPs linkage. We found a significant conservation of the 4 genes in SSA *Anopheles gambiae* s.l. populations. Fst values between species were globally low (<0.051, <0.146, <0.022 and <0.048 for *MISO*, *Vg*, *Lp* and *HPX15* respectively) with the highest divergence consistently observed in *An. arabiensis* populations, reinforcing the principal component analysis where genes in *An. arabiensis* slightly diverged from genes in *An. gambiae*, *An. coluzzii* and their intermediates. Among genes, *MISO* showed less structuration. The low nucleotide diversity within populations (>0.10) and negative Tajima's D values suggest a purifying selection. The observed heterozygosity did not significantly deviate from expectation and provided insights into the low genetic diversity within populations. No associated nsSNPs were found across *MISO* gene, while few low linked nsSNPs with ambiguous haplotyping were found in the other genes. Our results provide rare integrated findings on major malaria vectors' biological factors with their genetic features in natural populations and offer new insights for sustainable malaria control tools development. As reasonably conserved, *MISO*, *VG*, *LP* and *HPX15* could be good targets of small molecule inhibitors for controlling vector populations and lowering global malaria transmission.

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SPATIALLY-EXPLICIT SAMPLING OF *ANOPHELES GAMBIAE S.L* REVEALS FINE-SCALE POPULATION STRUCTURE AND MECHANISMS OF INSECTICIDE RESISTANCE

Sanjay Curtis Nagi[1], Nicholas-Ato Egyir[2], John Essandoh[3], Eric Lucas[1], Kwame Desewu[4], Alex Egyir-Yawson[2], David Weetman[1], Luigi Sedda[5], Martin J. Donnelly[1]
[1]Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [2]Department of Biomedical Sciences, University of Cape Coast, Cape Coast, Ghana., Cape Coast, Ghana, [3]Department of Biomedical Sciences, University of Cape Coast, Cape Coast, Ghana., Cape Coast, United Kingdom, [4]AngloGold Ashanti Malaria Control, Ashanti, Ghana, [5]University of Lancaster, Lancaster, United Kingdom

Progress in malaria control in sub-Saharan Africa is stalling, partly due to the spread of insecticide resistance in the Anopheline vector. Monitoring the evolution of insecticide resistance alleles and their spatial heterogeneity is important for malaria control programmes, and genomic surveillance has emerged as a pivotal tool for this purpose. Earlier genomics research has typically employed convenience-based sampling, and research has yet to be performed to optimise sampling regimens for malaria vector genomics. In this study, we developed a spatially explicit sampling framework that

considers the underlying ecology to enable sampling mosquitoes with reduced bias. We applied this framework to sample An. gambiae s.l mosquitoes in Obuasi, central Ghana, and performed whole-genome sequencing on 485 individual specimens. In this region, An. gambiae s.l have been documented as highly resistant to pyrethroid insecticides, with emergent resistance to other classes. Our sampling framework allowed us to explore fine-scale population structure at high resolution, detecting isolation-by-distance in An. coluzzii in Obuasi, and finding that at this scale, geographic distance, rather than the underlying ecology, drives population structure. We develop methods to estimate kinship between mosquitoes, finding that polymorphic chromosomal inversions impede the accuracy of established tools. We perform genome-wide selection scans and discover novel mutations in detoxification enzymes that are driving selective sweeps and look to be important for resistance, Gste2-F120L, and Cyp9K1-N225I. We also elucidate the continued evolution of the target of pyrethroid insecticides, the Voltage-gated sodium channel. Overall, we demonstrate that by sampling vectors strategically we can enhance our ability to perform effective genomic surveillance.

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MITOCHONDRIAL SEQUENCES OF *HAEMAOGOGUS* MOSQUITOES FROM TRINIDAD REVEAL PHYLOGEOGRAPHIC RELATIONSHIPS WITH SPECIES ENDEMIC TO THE AMAZON

Renee L.M.N. Ali[1], Satish Singh[2], Yoosook Lee[3], David W. Severson[4], Adesh Ramsubhag[2], Douglas E. Norris[1]

[1]The W. Harry Feinstone Department of Molecular Microbiology and Immunology, Johns Hopkins Malaria Research Institute, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [2]Department of Life Sciences, Faculty of Science & Technology, University of the West Indies, St. Augustine, Trinidad and Tobago, [3]Florida Medical Entomology Laboratory, Department of Entomology and Nematology, Institute of Food and Agricultural Sciences, University of Florida, Vero Beach, FL, United States, [4]Department of Biological Sciences and Eck Institute for Global Health, University of Notre Dame, Notre Dame, IN, United States

Haemagogus species are the primary vectors in the sylvatic cycle for yellow fever and the emerging Mayaro virus. Mosquitoes belonging to this genus are divided into two subgenera (*Haemagogus* and *Conopostegus*), with their distribution geographically restricted to forests of Central and South America and the Caribbean. While one primary vector *Haemagogus janthinomys* is well-studied, most other *Haemagogus* species are understudied. Likewise, there exists limited molecular data which can be used to better understand the taxonomy and evolutionary phylogenetics of this genus. Mitochondrial genomes (mitogenomes) have proved useful in determining phylogeographic associations, constructing phylogenies and taxonomic classification of an increasing number of Culicidae. Here we report the complete mitochondrial sequence of a putative new *Heamagogus* species from Trinidad. This mitogenome is 16, 615 bp long with 78% A-T content and 37 genomic features which is comparable to five *Haemagogus* mitogenomes available in NCBI's GenBank database. Bayesian inference and maximum likelihood using the concatenated 13 protein coding genes from all mitogenomes were analyzed to construct phylogenies and molecular dating estimations. Phylogenetic analyses revealed that the putative new Trinidad *Haemagogus* species diverged from *Hg. tropicalis*, a species restricted to the Brazilian Amazon region ~74 million years ago (MYA) and ~102 MYA from the albomaculatus section of the *Haemagogus* subgenera. This further supports previous taxonomic studies that placed mosquitoes in the subdivision of the *Haemagogus* subgenera into 3 sections (albomaculatus, spledens, tropicalis), with the putative new species being placed into the tropicalis section. These findings highlight the need for future investigations into the phylogenetics and evolutionary studies by encompassing more taxa from the endemic geographic regions where this mosquito genus is found.

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CHANGES IN *ANOPHELES STEPHENSI* DIVERSITY IN MAJOR HUB OF GENOMIC CONNECTIVITY IN ETHIOPIA

Elizabeth Waymire[1], Dejene Getachew[2], Isuru Gunarathna[1], Sarah Zohdy[3], Jeanne N. Samake[3], Tamar E. Carter[1]

[1]Baylor University, Waco, TX, United States, [2]Adama Science and Technology University, Adama, Ethiopia, [3]Centers for Disease Control and Prevention, Atlanta, GA, United States

Anopheles stephensi has been established in eastern Ethiopia since 2018, and evidence has shown that the *An. stephensi* in Dire Dawa, Ethiopia is a hub of genetic diversity for the Horn of Africa. *An. stephensi* has been implicated as the cause of a major malaria outbreak in 2021-2022 in Dire Dawa as well. The goal of this study is to elucidate regions of the genome that are changing over time, identify regions of the genome under selective pressure, and to understand what this means for malaria transmission in Dire Dawa. To do this, ten *An. stephensi* from Dire Dawa from 2018 and 2022 will be sequenced via Illumina short read sequencing. Single nucleotide polymorphisms (SNPs) will be identified in each dataset and compared via paired F_{ST} and Tajima's D, as well as principal component analysis. Preliminary data indicates 7.8 million SNPs across the genome in the 2022 dataset. There are 3.5 million SNPs concentrated to the second chromosome and 3.9 million concentrated to the third chromosome. Preliminary principal component analysis indicates that the 2022 population is significantly differentiated from the 2018 population. Ultimately, this data will provide a key look into the *An. stephensi* of Dire Dawa and what is happening to this population over time that could be responsible for increased cases of malaria.

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WHOLE GENOME SEQUENCE DATA REVEALS SELECTIVE SWEEP SIGNALS AROUND MAJOR INSECTICIDE RESISTANCE LOCI IN *ANOPHELES FUNESTUS* POPULATIONS FROM UGANDA

Lilian Namuli Kayondo[1], Sanjay C. Nagi[1], Joaniter I. Nakabirwa[2], Jonathan Kayondo[3], Samuel L. Nsobya[2], Mara K N Lawniczak[4], Moses Kamya[2], Martin J. Donnelly[1]

[1]Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [2]Infectious Diseases Research Collaboration, Kampala, Uganda, [3]4Department of Entomology, Uganda Virus Research Institute, Entebbe, Uganda, [4]Wellcome Sanger Institute, Hinxton, United Kingdom

Insecticide resistance threatens malaria control and elimination in Sub-Saharan Africa. To combat this, novel products, active ingredients and delivery methods are now coming to market and being evaluated in large cluster randomized trials (cRCTs). To maximize the efficacy and longevity of all vector control tools, early detection of resistance mutations in the *Anopheles* vector is crucial. The LLINEUP trial in Uganda (2017–2019), covering 40% of the country in 104 health sub-districts, evaluated the effectiveness of combined pyrethroid and synergist long-lasting insecticidal (PBO) nets. During LLINEUP, a shift in species composition was observed, with *An. funestus* becoming predominant in some regions. We embedded genomic surveillance within the trial with the aim of detecting and tracking insecticide resistance variants. We collected *An. funestus* mosquitoes using Prokopack aspirators and performed whole-genome sequencing on 1149 specimens, obtaining high-quality SNP and haplotype data. Genetic diversity and kinship analyses were indicative of a large and stable population throughout the intervention, this corroborated *An. funestus* density data collected during the trial. Standard approaches for describing genetic diversity e.g. F_{ST}, PCA revealed little genetic differentiation across Uganda. Genome-wide selection scans revealed strong signals of positive selection at a Cyp9k1 gene cluster (8 Mb-X chromosome) and CypP6a (8 Mb-2RL chromosome), both loci previously implicated in pyrethroid resistance. At both loci, haplotype clustering analyses showed a single haplotype shared across the entirety of Uganda. Weak signals of selection corresponding to the eye diacylglycerol kinase and Gste-2 were detected at approximately 13.5Mb on the X chromosome and 76Mb on the 2RL

chromosome respectively. The known in DDT and permethrin resistance associated variants in the *Gste2* locus, L119F and L119V, were identified. Embedding genomic surveillance in cRCTs enables the vector control community to discover putative resistance variants in a timely fashion and also can provide evidence of their impact on vector control tool efficacy.

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CONTINUOUS VITAL SIGN MONITORING OF INDIVIDUALS WITH ACUTE LASSA FEVER USING WEARABLE BIOSENSOR DEVICES

Brady Page[1], Matthias Pauthner[2], Raphaëlle Klitting[3], Foday Alhasan[4], Donald Grant[4], Kristian Andersen[2]

[1]University of California, San Diego, La Jolla, CA, United States, [2]Scripps Research Institute, La Jolla, CA, United States, [3]Aix-Marseille University, Marseille, France, [4]Kenema Government Hospital, Kenema, Sierra Leone

Lassa fever is a fulminant viral illness associated with high in-hospital mortality. In Sierra Leone, continuous monitoring of critically ill patients is hindered by a lack of equipment and personnel. In this study, we used wearable biosensor devices to remotely monitor hospitalized individuals with confirmed acute Lassa fever (n=30) in order to describe vital sign trends that may be associated with clinical outcome and to evaluate the feasibility of this approach in such a setting. A substantial amount of physiological data had to be discarded for low quality, yielding 8 patients with 558.4 hours of waveform data to be analyzed, with an average of 69.8 hours (12.6 - 133.3 hours) per patient. The median age of participants included in the analysis was 6.5 years (0.4-40 years), 50% were female, the median time from onset of symptoms to admission was 7 days (2-21 days), and rapid malaria test was positive in 50% of participants. The in-hospital mortality rate was 62.5%. Our results show that individuals who died (n=5) had higher mean heart rate (HR; 126 beats per minute) and respiratory rate (RR; 29 breaths per minute), as well as lower mean heart rate variability (HRV; 10 ms), compared to those that survived (63 beats per minute, 22 breaths per minute, and 59 ms, respectively). These findings are align with prior data regarding the relationship between HR, RR, HRV, and mortality in bacterial sepsis. Some interesting physiological phenomena were captured by the biosensors, including an episode of rapid atrial fibrillation that repeatedly was broken by cough-induced increase in vagal tone. Periods of clinical decompensation were able to be identified by captured vital sign changes. Although real-time monitoring of vital signs using wearable biosensors may have the potential to identify decompensations earlier than traditional bedside vital sign collection in a resource-limited setting, we encountered issues with device's data quality. Namely, there were issues with adhesion, connectivity, and rare spurious vital sign readings.

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STANDARDIZED BRIGHTON COLLABORATION CASE DEFINITIONS AND COMPANION GUIDES ON ADVERSE EVENTS OF SPECIAL INTEREST FOR HARMONIZED SAFETY MONITORING OF LASSA FEVER VACCINES

Wan-Ting Huang, Barbara Law, Dale Nordenberg, Ariel Zadok, Robert T. Chen

Safety Platform for Emergency vACcines (SPEAC) Project, Decatur, GA, United States

Lassa virus (LASV) is a zoonotic pathogen causing Lassa fever, a severe hemorrhagic disease endemic in West Africa. The WHO lists LASV as a priority pathogen needing new countermeasures. The Brighton Collaboration has advanced the science of vaccine safety since 2000. The Coalition for Epidemic Preparedness Innovations has funded Brighton's Safety Platform for Emergency vACcines (SPEAC) project to facilitate harmonized safety assessment of novel vaccine candidates for LASV and other priority pathogens with epidemic or pandemic potential. SPEAC conducted landscape literature reviews to generate a list of adverse events of special interest (AESIs) to be monitored for LASV vaccines, including those relevant for maternal immunization studies. These AESIs (https://speacsafety.net/tools/aesi-lists/lassa-fever/) are selected based on their association with immunization or vaccine platforms, theoretical links from animal models, or possible occurrences due to wild virus replication or virus-host immunopathogenesis. A prioritization of AESIs was conducted for the development of standardized case definitions, along with companion guides containing references on risk factors and background rates of these events; diagnostic codes; and structured data collection forms with algorithms to determine level of diagnostic certainty. Currently, 14 case definitions and 12 companion guides, including one for sensorineural hearing loss (SNHL), have been published. SPEAC is digitalizing the data collection forms and case logic algorithms into REDCAP forms and online Automated Brighton Classification (ABC) tools. Six case definitions, covering conditions such as anaphylaxis, generalized convulsion, Guillain-Barré syndrome and Fisher syndrome, myocarditis and pericarditis, SNHL, and thrombocytopenia, will be prioritized for this digitalization process. SPEAC is transforming knowledge and literature into practical tools that support harmonized safety assessment of LASV vaccines from clinical trials to postmarketing studies. All resources are publicly accessible on speacsafety.net and brightoncollaboration.org.

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SEROLOGIC EVIDENCE OF DENGUE AND CHIKUNGUNYA AMONG PATIENTS WITH ACUTE FEBRILE ILLNESS IN GHANA 2016 - 2018

Abigail Akua Abankwa

Noguchi Memorial Institute for Medical Research, Legon, Accra, Ghana

Dengue and Chikungunya Virus diseases are mosquito-borne tropical diseases, that has globally appreciated. These infections are characterized by febrile illness and a rash in most cases. Patients presenting with symptoms due to these infections in Ghana are usually screened for malaria, typhoid, or Yellow Fever virus. However, screening is rarely done for other possible causative agents such as Dengue fever and Chikungunya virus. A study was conducted in health facilities in seven selected regions in Ghana: namely, Ashanti, Greater Accra, Northern, Upper West, Volta, Western, and Western North regions. Patients who met the case definition were enrolled in the study. A total of 1105 blood samples were collected from patients from 2016 to 2018 and serological analysis of Dengue and Chikungunya viruses were performed with ELISA IgM and IgG commercial kits (Abcam, Cambridge, UK). However, for Chikungunya, only 1053 samples were tested. Analyzed results indicated that the Dengue fever virus and Chikungunya virus showed positivity rates of 61.63% and 40.27% respectively. Indicating the percentage of the study participants exposed to dengue fever virus and chikungunya virus. Greater Accra and Ashanti regions recorded the highest positivity for Chikungunya and Dengue fever viruses respectively. This study sought to establish a differential diagnostic system for Dengue and Chikungunya Viruses and to identify these viruses if they were in circulation in selected health facilities in Ghana.

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MAPPING THE GLOBAL BURDEN OF CHIKUNGUNYA

Miranda Erin Heilweil[1], James Ashall[2]

[1]Management Sciences for Health (MSH), Arlington, VA, United States, [2]London School of Hygiene and Tropical Medicine, London, United Kingdom

Chikungunya is a re-emerging arbovirus that presents a significant global health risk due to its potential to cause severe epidemics, including a recent outbreak in Paraguay. Harmonization of Chikungunya virus (CHIKV) surveillance and reporting systems is needed to improve prioritization of disease control interventions, including recently-approved vaccines. This study aimed to improve understanding of global Chikungunya risk and the burden of disease. Geospatial mapping of the global burden of CHIKV was performed utilizing surveillance data extracted from government and international health agency sources. A novel disease severity grading scale, including three additional high-risk classifications, was developed and applied to evaluate global and sub-national risk. This scale enabled high resolution geospatial modeling of CHIKV and was critical for modeling

subnational trends in Brazil, Paraguay, and India. Disability-adjusted life years (DALYs) were calculated for WHO regions and Americas subregions. An open-source risk map was produced showcasing the global distribution of CHIKV. Available data indicate that over 425,000 cases of Chikungunya occurred globally in 2022, a figure that is 17% higher than presented by recent international reports. CHIKV caused an estimated loss of at least 130,000 annualized global DALYs, with a greater proportion occurring in the WHO South-East Asia Region (SEARO) than previously suggested by the literature. Despite this, vaccine prioritization was low, even in high-burden countries. These estimates confirm CHIKV as an arbovirus that causes significant morbidity in the WHO Region of the Americas and SEARO regions. Disease distribution is spatially and temporally heterogeneous, with a shift in disease burden from the Caribbean to South America over the last decade. A Chikungunya vaccine is likely better targeted towards outbreak response compared to routine immunization.

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NOVEL RT-QPCR ASSAY FOR THE DETECTION AND QUANTIFICATION OF GROUP C ORTHOBUNYAVIRUS

Roger M. Castillo, Maria E. Silva, Alfredo A. Huaman, Megan A. Schilling

NAMRU SOUTH, WASHINGTON DC, DC, United States

Group C orthobunyaviruses (GRCVs) are an emerging group of arboviruses transmitted primarily by mosquitoes of the genus *Culex*. Infection with the GRCV causes malaise, fatigue, and loss of capacity for working; symptoms usually last about a week. For military forces deployed in endemic areas, particularly in tropical and subtropical areas of South and Central America, GRCV infections can reduce operational effectiveness and impact the ability to accomplish a mission. In recent years, climate change and demographic factors have been shown to foster the adaptation and proliferation of vector mosquitoes in urban areas, increasing the risk of arbovirus transmission. Hence, the transmission of emerging viruses that have the potential to cause outbreaks and epidemics in these regions, like GRCV, has increased. In previous years, GRCV detections were made by classical tools such as virus culture and serologic tests, which are expensive and time consuming. Currently, there is still lack of molecular tools to detect rapidly GRCV infections in human samples and vectors. Here, we describe a new TaqMan-based reverse transcription quantitative PCR (RT-qPCR) assay for the rapid detection of GRCV by using primers and probe targeting the S genome segment. The real-time PCR assay specificity was confirmed using five GRCV references and eighteen non-GRCV arboviruses. The limit of detection of GRCV was between 0.005 to 0.05 PFU/reaction. The effectiveness of this real-time PCR assay was determined by its ability to detect GRCV positives in acute-phase clinical samples from Iquitos, Peru. The results demonstrate that this newly established RT-qPCR assay may be useful for rapid detection of GRCV infections, allowing better management of the illness and investigation of the epidemiological factors of this emerging tropical pathogen.

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USE OF A NEW WORLD HANTAVIRUS RT-QPCR ASSAY TO DETECT MULTIPLE HANTAVIRUS IN HUMAN AND RODENT SAMPLES IN THE AMERICAS

Inna Krapiunaya[1], Maria E. Morales-Betoulle[1], Julia Brignone[2], Jacqueline Salazar[3], Roxana Loayza[4], Shannon Whitmer[1], Cinthia A. Ardaya[4], Jimmy Revollo[4], Nelly M. Loayza[4], Maria A. Morales[2], Maria L. Martin[2], Anabel Sinchi[2], Blas Armien[3], Jairo M. Rico[5], Lionel Gresh[5], Austin Leach[1], Leanna Sayyad[1], Tara Sealy[1], Elizabeth Shedroff[1], Caitlin M. Cossaboom[1], Trevor Shoemaker[1], John D. Klena[1], Joel M. Montgomery[1]

[1]CDC, Atlanta, GA, United States, [2]Instituto Nacional de Enfermedades Virales Humanas Dr. Julio I. Maiztegui, , Argentina., Pergamino, Argentina, [3]Instituto Conmemorativo Gorgas de Estudios de la Salud, Panama, Panama, Panama, [4]Centro Nacional de Enfermedades Tropicales, Santa Cruz de la Sierra., Bolivia, Santa Cruz, Bolivia, Plurinational State of, [5]Pan American Health Organization, Washington DC, USA, Washington, DC, United States

Hantavirus pulmonary syndrome (HPS) cases and non-HPS hantavirus infections are often overlooked in South and Central America. Multiple New World hantaviruses have been reported, however, hantaviruses remain underdiagnosed likely due to lack of awareness and testing in some endemic areas. Serological assays and end-point RT-PCR have been previously used to diagnose hantavirus infections. Since the COVID-19 pandemic, the capacity for laboratories in South and Central America to perform molecular diagnostic testing has significantly increased. The Pan American Health Organization and WHO Collaborating Centers in the US, Argentina, Panama and Bolivia, have identified a necessity to validate hantavirus molecular assays that could be implemented in the region. A New World hantavirus RT-qPCR assay (targeting the N gene) developed at the CDC, was tested using multiple New World hantavirus species in the US Centers for Disease Control and Prevention collection including Andes, Black Creek Canal, Convict Creek, Lechiguanas, Laguna Negra, Maciel and Sin Nombre virus, as well as the Old World hantaviruses, Seoul, Hantaan and Puumala viruses. The RT-qPCR detected all New World hantaviruses, while not detecting Old World hantaviruses. The exclusivity of the assay was confirmed by testing with other pathogens: arenaviruses, leptospira, rickettsia and malaria This assay detected hantavirus RNA in human samples collected from acutely ill individuals in Bolivia in 2018 and 2019. Of 14 patients with available clinical data, IgM and IgG antibodies were identified in 12 (86%) and 7 (50%) blood specimens, respectively; 11 (79%)were RT-qPCR positive. Metagenomic next generation sequencing identified Oran, Tunari, Alto Paraguay-like, Laguna Negra, and Lechiguanas hantaviruses from various geographic locations in Bolivia. We report here the New World hantavirus RT-qPCR results and the phylogenetic tree from Bolivia as well as recent data generated in Argentina and Panama with this assay. This work will help improve monitoring of hantavirus circulation in the Americas.

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SEROPREVALENCE OF LASSA AND OTHER EMERGING AND RE-EMERGING VIRUSES CIRCULATING IN HUMANS AND ANIMALS (DOGS AND RODENTS) IN LIBERIA

Varney M. Kamara

University of Hawaii, Honolulu, HI, United States

Liberia is among the low and middle-income countries that remain the home to Lassa Fever (LF). Like most emerging and re-emerging viral diseases, LF remains a problem due to the limited healthcare infrastructure and poor laboratory-based surveillance system necessary for proper management and control of diseases. Over 70 % of these emerging and re-emerging viral diseases have a zoonotic origin, with more than three-quarters of the emerging zoonosis pathogens originating from wildlife, constituting an ongoing threat to human and animal health, worldwide livelihoods, and economies. The objective of our study was to estimate the prevalence of LF and other emerging and re-emerging infectious diseases among people living in communities known as the Lassa belt in Liberia, their dogs, and rodents that live in close contact with them for seroreactivity. Approximately 6000 serum samples (200 healthy individuals with consent, 200 rodents, and 200 dogs) were collected between January and July 2021 from four regions/counties (Bong, Lofa, Nimba, and Grand Bassa counties), known as the Lassa fever belts. analyzed using a multiplexed MAGPIX assay to detect humans, dogs, and rodents IgG antibodies against a panel of virus antigens. The overall prevalence for LASV and other emerging and re-emerging diseases in the four regions was estimated at LASV, followed by SARS-CoV-2 S, CHIKV E2, and PUUV. The filoviruses, including EBOV, MARV, and SUDV, were extremely low in humans. A similar pattern was observed in the rodents. In the dogs, we observed low reactivity to all the antigens. There was a correlation between LASV GP & NP and SARS-CoV-2 S & NP. Interestingly, we see a correlation between LASV and

humans and rodents, clearly highlighting that there is an interlink between human and rodent transmissible diseases. This shows the importance of using the One Health approach in disease surveillance.

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EVIDENCE OF CO-TRANSMISSION OF ZIKA VIRUS DURING THE 2023 DENGUE OUTBREAK IN BANGLADESH

Anamul Hasan, Md Fahad Zamil, Afrida Tabassum Trina, Mohammad Sharif Hossain, Asifa Kumkum, Dilruba Ahmed, Mohammad Shafiul Alam

icddr,b, Dhaka, Bangladesh

Bangladesh saw its worst dengue outbreak in its history in 2023 when more than three hundred thousand people were hospitalized with a case fatality rate of 0.5% the highest in the world of that year. While Zika is a mosquito-borne virus transmitted by Aedes mosquitoes like dengue, it is primarily known for causing severe birth defects, including microcephaly, in babies born to infected mothers. Zika typically causes dengue like mild symptoms in adults. However, it can also lead to serious neurological complications like Guillain-Barré syndrome. Despite detecting the indirect evidence of the Zika virus in archived samples on rare occasions, the absence of a functional surveillance system prevented confirming its presence in any concurrent circulation. We identified a cluster of Zika cases during a diagnostic evaluation study for dengue. We enrolled 185 individuals coming at icddr,b diagnostic unit at Mohakhali, Dhaka from October 15 to December 31, 2023 with dengue-like symptoms including fever for 2-5 days coming for a confirmatory diagnosis. An NS1-based rapid diagnostic test (RDT) was performed as a routine test according to national guidelines. Regardless of their RDT test status, 152 samples were tested later with a commercial RT-PCR kit for the presence of dengue, Chikungunya and Zika viruses. Among them, 32.9% (50/152) were positive for dengue. However, among the negative samples, four were positive for Zika and one dengue-positive sample was found to be co-infected for a total of 3.3% (5/152) being positive for Zika. All of the identified cases were male, living within a kilometre radius of each other and without prior travel history outside of the country in the past two years indicating a local Zika virus transmission in the community. The identification of Zika virus co-circulating with dengue in an outbreak suggested a serious threat to public health in the coming years in Bangladesh. It is urgently needed to conduct emergency sero-surveillance to identify the spatiotemporal clusters of Zika virus and perform effective control measures to prevent the further spreading of Zika virus in Bangladesh.

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ASSOCIATION BETWEEN ANGIOTENSIN-CONVERTING ENZYME 2 SINGLE-NUCLEOTIDE POLYMORPHISMS AND RISK OF SARS-COV-2 INFECTION IN A GHANAIAN POPULATION

Alexander Owusu Boakye[1], Anthony Afum-Adjei Awuah[1], Christian Obirikorang[2], Evans Asamoah Adu[2], Doris Winter[3], Eva Lorenz[3], Nicole Gilberger[3], John Amuasi[1]

[1]Kumasi Centre for Collaborative Research in Tropical Medicine (KCCR), Kumasi, Ghana, [2]Department of Molecular Medicine, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, [3]Department of Infectious Disease Epidemiology, Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany

Angiotensin-converting enzyme 2 plays a pivotal role in the development of COVID-19, caused by the novel severe acute respiratory coronavirus 2. Individual susceptibility to COVID-19 has been strongly linked to single-nucleotide polymorphisms in the angiotensin-converting enzyme 2, which may alter its expression or binding affinity to the virus. This study examined a total of 749 SARS-CoV-2-specific IgG seronegative and 890 SARS-CoV-2-specific IgG seropositive individuals obtained from a population-based SARS-CoV-2-specific IgG seroprevalence study conducted between February 2021 and February 2022, using a highly specific and approved Enzyme-Linked Immunosorbent Assay to investigate the association between two important angiotensin-converting enzyme 2 single-nucleotide changes, hypothesized to downregulate angiotensin-converting enzyme 2 levels, (rs2285666-C>T and rs2106809- A>G) and the risk of COVID-19 infection among a Ghanaian population. Extracted host DNA from collected blood samples was genotyped using the Allele-Specific Oligonucleotide Polymerase Chain Reaction technique, with melting curve analysis. Associations between the single-nucleotide polymorphisms and COVID-19 were assessed using logistic regression models. Participants did not differ in terms of demographics and the distribution of allele and genotype frequencies, except for rs2106809 among males. The T-allele of rs2285666 was observed to significantly reduce SARS-CoV-2 infection risk among Ghanaian females. However, having two copies of the T-allele did not offer additional benefits. Interestingly, no association was observed for rs2106809. These findings provide preliminary evidence that suggests that variations in the angiotensin-converting enzyme 2 might influence COVID-19 infection among Ghanaians, aiding ongoing discussions on the COVID-19 genetic basis, which is important to inform strategies and policies for treatment, prevention, risk assessment, and diagnosis.

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CRYPTIC CIRCULATION, PERSISTENCE, AND POSITIVE SELECTION OF YELLOW FEVER VIRUS IN COLOMBIA

Lester J. Perez[1], Laura S. Perez-Restrepo[2], Karl Ciuoderis[2], Jaime Usuga[2], Isabel Moreno[2], Vanessa Vargas[2], Angela J. Arévalo-Arbelaez[2], Michael G. Berg[1], Gavin A. Cloherty[1], Juan Pablo Hernández-Ortiz[3], Jorge E. Osorio[4]

[1]Abbott Laboratories, Abbott Park, IL, United States, [2]GHI One Health Colombia, Universidad Nacional de Colombia, Medellín, Colombia, [3]Department of Materials and Nanotechnology, Universidad Nacional de Colombia, Madison, WI, United States, [4]Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin, Madison, WI, United States

Yellow fever virus (YFV) is a cause of acute febrile illness (AFI) endemic to tropical areas of South America and Africa and transmitted by *Aedes* mosquitoes. In Colombia, YFV causes periodic outbreaks, during 2020 to 2023, we conducted health facility based AFI surveillance in Leticia. The study enrolled 1,460 individuals with AFI of unknown etiology, from which 120 specimens were randomly selected for metagenomic next generation sequencing (mNGS). Complete genome coverage of YFV was obtained from the serum of a 23-year-old male presenting with fever, vomiting, chills, headache and body/muscle pain. It is unknown if the individual received prior yellow fever vaccination. Maximum likelihood tree of all YFV references indicated this strain (LET1450) branched with Bolivian sequences and formed a monophyletic clade within South American genotype II (SamII). This strain's ancestor emerged around 1989 and Skygrid analysis shows that despite a decline in YFV genetic diversity in 1951 coinciding with the beginning of global vaccination efforts, a slight rebound was observed between 1978 and 1985 which corresponded with the emergence of SamII. The subsequent formation of the Peru/Bolivia/Colombia cluster within SamII resulted from positive episodic selection. Examination of the envelope protein identified an A343S mutation under positive selection found exclusively in this SamII cluster. This mutation, situated in an exposed loop of domain III, suggests evasion of vaccine-mediated immunity. Discrete phylogeographic analysis undertaken to identify the putative origin and assess the geographic circulation of the LET1450 strain revealed two major importation events of YFV to Colombia from Peru and Bolivia, but it also revealed that Colombian strains were being transmitted back to Bolivia. Markov jump reconstruction further confirmed that the prevalence of YFV in Colombia is not due to repeated external introductions, but rather results from continuous, cryptic internal circulation. Our study highlights the value of mNGS for determining causes of AFI and for surveilling the emergence of potential vaccine evading strains.

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PERFORMANCE OF THE NG-TEST® IGG/IGM COVID-19 RAPID TEST FOR THE DIAGNOSIS OF SARSCOV2 INFECTION AMONGST HEALTHCARE WORKERS IN BAMAKO, MALI

Karamoko Tangara[1], Mathias Kamate[1], Merepen Dite Agnes Guindo[1], Drissa Konate[1], Bourama Traore[2], Fousseyni Kane[2], Housseini Dolo[1], Salimata Kante[1], Abdouramane Traore[1], Bourama Keita[1], Issoufi Y Maiga[1], Seydou Doumbia[3], Saidou Balam[1], Mahamadou Diakite[3]

[1]international Center For Excellence In Research, Bamako, Mali, [2]university Clinical Research Center (Ucrc), Bamako, Mali, [3]international Center For Excellence In Research, University Clinical Research Center (Ucrc), Bamako, Mali

Coronavirus 2019(COVID19) represents a worldwide public health emergency with an impact on economic and socio-cultural development. Although the PCR technique remains the gold standard of COVID-19 diagnosis, rapid diagnostic tests (RDTs) are an alternative in resource-limited countries such as Mali. However, adhesion of health care workers to the use of these RDTs still a problem due to the low performance. This study aimed to assess the performance diagnostic of NG-Test® IgG/IgM COVID19 rapid test to reinforce COVID19 case management in Mali. As part of a cohort study amongst health workers (HW), a cross-sectional survey was conducted in May 2022 amongst HW in six health centers and deux university hospitals of Bamako. Sociodemographic and clinical data, Nasopharyngeal swabs and blood sample were collected to determine SARS-CoV-2 infection by RT-qPCR and RDT (NG-Test® IgG/IgM). REDCap application was used for data management, Stata 14 software for data analysis and. KAPPA was used to determine the concordance between the results RT-qPCR (gold standard) and RDT with a significant level at 5%. A total of 917 health workers were included. The prevalence of SARS-CoV-2 infection was 1.3%. The sensitivity was 25%, specificity 76%, Predictive values of positive test 1,4%, Predictive values of negative test 98,7%, with a Kappa of 0.001 for RDT (IgM) compared to PCR. The sensitivity was 25%, specificity 75%, Predictive values of positive tests 1,3%, Predictive values of negative tests 99% with a Kappa of -0.0002 for RDT (IgG) compared to PCR. The NG-TEST® IgG-IgM COVID-19 test performance in the diagnosis of SARSCoV2 infection was very low compared to PCR.

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DETECTION OF NEUTRALIZING ANTIBODIES AGAINST ARBOVIRUSES FROM LIVER HOMOGENATES

Thais Alkifeles Costa[1], Matheus Soares Arruda[1], Gabriela Fernanda Garcia Oliveira[1], Erik V. Reis[1], Anna Catarina D.S. Guimarães[1], Gabriel Dias Moreira[1], Nidia E.C Arias[1], Marina do Vale Beirão[1], Nikos Vasilakis[2], Kathy A. Hankey[3], **Betania P. Drumond**[1]

[1]Federal University of Minas Gerais, Belo Horizonte, Brazil, [2]University of Texas - Medical Branch, Galveston, TX, United States, [3]New Mexico State University, Las Cruces, NM, United States

Yellow fever virus (YFV) circulates in a sylvatic cycle between non-human primates (NHPs) and arboreal mosquitoes in Brazil. Passive monitoring of ill or deceased NHPs is a key component of the Brazilian YF surveillance program. Samples from NHP carcasses are usually suitable for molecular tests but not for serological assays. As an alternative to the conventional plaque reduction neutralization test (PRNT) based on sera, we tested the utility of liver homogenates from experimentally infected (with YFV, Mayaro virus [MAYV], chikungunya virus [CHIKV], or mock) mice to quantify PRNTs. Although homogenates from mock-infected mice showed a low level of nonspecific virus neutralization against YFV, MAYV or CHIVK, homogenates from YFV-, MAYV- and CHIKV- infected mice demonstrated significantly higher levels of virus neutralization compared to controls. Receiver operating characteristic (ROC) curves analyses were performed using the median neutralization values of three technical replicates for each infected group separately or collectively. Results showed scores above equal or higher than 0.97 (95% CI equal or higher than 0.89-1.0) for the area under the curve at dilutions 1:20 to 1:80, suggesting that median virus neutralization values effectively differentiated YFV-, MAYV-, or CHIKV-infected groups from controls. Liver homogenates obtained from 25 NHP carcasses (collected during the 2017 YFV outbreak in Brazil) were also tested using both the adapted PRNT as well as rapid anti-YFV IgM immunochromatographic tests. Neutralization activity was detected in 6 NHP samples that were also positive by PCR and anti-YFV IgM tests and one sample that tested negative by PCR and IgM test. Our results demonstrate the feasibility of using liver homogenates as an alternative approach for serological investigation in viral epidemiologic surveillance. Carcasses are convenient samples that can be used for outbreak investigations, and the potential use of liver homogenates in serological tests expands the possibilities for the investigation of outbreaks and epizootics.

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A HIGH-THROUGHPUT LIVE-IMAGE REPORTER FLAVIVIRUS NEUTRALIZATION ASSAY PLATFORM FOR SEROSURVEILLANCE AND VACCINE EVALUATION

Bailey E. Maloney, Janae Raetz, Karen Boroughs, Alec Hutchison, Claire Huang

Centers for Disease Control and Prevention, Fort Collins, CO, United States

Virus neutralization tests, such as plaque (or viral focus) reduction neutralization tests (PRNT/FRNT), used to measure neutralizing antibodies elicited by virus infection or vaccination are critical tests for diagnostics, vaccine evaluation, and serosurveillance. To significantly improve the time/labor-intensive gold-standard PRNT for many medically important flaviviruses, we have engineered a panel of live-reporter flaviviruses to develop a high-throughput reporter-based micro-focus reduction neutralization test (R-mFRNT). The reporter flaviviruses express a strong tetrameric ZsGreen fluorescent protein within 24-28 hours post cell infection, and the intensive fluorescent viral foci can be accurately measured through a live-image cell cytometry plate reader. All reporter viruses were optimized for reporter stability after multiple cell passages, and validated by genome sequence, reporter RT-PCR, and dual-color (viral antigen and reporter) flow cytometry to be qualified for use in R-mFRNT. We have made more than 12 different reporter-flaviviruses for the R-mFRNT and verified that the neutralization antibody titers obtained from the R-mFRNT using the reporter viruses were equivalent to titers obtained by PRNT using wild-type viruses. The reporter WNV-based chimeric platform used to derive the reporter flaviviruses affords an identical high-throughput R-mFRNT workflow for multiple flaviviruses. So far, we have validated and used the assay for Zika vaccine studies, neutralizing IgM evaluation of dengue and Zika viruses, neutralizing antibody profiling after sequential flavivirus infections, and a serosurveillance of Powassan virus that required processing a large number of samples. We will report an update of the reporter flavivirus panel, including validation and characterization of several newly generated reporter viruses, the workflow of the R-mFRNT, and summary of study outcomes using the assay.

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PREDICTING THE IMPACT OF A POTENTIAL CHIKUNGUNYA OUTBREAK IN MIAMI AND THE IMPACT OF A CHIKUNGUNYA VACCINE

Adrianne M. de Roo[1], Bert Sloof[2], Martijn Boer[2], Gerard T. Vondeling[1]

[1]Valneva Austria GmbH, Vienna, Austria, [2]Asc Academics, Groningen, Netherlands

The chikungunya virus (CHIKV), an alphavirus transmitted primarily by *Aedes* mosquitoes, poses a significant global health threat. In the past two decades, we have seen a notable expansion of CHIKV's geographic reach due to climate changes and increased human mobility amongst others. In the United States (US), a total of 3,941 travel-acquired cases were reported between 2014 and 2016, and in June 2014, the first locally acquired CHIKV case was reported in Florida. A CHIKV outbreak in a big city such as Miami could have detrimental consequences, however, the potential impact has

not been studied so far. With this study, we aim to prepare public health authorities by quantifying both the potential size of an outbreak and the effectiveness of a reactive vaccination program. We developed a dynamic transmission model with a host-vector structure calibrated to incidence data from Puerto Rico using the Generalized Reduced Gradient (GRG) algorithm. The mosquito parameters within the model were climate-dependent and were derived from daily temperature, precipitation level, and relative humidity data specific to Miami. Model outputs suggest that with current routine vector control and climate conditions, approximately 10% of the local population of Miami could become infected with CHIKV in the first year after a potential outbreak. Implementing an emergency response vaccination program with 20% vaccine coverage would reduce the number of infections by 83%. Our model results show that an emergency response vaccination program can be effective during a CHIKV outbreak, especially if the outbreak is detected early on and the program is initiated promptly after detection. Future research should explore the applicability of these findings in other locations where *Aedes* mosquitoes are present.

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CHIKUNGUNYA INFECTION IN PERUVIAN PATIENTS WITH ACUTE FEBRILE ILLNESS: PREVALENCE AND CLINICAL CHARACTERISTICS

Miguel A. Aguilar-Luis[1], Hugh Watson[2], Carlos Holguin[3], Yordi Tarazona-Castro[4], Brenda Tapia[3], Wilmer Silva-Caso[1], Juana del Valle Mendoza[5]

[1]Universidad Peruana de Ciencias Aplicadas, Lima, Peru, [2]Evotec ID, Lyon, France, [3]Direccion Regional de Salud Piura, Piura, Peru, [4]Instituto de Investigacion Nutricional, Lima, Peru, [5]Universidad Peruana de Ciencias Aplicadas, lima, Peru

Chikungunya fever (CHIKF) is an emerging zoonotic disease that presents as an acute febrile illness (AFI), classically associated with arthralgia. Due to limitations regarding availability of diagnostic tests and health system access, cases may be underreported. Therefore, we performed a study to evaluate in the northern coast of Peru to measure its prevalence and describe their clinical manifestations. We conducted a 2-year cross-sectional study in Piura region, Peru, located in the north coast of Peru, neighboring Ecuador, and Colombia. Patients presenting with AFI to primary care clinics were included. Serum plasma collection was performed in all participants and evaluated for chikungunya virus (CHIKV) serology and molecular diagnosis with RT-PCR. Our study location was also endemic for Dengue virus (DENV); thus, IgM was also analyzed for coinfection evaluation. A total of 688 samples were collected and 669 were analyzed. CHIKV was detected in 60 (8.89%) samples through serology. Only 5% of the cases were identified with RT-PCR. CHIKV cases were most reported among participants aged 18-29 years old (30.0%) and the most common symptoms reported were headaches (68.0%), myalgias (54.4%) and arthralgias (50.8%). Coinfection with DENV was also reported (5.0%) among CHIKV samples. We report a significant prevalence of CHIKV in a northern coast of Peru and a considerable prevalence of CHIKV-DENV coinfection. These results highlight the need for improved surveillance of CHIKV, as it is a continuously transmitted pathogen in various parts of Peru. To accurately detect CHIKV, epidemiological surveillance should be strengthened using reliable diagnostic methods, as clinical symptoms may be unspecific.

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A COMPARISON OF THREE DIAGNOSTIC TESTS TO DETECT HUMAN PAPILLOMAVIRUS IN ASYMPTOMATIC WOMEN'S ENDOCERVICAL SAMPLES FROM 2022 TO 2023 IN A NORTHERN PERUVIAN REGION

Deysi Aguilar-Luis[1], Juana del Valle-Mendoza[1], Lorena Becerra Goicochea[2], Jorge Bazan-Mayra[3], Hans Huayta Campos[2], Jhony Barrantes Herrera[2], **Miguel A. Aguilar-Luis**[1]

[1]Universidad Peruana de Ciencias Aplicadas, Lima, Peru, [2]Hospital Regional Docente de Cajamarca, Cajamarca, Peru, [3]Direccion Regional de Salud Cajamarca, Cajamarca, Peru

Infection with the Human papillomavirus (HPV) is one of the causes of cervical carcinoma, which is on the rise. There has been no improvement in the development of research tools or testing methods. The Pap smear is the only method used to detect dysplasia and cervical cancer in developing countries. Therefore, we compared the concordance and reliability of three HPV tests and the Papanicolaou smear as primary cervical cancer screening methods. This study included 135 patients co-tested with HPV test and Pap smear simultaneously. The results of sensitivity, specificity, Positive predictive value (PPV) and Negative predictive value (NPV) and Cohen's Kappa index (agreement) were compared with the Gold standard. The tests were coded for statistical analysis (Sequencing "Test1", a commercial DNA_Flow kit "Test2", Multiplex PCR "Test3") and the Pap smear "Test4". We detect that 66.67% (90/135) were positive for HPV and 33.33% (45/135) were negative for HPV. The 17% (24/135) were positive PAP results with high-grade lesions, of which 25% (6/24) of women were HPV positive (6/24) and 75% (18/24) negative for HPV. The Test1, Test2 and Test3 detected; 82(60.74%), 97(71.85%) 91(67.41%), respectively. The sensitivity of Test2 (96.67%) was higher, and the specificity (77.78%) and NPV (89.69%) were lower compared to the other methods. Test1 presented greater specificity (100%) and NPV (100%) compared to the other methods. The Test1 showed one k=0.87, very good agreement, and the Test2 and Test3 showed k=0.78 and k=0.75, respectively, one moderate agreement. Also, we observed that higher rates of multiple infection (71.13%) and (70.33%) were obtained through Test2 and Test3, respectively. Infection rates were high in Test1. There is a difference between the Pap test and the HPV test. These tests must be accompanied by HPV tests. All HPV tests showed different sensitivities greater than 90% when compared. It is important to take these data into consideration to avoid false positives and negatives.

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PREVALENCE OF HEPATITIS D VIRUS INFECTION AND ASSOCIATED FACTORS AMONG HEPATITIS B VIRUS PATIENTS FROM SELECTED HOSPITALS IN ACCRA

Miriam Eshun[1], Joseph H. K Bonney[2], Francis Anto[3]

[1]Noguchi Memorial Institute for Medical Research, University of Ghana and School of Public Health, University of Ghana, Accra, Ghana, [2]Noguchi Memorial Institute for Medical Research, University of Ghana, Accra, Ghana, [3]School of Public Health, University of Ghana, Accra, Ghana

The most severe form of viral hepatitis infection is caused by co-infection of hepatitis B and D viruses (HBV and HDV) simultaneously or superinfection of hepatitis D virus on B infected patients. In 2021, total global estimate for HBV infections was 262,240000; out of which 1,994000 were newly diagnosed infections. However, in the same year, an estimated total of 69,512000 HBV infections was reported for Africa, and 187,000 were new infections. Magnitude of HDV infection is the fast progression of disease to liver cirrhosis, hepatocellular carcinoma (HCC) and high mortality rate, and no information known in our hospitals. Aim of the study was to determine the prevalence of HDV and its associated risk factors among HBV patients from selected hospitals in Accra. It was a cross sectional purposive study in four selected hospitals in Accra, Ghana. A total of 152 eligible participants were enrolled. Serum marker HBsAg was confirmed for each blood sample and the nucleic acid was extracted and purified from the positives. Molecular amplification assays were used on the positives and high yielding

nucleic acid positives were sequenced, and the phylogeny determined. Prevalence of HDV in the total number of participants screened was 1.4% (2/144). Out of the eligible participants who enrolled (152), 8 tested negative for serum marker, HBsAg. Genotype of the sequenced HDV positive was HDV-1. There was association between age, HBV and HDV infections with their p-values 0.022 and 0.037 respectively. An association was established between multiple sexual partners and HDV infection with a p-value 0.039. Males were mostly infected with the HBV; 9 of them having HCC. Among the age groups, 30 to 39 years were the most infected with HBV (28.3%). The 2 male HDV positives were within this age group; and had multiple sexual partners. The 1.4% prevalence of the HDV infection suggest the presence of the virus among the participants and presents a public health threat in the study communities which are endemic for HBV. We advocate awareness creation through education, HBV screening and vaccination for all, especially, young adults in HBV hyperendemic countries.

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ACCURACY OF PHYSICIANS' CLINICAL DIAGNOSIS OF DENGUE AMONG PATIENTS PRESENTING TO EMERGENCY ROOMS — PUERTO RICO, 2012-2022

Joshua M. Wong[1], Zachary J. Madewell[1], Alfonso Hernandez-Romieu[2], Janice Perez-Padilla[1], Liliana Sánchez-González[1], Diego Sainz[3], Jorge Bertran[3], Jorge Munoz[1], Vanessa Rivera-Amill[4], Gabriela Paz-Bailey[1], Laura E. Adams[1]

[1]Centers for Disease Control, San Juan, PR, United States, [2]Centers for Disease Control, Atlanta, GA, United States, [3]Auxilio Mutuo Hospital, San Juan, PR, United States, [4]Ponce Health Science University, Ponce, PR, United States

Clinically diagnosing dengue is challenging due to overlapping symptoms with other febrile illnesses. Warning signs predict progression to severe disease & warrant admission for treatment with protocolized therapy. The Sentinel Enhanced Dengue Surveillance System enrolls patients presenting with fever or respiratory symptoms to 3 emergency departments & urgent care clinics in Puerto Rico, collecting clinical data & samples for pathogen identification. We compared the accuracy of physicians' clinical diagnosis to laboratory-confirmed dengue, defined as a positive RT-PCR ≤7 days or a positive IgM, with concurrent negative Zika testing, ≥4 days after symptoms onset. We considered "dengue" a correct diagnosis. Non-dengue or nonspecific diagnoses were considered incorrect. Among 43,608 participants, 1,432 (3.3%) had laboratory-confirmed dengue. Clinical dengue diagnosis had a sensitivity, specificity, positive predictive value, & negative predictive value of 40.7%, 98.2%, 43.9%, & 98.0%, respectively. Sensitivity was highest among children 10-19 yr (53.5%) & lowest among children 1-4 yr (17.2%) & adults ≥50 yr (20.9%). More dengue warning signs correlated with higher clinical diagnosis sensitivity ($p<0.001$), reaching a maximum sensitivity of 64.0% with ≥5 warning signs. Sensitivity did not vary by specific warning signs, comorbidities, or seasonality. Sensitivity was similar during dengue epidemic years (2012-2013, 44.8%) compared to non-epidemic years with dengue transmission (2019-2022, 37.9%, $p = 0.06$). Among participants with dengue & ≥1 warning sign, hospitalization rates were higher in participants with correct diagnoses vs. incorrect (77.8% vs. 23.5%, $p <0.001$). Among 849 participants with dengue & incorrect diagnoses, the most common misdiagnoses were "viral syndrome" (n = 438, 51.6%), "fever not otherwise specified" (n = 227, 26.7%), & "pneumonia" (n = 75, 8.8%). Limited sensitivity in physicians' clinical diagnosis compared to laboratory testing resulted in inappropriate triage. Clinical training & dengue rapid diagnostic testing could improve diagnosis & increase rates of appropriate care.

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GENETIC CHARACTERIZATION OF INFLUENZA AND SARS-COV-2 IN THE DEPARTMENT OF DEFENSE BENEFICIARIES DURING THE 2023-2024 SEASON

William Gruner, Anthony Fries, Deanna Muehleman, Jimmaline Hardy, Jennifer Meyer, Kelsey Lanter, Padraic Fanning, James Hanson, Peter Wasik, Elizabeth Macias

DCPH-Dayton, Wright-Patterson AFB, OH, United States

The Department of Defense (DoD) Global Respiratory Pathogen Surveillance Program conducts testing on respiratory specimens from a worldwide network of sentinel sites using PCR-based assays and next-generation sequencing (NGS) to detect and characterize respiratory pathogens. Analyses aid in the annual selection of influenza vaccine strains and help define the impact of influenza and SARS-CoV-2 in the DoD. The program collects respiratory specimens and metadata from DoD active duty and beneficiaries with influenza-like or COVID-19-like illness symptoms across 100+ global sentinel sites. PCR confirmed influenza and SARS-CoV-2 positive specimens are further characterized by NGS. In combination with partner laboratory data, phylogenetic analyses and lineage determinations are performed to assess the genetic changes occurring in these viruses. 487 influenza viruses were analyzed, including 257 A(H1N1)pdm09, 151 A(H3N2), and 79 B/Victoria. Among A(H1N1)pdm09 viruses, 70 were clade 5a.2a and 187 were 5a.2a.1. For A(H3N2), one was clade 2a.3a and the remaining 150 were 2a.3a.1. All B/Victoria viruses were clade V1A.3a.2. For SARS-CoV-2, 685 specimens were sequenced; lineages identified included one BA.2, 36 BA.2.86, two CH.1.1, 125 EG.5, 120 HV.1, 195 JN.1, 74 XBB.1.16, 57 XBB.1.5, 38 XBB.1.9, 24 XBB.2.3, and 13 other recombinant viruses. Influenza activity was moderate for the 2023-2024 season. Circulating strains for A(H1N1)pdm09 and B/Victoria closely matched the vaccine strains genetically and antigenically, however the A(H3N2) strains drifted slightly therefore the influenza vaccine A(H3N2) strain was changed from a clade 2a virus to a 2a.3a.1 virus. SARS-CoV-2 activity was also moderate with lineage diversity remaining high throughout the season. Lineage EG.5 predominated early in the season while JN.1 predominated later in the season.

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RECONCILING HETEROGENEOUS DENGUE VIRUS INFECTION RISK ESTIMATES FROM DIFFERENT STUDY DESIGNS

Angkana T. Huang[1], Darunee Buddhari[2], Surachai Kaewhiran[3], Sopon Iamsirithaworn[3], Direk Khampaen[3], Aaron Farmer[2], Stefan Fernandez[2], Stephen J. Thomas[4], Isabel Rodriguez Barraquer[5], Taweewun Hunsawong[2], Anon Srikiatkhachorn[2], Gabriel Ribeiro dos Santos[1], Megan O'Driscoll[1], Marco Hamins-Puertolas[5], Timothy Endy[6], Alan L. Rothman[7], Derek A. T. Cummings[8], Kathryn Anderson[4], Henrik Salje[1]

[1]University of Cambridge, Cambridge, United Kingdom, [2]Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand, [3]Ministry of Public Health, Nonthaburi, Thailand, [4]State University of New York Upstate Medical University, Syracuse, NY, United States, [5]University of California, San Francisco, San Francisco, CA, United States, [6]Coalition for Epidemic Preparedness Innovations, Washington, DC, DC, United States, [7]University of Rhode Island, Kingston, RI, United States, [8]University of Florida, Gainesville, FL, United States

Revealing rates at which susceptible individuals contract a pathogen, known as the force of infection (FOI), is crucial for evaluating transmission risk and reconstructing the distribution of immunity within populations. For dengue virus (DENV), reconstructing exposure statuses is particularly important due to the strong association between prior exposure and severe disease risk, and potential detrimental effects from vaccinating DENV-naive individuals. Various study designs can be employed to measure FOI. Longitudinal cohort studies are considered the gold standard, directly tracking the transition of individuals from seronegative to seropositive states due to incident infections (seroincidence). Cross-sectional studies can provide FOI estimates by comparing seroprevalence

across different age groups, while FOI can be inferred from the ages of reported cases. However, agreement between these methods has not been adequately assessed. Drawing from 26 years of data obtained from cohort studies and hospital-attended cases in Kamphaeng Phet province, Thailand, we estimated annual FOI between 1994 and 2019 of the same population. We observed highly inconsistent FOI estimates from the three sources (seroincidence, seroprevalence, and case counts). Annual FOI estimates derived from seroincidence were 1.94 to 3.77 times higher than those derived from reported cases. Although the correlation between seroprevalence-derived and case-derived FOI was low (correlation coefficient=0.20), no systematic bias was detected. Through comprehensive simulations and theoretical analyses, we demonstrate that discrepancies between the estimates can arise from failing to account for complexity in anti-DENV antibody kinetics, assay noise, and variations in infection risk across ages. Extending standard inference models to incorporate these factors reconciled the FOI and susceptibility estimates. Our findings underscore the value of comparing inferences across multiple data types to uncover additional insights that may not be attainable through single data type analyses alone.

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EXPANSION FACTOR ESTIMATES OF DENGUE UNDERREPORTING IN ENDEMIC COUNTRIES: A SYSTEMATIC REVIEW

Justin J. O'Hagan[1], Eleanor Lucas[2], Nikolina Boskovic[3], Rosybel Drury[4], Morgan A. Marks[1], Beth Lesher[2]

[1]Merck & Co., Inc., Rahway, NJ, United States, [2]OPEN Health, Bethesda, MD, United States, [3]OPEN Health (at time of study), Bethesda, MD, United States, [4]MSD, Puteaux, France

There is a substantial burden of dengue, with the 3 largest annual epidemics globally reported since 2019. Dengue is commonly underreported in passive surveillance systems, and expansion factors (EF) are often used to correct for this. An expansion factor of 10 means that for every 1 reported case there are a total of 10 cases, including 9 unreported cases. We conducted a systematic literature review of the dengue literature to identify references calculating EF or reporting unique EF or the data needed to derive EF. We searched Embase, MEDLINE, Cochrane Library, Latin American and Caribbean Health Sciences Literature from 1995-2022, and the gray literature to identify dengue EF for endemic countries using pre-defined search terms and inclusion/exclusion criteria. We identified 31 references from 22 countries (Latin America [LATAM], n=9; Asia Pacific [AP], n=13). In total, 290 EF were identified (LATAM, n=106; AP, n=185), 87% (n=252) of all EF were calculated using country-specific empirical data, whereas 13% (n=39) were derived using expert opinion or by extrapolation. The most EF identified were for Thailand (n=45), Cambodia (n=32), and the Philippines (n=28). Of identified EF, 48% (n=140) pertained to symptomatic cases (EF range: 0-265), 21% (n=61) to hospitalized cases (EF range: 0.3-10.6), 13% (n=37) to combinations of hospitalized and outpatient cases (EF range: 2-288), 10% (n=29) to asymptomatic infections (EF range: 3-318), and 7% (n=20) to outpatient cases only (EF range: 3.7-178.8). Just 1% (n=3) of EF pertained to fatal cases. Only 3 studies (2 involving expert opinion) stratified results by a public or private facility, and all reported higher EF in private settings. In summary, we identified EF for only 22 of >100 dengue endemic countries and observed variability with how EF are derived across studies.

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HIGH PREVALENCE OF HEPATITIS B VIRUS AMONG PREGNANCY WOMEN IN GUINEA

Mamady Cisse[1], Timothe Guilavogui[1], Nene Oumou Diallo[1], Abdoulaye Toure[2], Mamoudou Conde[1], Youssou Koita[1], Laye Kaba[1], Moustapha Grovogui[1], Alimou Camara[1], Noumouke Kaba[1], Noel Tambada Guilavogui[1]

[1]ministère De La Santé Et De L'hygiène Publique, Conakry, Guinea, [2]université Gamal Abdel Nasser Conakry, Conakry, Guinea

Hepatitis B virus (HBV) infection poses a significant global challenge, particularly in developing countries. While efforts to control HIV/AIDS, tuberculosis, malaria, and neglected tropical diseases progress, hepatitis B emerges as a major public health issue. HBV infection during pregnancy carries a high risk of vertical transmission, negatively affecting both mother and child. Our study aimed to assess the prevalence of HBV in pregnant women and identify associated risk factors. We conducted a cross-sectional observational study from 1 July 2020 to 30 August 2021, involving a random sample of 5,000 pregnant women attending antenatal clinics across the country, encompassing rural and urban areas. The study, funded by a World Bank health project, collected capillary whole blood (50 µL) from the fingertip using a pipette for serum testing. The Elisa test served as a confirmatory method. We gathered data on socio-demographic characteristics and risk factors via a questionnaire developed with the Open Data Kits (ODK) application, analyzing samples using chemiluminescence. Statistical analysis utilized Pearson's chi-square or Fisher's exact test, with a significance level set at $p < 0.05$. We employed stepwise multiple logistic regression to identify HBV risk factors in pregnant women. The surveyed pregnant women, predominantly under 35 years old (90.2%), with 30.7% under 21 and 59.5% between 21 and 34, were mainly illiterate (61.8%) and married (95.8%). The study revealed HBsAg prevalence at 19.64% (95% CI [18.53%, 20.80%]) and HBeAg prevalence at 2.49% (95% CI [2.08%, 2.97%]). Significant risk factors for HBV infection included place of residence, education level, hepatitis B vaccination history, number of parities, and history of endoscopic examinations. The study indicates a high prevalence of HBsAg among pregnant women in Guinea, underscoring the need for the PMTCT programme to enhance systematic screening and vaccination against HBV during antenatal visits.

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INFORMING AN INVESTMENT CASE FOR JAPANESE ENCEPHALITIS VACCINE INTRODUCTION IN BANGLADESH

Mariana Perez Duque[1], Abu M. Naser[2], Gabriel Ribeiro dos Santos[1], Megan O'Driscoll[1], Kishor K. Paul[2], Mahmudur Rahman[3], Mohammad Shafiul Alam[2], Hasan Mohammad Al-Amin[2], Mohammed Z. Rahman[2], Mohammad E. Hossain[2], Repon C. Paul[4], Stephen P. Luby[5], Simon Cauchemez[6], Jessica Vanhomwegen[7], Emily S. Gurley[8], Henrik Salje[1]

[1]Pathogen Dynamics Unit, Department of Genetics, University of Cambridge, Cambridge, United Kingdom, [2]International Centre for Diarrheal Diseases Research, Bangladesh (icddr,b), Dhaka, Bangladesh, [3]Institute for Epidemiology, Disease Control and Research (IEDCR), Dhaka, Bangladesh, [4]Centre for Big Data Research in Health, University of New South Wales, Sydney, Australia, [5]Division of Infectious Diseases and Geographic Medicine, Stanford University, Palo Alto, CA, United States, [6]Mathematical Modelling of Infectious Diseases Unit, Institut Pasteur, Université Paris Cité, UMR 2000 CNRS, Paris, France, [7]Emerging Diseases Unit, Institut Pasteur, Paris, France, [8]Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States

Japanese encephalitis virus (JEV) circulates endemically in Bangladesh and the country is set to introduce JEV vaccine. However, knowing how best to deploy the vaccine has been hampered by an insufficient understanding of key aspects of JEV ecology, including the spatial heterogeneity in risk, and risk factors for infection. We conducted a national seroprevalence study where we visited 57 communities around the country and collected blood from randomly selected individuals (N=2,938). The blood was tested for anti-JEV IgG antibodies using a novel Luminex platform assay that

limits cross-reactivity with dengue virus. We found 3.4% (95%CI: 2.8-4.1) of participants had antibodies against JEV. We used spatially explicit mathematical models to predict risk for infection and estimated that on average 215,000 (95% CI:160,000-300,000) people get infected each year. Infection risk was greatest around pig-raising communities. For each 10 additional pigs within a 5km radius we found an increase of 1.02 in the odds of being seropositive (95%CrI:1.01-1.03). This study provides the basis to identify regions in the country where vaccine deployment would be most beneficial and, will allow us to assess the impact of different approaches in terms of health outcomes (infections, cases, deaths, and disability life-years averted) per doses used.

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DENGUE IN AMAZONAS: UNDERSTANDING SPATIOTEMPORAL DYNAMICS AND SEROTYPE CIRCULATION

Fátima Burgos[1], Milagros Saavedra-Samillán[1], Lourdes Ramírez-Orrego[1], Jesús Rascón[2], Luis M. Rojas[3], Pershing Bustamante-Chauca[3], Stella M. Chenet[1]

[1]Instituto de Investigación de Enfermedades Tropicales, Universidad Nacional Toribio Rodríguez de Mendoza de Amazonas (UNTRM), Chachapoyas, Peru, [2]Instituto de Investigación para el Desarrollo Sustentable de Ceja de Selva (INDES-CES), Universidad Nacional Toribio Rodríguez de Mendoza de Amazonas (UNTRM), Chachapoyas, Peru, [3]Dirección Regional de Salud de Amazonas (DIRESA), Chachapoyas, Peru

Peru is currently facing its worst dengue outbreak on record, with Amazonas among one of the most severely affected regions, reporting 3195 cases in five of its seven provinces in 2023. This study analyzed the spatiotemporal dynamics of dengue and climate effects in Amazonas from 2000 to 2023 and identified the circulating serotypes utilizing 420 serum samples. Climate data was acquired from NASA MERRA-2, a global high-resolution dataset. Serotype detection was performed using multiplex reverse transcription polymerase chain reaction (RT-PCR). Statistical analysis was executed with R software v.4.3.1. According to the data, major dengue outbreaks were reported in Bagua, Utcubamba, and Condorcanqui provinces between 2008 and 2011. In 2020, after the introduction of Cosmopolitan DENV-2, cases increased dramatically and expanded to Bongará (Jazán) and Chachapoyas (Balsas). By 2021, DENV-1 (46.19%) and DENV-2 (53.81%) were co-circulating across all five provinces, and the latter serotype was associated with complex clinical manifestations of the disease ($p = 0.004$). Although Bagua, Condorcanqui, and Utcubamba showed no significant correlation between incidence and climatic variables, principal component analysis (PCA) revealed these endemic regions have favorable conditions for year-round transmission. On the other hand, Chachapoyas showed a weak association between incidence and minimum temperature ($rho = 0.17$, $p = 0.03$), relative humidity ($rho = 0.15$, $p = 0.05$), and precipitation ($rho = 0.18$, $p = 0.01$) at 0-1-month lag, this implies that factors beyond climate, disrupting sanitation and facilitating population movement, played a more significant role in dengue dynamics. In conclusion, the increase in incidence and cases with warning signs were associated with the DENV-2 serotype and other factors such as hydrogeological events could have contributed to the expansion of the disease. This underscores the need for local control and prevention programs to grasp the extent of the disease and factors influencing vector establishment and arbovirus transmission.

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NEURODEVELOPMENTAL OUTCOMES IN CHILDREN WITH AND WITHOUT ZIKA, DENGUE, AND OTHER FLAVIVIRUS EXPOSURE, ZIKA EN EMBARAZADAS Y NIÑOS, COHORT, COLOMBIA

Hanvit Oh

CDC, Atlanta, GA, United States

Congenital Zika virus (ZIKV) infection is associated with severe birth defects and adverse neurodevelopmental outcomes; there is less evidence for these outcomes for dengue virus (DENV) and other flaviviruses. We aim to describe neurodevelopmental outcomes up to 18 months of age among children in Colombia by confirmed ZIKV, DENV and other flavivirus exposure *in utero* and by reported microcephaly or any brain or eye abnormality. Data are from the prospective cohort study Zika en Embarazadas y Niños, in which pregnant women were enrolled 2017-2018 and their children followed until 2020. Pregnant women were tested for ZIKV, DENV, and other flaviviruses using the Trioplex Real-time RT-PCR Assay, ZIKV *Detect* 1.0 or 2.0 IgM Capture ELISA Kit assays, and/or the Panbio Dengue IgM Capture ELISA assay. Child development in cognitive, language, and motor areas was assessed at four timepoints (6, 9, 12, 18 months) using the Bayley Scales of Infant and Toddler Development (BSID-III). Confirmed developmental delay was defined as BSID-III scores > 2SD below the mean in one area or > 1.5SD below the mean in two or more areas; "at risk" was defined as >1.5 - <= 2SD below the mean in one area. Of 732 children enrolled with at least one follow-up visit and a BSID-III result, 67 (9%) were exposed to ZIKV *in utero,* 11 (2%) were exposed to DENV or other flavivirus infections. Among ZIKV-exposed children, 19% had a confirmed delay and 39% were at risk for delay. Among DENV- or other flavivirus-exposed children, 9% had a confirmed delay and 45% were at risk. Among unexposed children, 19% had a confirmed delay and 43% were at risk for delay. Among 35 children with microcephaly or any brain or eye abnormality, 37% had a confirmed delay. Among 697 children without these birth defects, 18% had a confirmed delay. Similar proportions of developmental delay were observed between children who were exposed vs. unexposed to flaviviruses. There was a higher prevalence of developmental delays among children with certain birth defects. All young children, including those with infectious disease exposure in utero, should receive recommended screenings and prompt referrals for developmental delays.

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QUANTIFYING THE IMPACT OF MASS DOG VACCINATION ON PUBLIC HEALTH OUTCOMES IN TANZANIA

Eleanor Rees[1], Kennedy Lushasi[2], Elaine A. Ferguson[1], Ahmed Lugelo[2], Anna Czupryna[1], Danni Anderson[1], Machunde Bigambo[3], Joel Changalucha[2], Lwitiko Sikana[2], Felix Lankester[4], Katie Hampson[1]

[1]University of Glasgow, Glasgow, United Kingdom, [2]Ifakara Health Institute, Ifakara, Tanzania, United Republic of, [3]Global Animal Health Tanzania, Arusha, Tanzania, United Republic of, [4]Washington State University, Pullman, WA, United States

Dog-mediated rabies causes ~59,000 human deaths annually, mostly children, with the highest disease burden in Africa and Asia. Rabies is fatal upon onset of symptoms, but disease progression can be halted with prompt use of post-exposure prophylaxis (PEP) following a high risk bite. 'Zero by 30' is a global strategic plan that aims to end human deaths from dog-mediated rabies by 2030 using a One Health approach, and encompasses the use of PEP for human bite victims and mass dog vaccination (MDV) to interrupt dog-to-dog transmission. The strategy also advocates for the use of integrated bite case management (IBCM) as a One Health surveillance approach that promotes inter-sectoral collaboration between human and animal health workers. IBCM has been implemented across four regions in Tanzania since 2018, and using this data we describe the epidemiology of rabies in Tanzania over a six-year period, with detailed information on high risk bites, PEP use and animal investigations. In addition, the implementation of a large MDV trial across Mara region offers the opportunity to explore the impact of MDV on public health outcomes such as PEP use and human rabies deaths, by comparing MDV trial districts prior to and post MDV implementation and by comparing regions with and without sustained MDV. Specifically, in Mara region the human rabies mortality rate is significantly lower at 0.14 (0.06 - 0.21) per 1,000 dogs compared with other regions [Morogoro: 0.49 (0.32 - 0.65); Lindi 0.98 (0.52 - 1.42) ; Mtwara 0.74 (0.40 - 1.08)], and demonstrates the impact of MDV on reducing human rabies deaths. MDV is known to interrupt dog-to-dog rabies transmission, however, the impact of MDV on public health outcomes has rarely been demonstrated at scale in African settings. As we approach 2030, robust evidence on the effectiveness of MDV is required if countries are to implement rabies elimination strategies to achieve this

goal. Public health policies often focus on interventions to prevent rabies onset in bite victims through PEP, however, ending human deaths from dog-mediated rabies will only be achieved by a One Health approach to eliminate the disease in the source population.

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CHARACTERIZING DENGUE SEROPREVALENCE AND HETEROGENEITIES IN TRANSMISSION INTENSITY IN GHANA

Anthony Afum-Adjei Awuah[1], Anna Vicco[2], Evans Adu Asamoah[1], Eric Ebenezer Boham[1], Hakim Alani[1], Akua Pomaah Wiredu[1], Louis Adu-Amoah[1], Belen Pedrique[3], Martine Guillerm[3], Christian Obirikorang[1], Shubham Shrivastava[4], Nicole S. Struck[5], Isabela Ribeiro[3], John H. Amuasi[1], Ilaria Dorigatti[2]

[1]Kumasi Centre for Collaborative Research in Tropical Medicine (KCCR), Kumasi, Ghana, [2]School of Public Health, Imperial College London, London, United Kingdom, [3]Drugs for Neglected Disease initiative (DNDi), Geneve, Switzerland, [4]Research School for Health Affairs (IRSHA), Pune, India, [5]Bernhard Nocht Institute for Tropical Medicine (BNITM), Hamburg, Germany

There has been no confirmed case of dengue in Ghana to-date, and the risk of infection is unknown. This is largely on account of limited dengue surveillance in the country. To determine the historical circulation of dengue and reconstruct the immunity profile of the population, we conducted an age-stratified seroprevalence study using archival samples obtained from a representative SARS-CoV-2 serosurvey in three major cities.
An Enzyme-Linked Immunosorbent Assay (ELISA) was used to measure IgG antibody levels against purified dengue particles (ELISA-1). A subset of samples was also tested by ELISA to detect IgG against the recombinant nonstructural protein (NS1) of dengue (ELISA-2) (n=200) and Plaque Reduction Neutralization Test (PRNT) for all 4 dengue serotypes (n=69). We used a Bayesian approach to reconstruct all results obtained in the study and estimate the force of infection of dengue assuming a time-constant transmission.
1486 plasma samples were tested from Kumasi (n= 477), Accra (n= 490), and Tamale (n= 519). The estimated sensitivity and specificity of the IgG ELISA -1 assay compared to the PRNT were respectively 83% (95% CrI 78-88) and 89% (95% CrI 80-97), while the IgG ELISA-2 assay had a sensitivity of 20% (95% CrI 12-30), and a specificity of 99% (95% CrI 94-1). We estimated large heterogeneities in dengue transmission intensity across locations. A higher average annual per-capita risk of dengue infection was estimated in Tamale [0.071 (95% CrI 0.056-0.096)] compared to Accra [0.026 (95% CrI 0.021-0.033)] and Kumasi [0.005 (95% CrI 0.001-0.008)]. On average, we estimated that respectively 43%, 11%, and 70% of the Accra, Kumasi, and Tamale populations have been exposed to dengue. This study provides evidence that dengue has been circulating at different endemic levels across Ghana, with higher circulation in locations close to Burkina Faso, which has recorded Africa's largest dengue outbreaks to date. There is hence the need for enhanced passive and active surveillance to monitor the circulation and potential emergence of dengue outbreaks in the country.

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LESSONS LEARNED FROM GEOGRAPHIC INFORMATION SYSTEMS FOR INFECTIOUS DISEASES RESEARCH AND SURVEILLANCE

Tippa Wongstitwilairoong[1], Darunee Buddhari[1], John Mark Velasco[2], Paula Corazon Diones[2], Maria Theresa Alera[3], Sanjaya K. Shrestha[4], Taweewun Hunsawong[1], Chonticha Klungthong[1], Tipawan Kangvanrattana[1], Kathryn McGuckin Wuertz[1], Aaron Farmer[1]

[1]Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand, [2]Philippines-Armed Forces Research Institute of Medical Sciences Virology Research Unit, Manila, Philippines, [3]Philippines-Armed Forces Research Institute of Medical Sciences Virology Research Unit, Cebu, Philippines, [4]Walter Reed-Armed Forces Research Institute of Medical Sciences Research Unit-Nepal, Kathmandu, Nepal

WRAIR-Armed Forces Research Institute of Medical Sciences (WRAIR-AFRIMS) collaborates closely with public health partners in Nepal, the Philippines, Thailand and other areas in Southeast Asia, forming an infectious disease research and surveillance network. Medical research and surveillance often entail the collection of vast amounts of data, which are then analyzed for clinical and statistical significance, as well as for generating hypotheses. Geographic Information Systems (GIS) technology emerges as a valuable tool for researchers and epidemiologists, facilitating the graphical representation of infectious disease outbreak results in a universally understandable manner, displaying both temporal and spatial aspects. WRAIR-AFRIMS utilizes GIS-based procedural visualization for conducting infectious disease research and surveillance, generating crucial insights necessary for decision-making at local, national, and international levels. WRAIR-AFRIMS' Virology department integrates clinical and laboratory data related to respiratory illnesses, SAR-CoV2, febrile and vector-borne infections (FVBI) including geolocation data on thousands of samples collected annually. Employing GIS software such as ArcGIS and QGIS, they create visual maps illustrating the spread of infectious diseases over time and surveillance areas such as FVBI and respiratory surveillance. These maps facilitate the analysis of spatial patterns encompassing disease incidence, prevalence, and distribution. The resulting data enables authorities to monitor disease trends, detect anomalies, and allocate resources effectively for targeted interventions. Through collaborations facilitated by WRAIR-AFRIMS, GIS technology has advanced in monitoring infectious diseases in real-time, enhancing the accuracy of disease risk assessments, and supporting decision-making processes. These efforts contribute to improved communication of surveillance findings to stakeholders and the public, ultimately bolstering public health responses and outcomes.

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THE IMPACTS OF COVID-19 ON THE TREND OF MEASLES OUTBREAK IN NIGERIA

Promise Ayooluwa Ajala, Ayooluwa Oluwaseun Ajayi, Funmilayo Grace Adelakun, Favour Mofiyinfoluwa Abiona, Oluwatomisin Oluwadamilola Olawoye

College of Medicine, University of Ibadan, Ibadan, Nigeria

The COVID-19 pandemic that started in 2019 is one of the worst pandemics that affected both developing and developed countries. Measles is a vaccine-preventable disease caused by a virus. It occurs more in children. This study aims to look at the implications of the COVID-19 pandemic on the reportage of Measles and on the uptake of the Measles vaccine in Nigeria. Data was extracted from the Nigeria Centre for Disease Control (NCDC) Measles situation report. Measles situation reports from 2018-2023 and COVID-19 reports for 2020-2022 were extracted and used in this study. The total number of confirmed cases of Measles in Nigeria was 5,067 in 2018, 28,440 in 2019, 9,316 in 2020, 10,096 in 2021, 11,433 in 2023. There was a 67.3% decrease in the number of confirmed cases in 2020 with a subsequent gradual increase of up to 19% as of 2023. The percentage of confirmed cases that were not vaccinated was 53.3% in 2020 with a significant rise to 82.2% in 2021 and a subsequent decline to 73% in 2023. The percentage of children between age of 9-59 months that were confirmed with measles was 58.7% in 2020 with an increase to 75% in 2021 and a decline to 64% in 2023. In 2020, a total of 88,414 cases of COVID-19 had been recorded. In 2021, there was a rise in the figure by 168.7% to 237,561 and in 2022, the number of confirmed cases rose by only 12% to 266,415. While COVID-19 was on the rise, there was a decrease in the number of confirmed cases of Measles. This could be due to the under-reportage of the incidence of measles at this period among other reasons. There was also a significant increase in the percentage of unvaccinated Children who were affected by the disease. This could be due to decrease in the uptake of vaccines during the lockdown in Nigeria at the time of the COVID-19 pandemic. The gradual increase to 73% in

2023 could be because of the gradual increase in access to vaccines after the lockdown. The concurrent incidence of COVID-19 and Measles greatly reduced the reportage and the vaccine uptake for Measles. Health agencies should be equipped to manage both infections concurrently; making vaccines accessible and also putting up measures to ensure adequate reportage of other tropical diseases.

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PREVALENCE OF PREVIOUS DENGUE INFECTION AMONG SCHOOL CHILDREN IN GRADES 3-10— AMERICAN SAMOA, SEPTEMBER-OCTOBER 2023

Sandra Kiplagat[1], Noelle Tavale[2], Adam Konrote[2], Astrid M. Johansson[2], Angelynn Papu[2], Joshua M. Wong[1], Janice Perez-Padilla[1], Forrest K. Jones[1], Hans Desale[1], Annette F. Ilimaleota[2], Jacki M. Tulafono[2], Mark Delorey[3], Emma Jones[3], Emi Chutaro[4], Janet Camacho[4], Freddy Medina[1], Rafael Tosado-Acevedo[1], Jorge L. Munoz-Jordan[1], Gabriela Paz-Bailey[1], Laura E. Adams[1], Motusa T. Nua[2], Scott Anesi[2]

[1]Centers for Disease Control and Prevention, San Juan, PR, United States, [2]American Samoa Department of Health, Pago Pago, American Samoa, [3]Centers for Disease Control and Prevention, Fort Collins, CO, United States, [4]Pacific Island Health Officers' Association, Honolulu, HI, United States

Dengue has caused multiple outbreaks in American Samoa, with >660 reported cases during 2016-2018. In the U.S., the Dengvaxia dengue vaccine is recommended for children aged 9-16 years with a previous dengue infection and living in areas where dengue is endemic, including American Samoa. Since previous infection must be confirmed with laboratory testing to determine eligibility, seroprevalence estimates in the vaccine age-eligible population are critical to inform resource planning and ensure safe vaccine implementation. To determine dengue seroprevalence in this population, we conducted a serosurvey with a single-stage cluster sampling strategy, stratified by elementary and high schools. Among a total of 36 schools in American Samoa, we selected 7 and invited all children in grades 3-10 with parental permission to participate. We tested participants with the CTK Onsite Dengue IgG rapid test with a sensitivity and specificity of 89.6% and 95.7%, respectively. We computed estimates of seroprevalence and 95% confidence intervals (CIs) using design weights. Among 2267 children invited to participate, we tested 887 (39%). Median age was 11 (range: 7-16) years, 492 (56%) were positive, 371 (42%) were negative, and 24 (3%) had uninterpretable results. The estimated seroprevalence for all ages tested was 59% (95% CI: 47-71) and 60% (95% CI: 48-72) for individuals aged 9-16 years. The seroprevalence was lowest for children aged 10 years (53%; 95% CI: 25-81) and highest among children aged 13 years (72%; 95% CI: 56-88). Dengue seroprevalence among vaccine age eligible children in American Samoa exceeded the 20% seroprevalence threshold in vaccine recommendations for use. Dengue vaccination could be safely implemented as part of a comprehensive dengue control and prevention strategy in American Samoa.

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INTERACTIONS AMONG ACUTE RESPIRATORY VIRUSES IN PUERTO RICO, 2013-2023

Zachary J. Madewell[1], Joshua M. Wong[1], Vanessa Rivera-Amill[2], Gabriela Paz-Bailey[1], Laura E. Adams[1], Yang Yang[3]

[1]CDC, San Juan, PR, United States, [2]Ponce Health Sciences University, Ponce, PR, United States, [3]University of Georgia, Athens, GA, United States

Respiratory viral infections can modify immune responses to subsequent unrelated respiratory viral infections via non-specific immunity or increased immunopathology. Limited data exist on how infection with one virus affects the immediate course of another at the population level, especially in the tropics. We analyzed 2013–2023 data from the Sentinel Enhanced Dengue Surveillance System in Puerto Rico, which enrolls patients with fever or respiratory symptoms at 3 emergency departments or urgent care clinics and collects nasopharyngeal samples for pathogen identification using a panel of 7 acute respiratory viruses: influenza A (IAV) and B (IBV); respiratory syncytial virus (RSV); human parainfluenza virus 1 (HPIV-1) and 3 (HPIV-3), adenovirus (HAdV), and metapneumovirus (HMPV). We used a multivariate Bayesian hierarchical model to evaluate correlations in monthly infection prevalence between virus pairs, adjusting for demographics, temporal autocorrelations, seasonality, long-term trends, and multiple comparisons. Among 43,385 enrolled participants, 13,315 (30.7%) tested positive for any acute respiratory virus and 223 (0.5%) were coinfected with >1 virus. Our model identified zero virus pairs with negative correlations and five with positive correlations, indicating increased likelihood of detection when one is present, with 0 and 1 indicating no and perfect correlation: HMPV/HPIV-1 (ρ = 0.51), RSV/HPIV-3 (ρ = 0.45), IBV/HAdV (ρ = 0.39), IBV/HMPV (ρ = 0.35), and IAV/IBV (ρ = 0.31). These interactions can influence disease severity, transmission, immune response, and vaccine effectiveness. Identifying correlations between respiratory viruses can inform public health strategies. For example, a positive correlation of 0.51 between HMPV and HPIV-1 means that when HMPV prevalence deviates from its expected seasonal trend, HPIV-1 prevalence tends to deviate in the same direction and by a moderate degree. A surge in HMPV cases might signal a coming increase in HPIV-1 cases, allowing hospitals to prepare for a potential rise in patients with croup and bronchiolitis, both complications associated with HPIV-1 infection.

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HUMAN SEROPREVALENCE OF ANTIBODIES TO FILOVIRUSES CAUSING OUTBREAKS IN SUB-SAHARAN AFRICA: A SYSTEMATIC REVIEW AND META-ANALYSIS

Christopher S. Semancik[1], Christopher Cooper[2], Thomas S. Postler[2], Matt Price[1], Heejin Yun[3], Marija Zaric[3], Monica Kuteesa[1], Nina Malkevich[1], Andrew Kilianski[1], Swati B. Gupta[1], Suzanna C. Francis[1]

[1]International AIDS Vaccine Initiative, New York, NY, United States, [2]International AIDS Vaccine Initiative, Vaccine Design and Development Laboratory, Brooklyn, NY, United States, [3]IAVI Human Immunology Laboratory, Imperial College London, London, United Kingdom

Recent outbreaks of Ebola virus disease and Marburg virus disease in sub-Saharan Africa illustrate the urgent need to better understand animal reservoirs, natural causes, burden of disease, and human transmission of filoviruses. We conducted a systematic literature review to assess the seroprevalence of antibodies against filoviruses that cause human outbreaks in sub-Saharan Africa. Titles, abstracts, and full texts resulting from a search of PubMed, Embase, and Web of Science were reviewed for inclusion by a primary reviewer and a team of three secondary reviewers. Data were extracted using a pre-specified and piloted data extraction form. The review included human cross-sectional studies, cohort studies, and randomized controlled trials conducted in sub-Saharan Africa and published before March 13, 2024. We stratified seroprevalence by virus species and sample population, and presented seroprevalence in forest plots with 95% confidence intervals. For strata containing five or more studies, data with an I^2 value ≤75% were pooled. All included studies were assessed for risk of bias using the JBI Prevalence Critical Appraisal Tool. We identified 4,870 records, from which 74 studies were included. Ebola virus (species *Orthoebolavirus zairense*) seroprevalence in 31 studies ranged broadly from 1%-18% in the general population, 1.4%-15.3% in asymptomatic individuals, and 2.6%-32% in close contacts. For Marburg virus (species *Orthomarburgvirus marburgense*), seroprevalence in 13 studies ranged from 0%-2.4% in the general population and 0%-18.6% in symptomatic individuals. In the risk of bias appraisal, five studies were rated very low risk of bias, 47 low, and 22 moderate. This systematic review identifies gaps in filovirus clinical research, such as an apparent lack of knowledge about asymptomatic infection and the lack of a gold standard assay or processes to establish cutoff for relevant assays used for diagnosis of exposure in humans. Thus, this work provides comprehensive literature research information that may contribute to the improvement of clinical trial design and standardized cross-sectional seroprevalence studies.

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OUTBREAK OF MONKEYPOX IN BENIDORM, ALICANTE (SPAIN)

Javier Ena, Francisco Pasquau, Carmen Martinez-Peinado, Concepción Amador, Jose Vicente Pascual Martinez
Hospital Marina Baixa, Villajoyosa, Spain

Spain has been one of the countries with the highest reported cases of mpox. Benidorm City in the East Coast of Spain is a tourist area attracting numerous visitors. From June 2022, up to date, we reviewed the cases of mpox diagnosed in our area. Mpox infection was confirmed in samples of skin exudates or mucosal lesions by RT-PCR (RealStar Orthopoxvirus PCR kit 1.0 Altona Diagnostics GmbH, Hamburg). We diagnosed 35 cases in males (100%) with a mean age of 41 +/- 11 years, of whom 26 were European Caucasian and 9 Latin American, none of them with a history of travel to Central Africa. A total of 15 patients had HIV infection (CD4 range 212-1542 cells/mm3) and 7 were receiving PrEP. Syphilis was detected in 14 (40%), and RT-PCR screening for other STIs was negative. Patients presented with fever (48%), malaise or fatigue (94%), and odinophagia 22%. All patients showed skin lesions in vesicles or pustules distributed over the pubic region, genitals extremities, face and body. Three patients required admission to the hospital due to the severity of symptoms, with high fever, proctitis, and odinophagia. There were no cases of encephalitis, pneumonia, or corneal involvement. All patients evolved favorably with symptomatic treatment. Cases peaked in the summer 2022 and rapidly decreased in the following months. The outbreak was controlled before the routine availability of mpox vaccine programs and antivirals like tecovirimat. The infection control program and the information on preventive measures for people at risk were important factors in controlling the outbreak.

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SPATIO-TEMPORAL DISTRIBUTION OF CRIMEAN CONGO HEMORRHAGIC FEVER AND ITS RELATIONSHIP WITH CLIMATE FACTORS IN PAKISTAN: A DECADE-LONG EXPERIENCE FROM TERTIARY CARE LABORATORY NETWORK

Muhammad Abbas Abid, Joveria Farooqi, Rabiya Owais, Ayesha Sadiqa, Najia Ghanchi, Humaira Shafaq, Erum Khan
Aga Khan University, Karachi, Pakistan

Crimean Congo Hemorrhagic Fever (CCHF) has a case fatality rate as high as 80%. Pakistan shares borders with high incidence countries for CCHF, placing Pakistan at risk for outbreaks. There is limited knowledge about the total burden and spatio-temporal distribution of CCHF in Pakistan. We aim to study the spatio-temporal distribution of CCHF, using laboratory data from over 100 cities across Pakistan from 2012 to 2023 and observe correlation of CCHF cases with seasonal variation and climatic factors. Data was extracted of test requests generated for CCHF at country-wide patient sample collection points (n=307) across Pakistan from January 1st, 2012 to May 31st, 2023. Average monthly temperature and precipitation data was used in the Poisson regression method to examine the effect on the number of cases. Total 2,559 patients were clinically suspected with 547 samples confirmed positive for CCHF using real-time PCR assay, with a positivity rate of 21.37% and a male predominance (84.6%). A linear increase in cases was noted year-wise. More than half the cases (57.6%, n=315) were detected between 2016 and 2019 while 97.4% (n=533) were detected in 3 cities. Highest number of cases were reported during summer ($p < 0.001$) with 41.13% confirmed cases reported in the months of August and September. A positive correlation of suspected cases was observed with temperature of zero-month lag (p=0.000), and a negative correlation was observed with precipitation with a 2-month lag (p=0.000). Case fatality rate for CCHF patients admitted at Aga Khan University Hospital was 45.8%. CCHF is on the rise in Pakistan. Positive cases are concentrated within 3 big cities where human and animal migration rates are high. Number of cases in these cities positively correlate with summer season and temperature, and negatively correlate with precipitation. Outbreak situations occur when multiple factors coincide. Seasonal and climatic patterns can be used as predictors of disease by policymakers for strict implementation on animal regulation, transport, and surveillance of animal migration to curtail outbreak situations in Pakistan.

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SPATIAL DRIVERS OF DENGUE TRANSMISSION INTENSITY IN COASTAL ECUADOR, 2015-2016

Phong Hong[1], Savannah Colston[1], Veronica Berrocal[2], Leah C. Katzelnick[3], Varsovia Cevallos[4], Josefina Coloma[5], Joseph N.S. Eisenberg[1]
[1]School of Public Health, University of Michigan, Ann Arbor, Ann Arbor, MI, United States, [2]Department of Statistics, University of California, Irvine, Irvine, CA, United States, [3]Viral Epidemiology and Immunity Unit, Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States, [4]Centro de Investigación en Enfermedades Infecciosas y Vectoriales (CIREV), Instituto Nacional de Investigación en Salud Pública (INSPI), Quito, Ecuador, [5]School of Public Health, University of California, Berkeley, Berkeley, CA, United States

Dengue, predominantly associated with urban areas, has also emerged in rural settings. This challenges the historical perception and underscores a knowledge gap regarding the influence of rural and urban spatial characteristics on dengue virus (DENV) transmission dynamics. This study examines spatial drivers of dengue dynamics at a parish-level in coastal Ecuador, aiming to identify the most influential aspects of urbanicity for characterizing dengue risk. We employ a spatial generalized linear mixed model (GLMM) to compare how four metrics of urbanicity influence our model's ability to explain fine-scale spatial patterns of dengue force of infection (FOI). These four urbanicity predictors are i) population density, ii) a binary urban census assignment, iii) an EU Organization for Economic Co-Operation and Development's Functional Urban Areas (FUA) assignment, and iv) a composite urbanicity index. This composite index is constructed from Ecuador's 2010 census variables, chosen for their variation across parishes and their relevance to dengue risk, including percentage of households with modern roofs, piped water inside home, access to public water, paved roads, and trash collection services. Between January 2015 and December 2016, Ecuador's passive surveillance system reported 1609 severe dengue and dengue with warning signs (DwWS) cases with associated ages across 266 coastal parishes within El Oro, Esmeraldas, Guayas, Manabí, and Santa Elena provinces. FOI increased geometrically as a function of our urbanicity metric. In comparison with the politically defined dichotomous urban/rural variable, we found that 4% of urban parishes were classified with low dengue risk and 21% of the rural parishes were classified with high dengue risk. Our findings highlight a need for a more nuanced characterization of spatial and conceptual aspects of urbanicity as they apply to DENV transmission — beyond an urban/rural dichotomy — to enhance the identification and management of existing and emerging high burden areas in other dengue-endemic countries with resource-limited surveillance systems.

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THE BURDEN OF DENGUE IN LATIN AMERICA AND ASIA: EPIDEMIOLOGICAL DATA OVER 57 MONTHS OF FOLLOW-UP IN A PHASE 3 TRIAL

Randee Kastner[1], Eduardo López-Medina[2], Chukiat Sirivichayakul[3], Shibadas Biswal[4], Ian Escudero[4], Nicolas Folschweiller[1], Nicholas Roubinis[1], Vianney Tricou[1], Suely Tuboi[5], John Weil[1]
[1]Takeda Pharmaceuticals International AG, Glattpark-Opfikon (Zürich), Switzerland, [2]Centro de Estudios en Infectologia Pediátrica (CEIP), Universidad del Valle and Clínica Imbanaco Grupo Quironsalud, Cali, Colombia, [3]Department of Tropical Pediatrics, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand, [4]Takeda Vaccines, Inc., Cambridge, MA, United States, [5]Takeda Pharmaceuticals Brazil, São Paulo, Brazil

The placebo arm data from Takeda's phase 3 trial, DEN-301 (NCT02747927), offer a rare opportunity to assess the standardized burden

of febrile illness, serotype-specific virologically confirmed dengue (VCD), dengue hemorrhagic fever, dengue shock syndrome, and severe dengue across time, countries, and regions. The trial enrolled 20,099 participants aged 4-16 years in Asia (the Philippines, Sri Lanka, Thailand) and Latin America (Brazil, Colombia, Dominican Republic, Nicaragua, Panama), of which 6,687 were randomized to placebo. Of these, 91% completed the study follow-up of ~57 months (30,620.6 person-years [p-y]). A total of 9,698 febrile illnesses were recorded through active surveillance, of which 98% were tested for dengue virus (DENV) using RT-PCR, and 5.8% (560 cases) were confirmed as dengue. This proportion increased with age (among those 4-5 years: 4.7%, 6-11 years: 5.8%, 12-16 years: 6.9%). By region, the incidence of dengue was more than twice as high in Asia (incidence rate [IR]=2.7/100 p-y) than Latin America (IR=1.2/100 p-y). Over 25% of VCD cases were hospitalized (IR=0.5/100 p-y). The decision to hospitalize was per local standard of care and rates of hospitalized VCD ranged widely by country (eg, 4.4% in Panama, 8.3% in Brazil, 68.0% in Sri Lanka). From December 2018 (study month 28) through ~57 months of follow-up, there was a decrease in VCD incidence/100 p-ys (months 28-39: 3.0, months 40-51: 0.7, months 52-57: 0.4). Of the 560 VCD cases over 57 months, DENV-1 was the most predominant serotype detected (41.1%), followed by DENV-2 (34.5%), DENV-3 (20.4%), and DENV-4 (4.1%). These data allow a standardized comparison across populations and time (2016-2022). The burden of dengue in children aged 4-16 years was higher in Asia than in Latin America. Different local standards of care may explain the variable hospitalization rates. The observed decrease in dengue incidence may reflect epidemiological trends or more probably the impact of social restrictions during the COVID-19 pandemic. These findings may help validate assumptions needed to design public health interventions such as vaccination programs.

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SEROPREVALENCE AND SEROINCIDENCE OF LASSA FEVER VIRUS INFECTION IN A POPULATION-BASED COHORT STUDY IN SIERRA LEONE (IAVI X100)

Donald Grant[1], **Matt A. Price**[2], Nell Bond[3], Celia R. Glezer[3], Emily J. Engel[3], Robert F. Garry[3], Robert Samuels[1], Crystal Zheng[3], Mambu Momoh[1], Lansana Kanneh[1], John S. Schieffelin[3], Jeffrey G. Shaffer[3], Suzanna C. Francis[2], Marija Zaric[2], Patricia E. Fast[2], Swati Gupta[2]

[1]Kenema Government Hospital, Kenema, Sierra Leone, [2]IAVI, New York City, NY, United States, [3]Tulane University, New Orleans, LA, United States

An improved understanding of Lassa fever virus (LASV) epidemiology is needed to inform the design of efficacy trials and delivery strategies for LF vaccines. Investigators at Kenema Government Hospital (KGH) in Sierra Leone, Tulane University, and IAVI implemented a prospective study (X100) to obtain the seroprevalence and seroincidence of LASV. Three districts were selected based on confirmed case frequencies presenting at the KGH National Viral Hemorrhagic Fever Ward: high (Kenema District), emerging (Tonkolili District), and low (Port Loko District). Residents of 26 villages across the 3 districts were enrolled. Demographic data, medical history, and blood were collected using finger sticks and dried blood spots at two time points (baseline and 18-24 months later). Dried blood spots were tested by pan-LASV-NP IgG ELISA (Zalgen Labs, MD, USA). Between April 2021 and May 2022, 8,237 residents aged ≥2 years were enrolled; baseline and follow-up data were available for analysis for 6,447 (78.3%) participants from 803 total households across all 26 villages. Of the 6,447 participants, 3,255 (50.5%) were female, 1,634 (25.4%) were children aged 2-10 years, and 1,532 (23.8%) were adolescents aged 10-19 years. Baseline LASV seroprevalence was 1,832/6,447 (28.4%). Among 4,615 seronegative participants we observed 528 cases of incident infection for an incidence of seroconversion of 6.9 cases/100 person years (95% CI: 6.3-7.5). The incidence of reinfection among seroprevalent participants, defined as a 4-fold increase in ELISA antibody titres from baseline to follow up, was significantly lower, at 4.2 cases/100PY (3.5-4.9, p<0.001). We observed high rates of seroincidence in this rural, population-based cohort of Sierra Leonians at risk of LASV infection. These data are crucial for understanding the populations most at risk for incident LF disease, which will inform efficacy trial designs and future vaccine delivery strategies.

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CHARACTERIZING THE INTRA-HOST PLAQUE VARIANTS AND GROWTH KINETICS OF GLOBAL ZIKA VIRUS STRAINS

Millicent Sifuna[1], Alexander T. Ciota[2], Maria Onyango[1]

[1]Texas Tech University, Lubbock TX, Lubbock, TX, United States, [2]Wadsworth Center, Albany, NY, United States

Zika virus (ZIKV), originally isolated from Zika forest in Uganda has two primary lineages: African and Asian. The ZIKV strains that circulate in the Americas, Pacific, and Southeast Asia compose the Asian lineage while the African lineage is associated with the strains that circulate in Africa. The Asian lineage is responsible for all human outbreaks caused by ZIKV strains while the African lineage has not been associated with any epidemic transmission. We hypothesize that intra-host viral mutations exist, within each ZIKV strain, and these variants drive the geographical disparities in pathology of disease resulting from infection with ZIKV. To address our hypothesis, we characterized the plaque morphology and growth kinetics of the Dakar, Uganda, Nigeria, Honduras, and Thailand ZIKV isolates, purified single plaques of Dakar and Honduras isolates, and assessed their growth kinetics. The global ZIKV isolates demonstrated unique plaque variants associated with distinct plaque morphology in the parental population. Dakar isolate had plaque morphologies different from the other isolates belonging to the African lineage (Nigeria and Uganda) but were identical to the isolates in the Asian lineage (Honduras and Thailand). Further, the Dakar isolate displayed the most significant cytopathic effect on the cell monolayers. Interestingly, Honduras and Thailand isolates did not clear the cell monolayer at the point of infection as the African strains did. The Dakar strain manifested dual host tropism compared to the other isolates. Purification of single plaques resulted in a significant reduction in growth kinetics compared to the parental wild type, suggesting a cooperative behavior of swarm mutants to achieve virulence. Our results provide important information on lineage-specific viral biological characteristics. The observation of intra-host plaque variants suggests that the virus could evade immune system response and make it more difficult to develop vaccines that can effectively target all ZIKV strains.

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MAPPING RESPIRATORY VIRUS EVOLUTION AND OUTBREAKS IN CAMBODIA USING PATHOGEN GENOMICS

Sreyngim Lay[1], Mengheng Oum[1], Jessalyn Sebastian[2], Lyhourng Long[1], Sophana Chea[1], Piseth Ly[1], Sokna Ly[1], Ratanak Sath[1], Savuth Chin[3], Darapheak Chau[3], Heng Seng[3], Sovann Ly[3], Daniel M. Parker[2], Volodymyr M. Minin[2], Jessica E. Manning[4], Chanthap Lon[1], **Christina Yek**[4]

[1]International Center of Excellence in Research Cambodia, Phnom Penh, Cambodia, [2]University of California Irvine, Irvine, CA, United States, [3]Ministry of Health, Phnom Penh, Cambodia, [4]National Institute of Allergy and Infectious Diseases, Rockville, MD, United States

Genomic surveillance can guide early detection and response to emerging epidemics. We applied metagenomic sequencing to investigate respiratory virus outbreaks in Cambodia. Nasopharyngeal swabs were collected from subjects aged 6 months to 65 years with respiratory symptoms and fever in 4 Cambodian hospitals. Geographic coordinates for subject home villages were recorded. We performed shotgun short-read RNA sequencing and aligned sequences of Influenza A virus H3N2, human parainfluenzavirus 3 (PIV3), and human respiratory syncytial virus (RSV) strains A and B. We performed Bayesian inference of phylogenetic trees for each pathogen, where branch lengths were measured in calendar time, which allowed us to compute posterior medians and highest posterior density (HPD) intervals for all divergence times, including time to most recent common ancestor (TMRCA). From December 2020 to August 2023, 1,118 subjects with acute respiratory febrile illness were enrolled. Sequencing detected 25 distinct respiratory virus species among 502 (44.9%), most commonly within genus

Enterovirus (N=189), Betacoronavirus (N=94), Orthopneumovirus (N=65), Respirovirus (N=47), and Alphainfluenzavirus (N=41). Discrete time-clusters were noted of H3N2 (September 2022), PIV3 (March 2021 and July 2022), RSV-A (November 2021), and RSV-B (August 2022). The posterior median of TMRCA ranged from 1.5 years (95% HPD 0.8-2.7) for H3N2 HA segment and 2.6 years (1.8-3.7) for RSV-A, to 5.5 years (1.6-18.3) for RSV-B and 12.9 years (7.5-21.9) for PIV3. Within time-clusters, pairwise genetic and physical distances at strain (for all 4 viruses) and clade (for RSV-B and PIV3) levels were not correlated. Sequencing detected diverse respiratory viruses among Cambodians with febrile respiratory illness during the COVID-19 pandemic. Peaks in RSV-B and PIV3 cases initially suggested discrete outbreaks in summer 2022, but genomic data revealed co-circulation of several clades, potentially arising due to lifting pandemic restrictions. While H3N2 and RSV-A outbreaks may have resulted from single introductions, sampling was insufficient to map transmission networks.

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ANALYSIS OF SARS COV2 VARIANTS IN WASTEWATER OF THE METROPOLITAN DISTRICT OF QUITO USING A PASSIVE SAMPLING 3D PRINTED DEVICE

Paula Estefania Mejia, Paúl Cárdenas, Rommel Guevara, Erika Muñoz, Dennisse Benitez, Patricio Rojas, Verónica Barragán, Gabriel Trueba

San Francisco of Quito University, Quito, Ecuador

SARS CoV2 infection in humans produces viral RNA elimination mainly through respiratory droplets but also actively through fecal matter. Wastewater surveillance of pathogens has become an essential tool for epidemic preparedness. It has allowed tracking of the SARS CoV2 emerging variants in populations with low clinical diagnosis rates. For this reason, we implemented a passive sampling approach to determine SARS CoV2 variants in Quito, Ecuador, during 2023 and 2024 and to correlate it with the results from the national genomic surveillance performed in patients in hospitals in Quito. A passive torpedo-type sampling equipment was used to collect the samples, which contained a nylon membrane, gauze, and a swab; the device was placed in wastewater collectors for 24 hours weekly. This method is a more straightforward approach to wastewater sampling than the conventional one that requires several liters of collection and spin-down/PEG concentration. RNA was concentrated with PEG. RNA viral extraction was performed, followed by real-time PCR for virus detection and multiplex PCR for sequencing with Oxford Nanopore Technology. Bioinformatics data was analyzed in Freyja. A predominance of omicron variant with lineages HN.1, EG.5.1.6, XBB.1.5.1.5, and HV.1 were encountered during the infection peaks in Quito on August 2023 and January 2024. The same variants and in similar proportions were encountered in our genomic surveillance in infected humans during both peaks. Strikingly variant JN.1 was not the leading cause of infections during this time compared with its northern hemisphere predominance. SARS CoV2 wastewater epidemiological surveillance is a straightforward approach for genomic characterization of circulating variants and viral abundance quantification.

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DENGUE VIRUS SEROTYPE 3 ORIGINS AND GENETIC DYNAMICS, JAMAICA

Shanice A. Redman

University of the West Indies, Mona Campus, Kingston, Jamaica

Dengue is hyperendemic in Jamaica with increasingly frequent epidemics. Due to the limited number of whole or near whole genome sequences it remains unclear as to the temporal origin of dengue viruses introduced into Jamaica and their subsequent transmission to other countries. In this study we examined the molecular epidemiology of dengue virus serotype 3 (DENV3) in Jamaica during 2016-2020, a time period inclusive of the 2016 and 2019 epidemics. Residual dengue virus NS1 positive serum samples collected from patients seeking care for dengue during 2019-2020 at the University Hospital of the West Indies were sequenced with a target enrichment approach using the Comprehensive Viral Research Panel probe set. Five whole genome (100% coverage), 7 near whole genome (91-99% coverage), and 3 partial genome (28-65% coverage) sequences were obtained. Ten additional publicly available dengue virus sequences from 2016-2019 were also included for analysis. Sequences were aligned to reference DENV sequences obtained from Nextstrain using MAFFT. Phylogenomic and phylogeographic analyses of the sequences were completed using IQTREE2 and BEAST, respectively. All 25 samples analyzed were identified as DENV3 genotype III, with a *skygrid* reconstruction showing a stable evolutionary rate of 1.78×10^{-3} substitutions/site/year. Positive selection of mutations within the E gene accumulated over time after introduction of DENV3 genotype III, allowing for continuous circulation of this genotype in Jamaica. Phylogeographic analysis indicated that DENV3 most likely originated from India and China in 2014, two years prior to the initial detection of DENV3 during the 2016 dengue epidemic. DENV3 genotype III circulating in Jamaica was subsequently transmitted to Saint Lucia and potentially other unsampled countries. Our investigation yielded a wealth of information about the molecular epidemiology of dengue virus in Jamaica, previously not available to local public health officials and providing a greater understanding of the circulation of dengue worldwide.

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RECOVERY OF COMPLETE GENOME SEQUENCES OF CRIMEAN-CONGO HAEMORRHAGIC FEVER VIRUS THROUGH TARGETED NEXT-GENERATION SEQUENCING APPROACHES: A COMPARATIVE STUDY BETWEEN MULTIPLEX TILING PCR AND PROBE HYBRIDIZATION CAPTURE

Jake D'Addiego[1], Sonal Shah[2], Nazif Elaldi[3], Baris Yildiz[3], Ayse Nur Pektas[3], Binnur Bagci[3], Tuba Nur Tasseten[3], Zati Vatansever[3], David Allen[2], Roger Hewson[1]

[1]UK Health Security Agency, Salisbury, United Kingdom, [2]London School of Hygiene and Tropical Medicine, London, United Kingdom, [3]Sivas Cumhuriyet University, Sivas, Turkey

Crimean-Congo haemorrhagic fever (CCHF) is the most prevalent human tick-borne viral disease, with a reported case fatality rate of 30% or higher. The disease is caused by the CCHFV virus (CCHFV), an orthonairovirus within the *Nairoviridae* family (order *Bunyavirales*), and is endemic to vast geographical areas spanning Africa, Europe, Middle East and Asia. There are currently no licensed vaccines or effective therapeutics against CCHF. The geographical expansion of tick vectors coupled with the fast mutation rate of RNA viruses has made the investigation of tick-borne pathogens a public health priority. Although modelling data from Türkiye has shown that anthropogenic factors such as fragmentation of agricultural land interspersed with forest and shrub-type vegetation play a role in increased CCHFV transmission, other studies have shown that climatic factors such as reduced rainfall and increases in temperature will cause a northward expansion of the suitable habitat for several tick species including *Hyalomma marginatum*, the most common CCHFV vector. With the predicted expansion of the habitat for many tick species including CCHFV vectors, the development of enrichment strategies to recover CCHFV genomic sequences from genetically diverse viruses will be of paramount importance to not only detect the presence of the virus in potentially new areas, but also for public health laboratories actively involved in CCHFV molecular surveillance to rapidly detect/diagnose and characterize currently circulating strains. We have developed a novel probe hybridisation capture method and successfully recovered near complete genome sequences for different CCHFV genetic lineages including Europe 1, Europe 2, Africa 2 and Africa 3. The presented methodology could be valuable in CCHFV endemic regions in which circulating viruses have not yet been characterised or their presence is still unknown.

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MULTIPLE GENOTYPES AND CLADES OF DENGUE VIRUS IDENTIFIED DURING 2022 AND 2023 IN CENTRAL NEPAL

Margaret Chi, Nishan Katuwal, Aastha Shrestha, Dipesh Tamrakar, Rajeev Shrestha

Dhulikhel Hospital Kathmandu Univeristy Hospital, Dhulikhel, Nepal

Dengue virus (DENV), a virus of the *Flaviviridae* lineage, causes one of the most widespread arboviral diseases with 100-400 million cases occurring each year. DENV exists in four antigenically distinct serotypes which can be further subclassified into 3-6 genotypes and numerous clades. In Nepal, Dengue has been a disease of public health concern since its first case in 2004. Originally limited to the lowland "Terai" regions of Nepal, the at-risk region for DENV has been steadily expanding into higher elevation regions, with the last two years each recording over 50,000 cases. We analyzed 90 NS1-seropositive serum samples with qPCR from patients presenting with dengue-like illness at Dhulikhel Hospital, a tertiary care hospital, in 2022 and 2023. Among 90 seropositive samples, 80 were positive by qPCR. We utilized the iSeq100 to perform amplicon-based whole genome sequencing on DENV from qPCR-positive serum (Ct<35.00). Of the 41 complete genomes obtained, 9 were identified as DENV-1, 15 as DENV-2 and 17 as DENV-3. Phylogenetic analysis revealed a clade of DENV-1 sequences within genotype III clustering with 2019 Indian strains and 2022 Nepali strains. This represents a shift in the predominant genotype of DENV-1 in Nepal from genotype V since 2017. All DENV-2 sequences fell into the Cosmopolitan genotype. DENV-2 sequences did not cluster into discrete clades, but were related to sequences from India, Singapore, Bangladesh, and China from 2018-2022, Nepali sequences from 2017, and recent isolates from the US. Though historically outbreaks have been dominated by DENV-1 and -2 in Nepal, we identified numerous DENV-3 sequences. These were related to Indian strains from 2019 and Nepali strains from 2022 and were identified as genotype III. Our study showed a dominance of Indian DENV populations on Nepali DENV-1 and -3. Moreover, the findings have demonstrated the shift of DENV-1 genotype from V to III with notable genetic diversity within the cosmopolitan genotype of DENV-2 in Nepal, linking cases to those in South and Southeast Asian countries and North America.

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PHYLOGENETIC ANALYSIS OF CRIMEAN CONGO HEMORRHAGIC FEVER VIRUS STRAINS CIRCULATING IN PAKISTAN DURING 2022-2023

Sabiha Yousuf, Aisha Ishauqe, Yusra Rehman, Erum Khan, Kehkashan Imtiaz, Najia Ghanchi

The Aga Khan University, Karachi, Pakistan

Crimean-Congo haemorrhagic fever (CCHF) is a tickborne viral disease endemic in regions of Asia, Africa, and Europe, with case fatality rate of 10-40%. Pakistan is considered endemic region for CCHFV, while limited studies have been conducted in the country to provide insights into the genetic diversity and evolution of CCHFV. In this study we aimed to perform whole-genome sequencing of CCHFV strains and its phylogenetic analysis for better understanding of epidemiology and evolutionary relationship of indigenous strains during 2022-2023. Thirty-one CCHFV positive serum samples were collected from the Aga Khan University Hospital laboratory during 2022 to 2023. RNA was isolated and sequencing was performed on Illumina MiniSeq platform. Raw reads were aligned to reference genome using BWA tool followed by variant calling and generating consensus sequence using iVar tool. Inclusion criteria for phylogenetics analysis was set at a depth of equal to or greater than 10X and more than 95% coverage. Phylogenetic trees of all segments were constructed using Fasttree, visualization and annotation of trees was done in iTOL. A total of eight samples were sequenced successfully. All three segments (S, M, and L) were clustered in Clade-IV (Asia-1), along with other regional strains from Afghanistan, India, and Iran, as well as reported sequences from Pakistan. Detailed mutation analysis was conducted, and samples were compared with publicly available whole genome sequences in BV-BRC database. We found that all segments contain unique mutations 1, 10 and 30 in S, M and L segments, respectively. The study provides valuable insight into the genomic diversity and evolutionary history of CCHF strains circulating in Pakistan during 2022-2023. While similarities with neighboring country strains show that climatic conditions of the region play a role in disease transmission, as vector proliferation is dependent on climate and animal movement between borders.

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WEST NILE VIRUS INFECTIOUS UNITS CONTAIN MULTIPLE VIRUS PARTICLES

Marina Fujii, Emily Fitzmeyer, Bradly Burke, Emily Gallichotte, Marcela Henao-Tamayo, Brian Geiss, Gregory D. Ebel

Colorado State University, Fort Collins, CO, United States

A virus infectious unit is widely considered to consist of a single virus particle that enters a cell to initiate the infectious cycle. However, a growing body of research suggests multiple alternate mechanisms through which viruses may initiate infection. For example, Zika virus plaques tend to be initiated by aggregates containing a mean of 10 virus particles, with single-particle infections occurring only very infrequently. To define the extent to which West Nile virus (WNV) infections are similarly initiated by infectious units consisting of multiple aggregated virus particles, we assessed the genomic content of single WNV plaques using a molecularly barcoded WNV. Multiple vertebrate cell lines were infected at a low multiplicity of infection and well-isolated plaques were allowed to develop. From these plaques, we extracted RNA and analyzed viral barcode diversity using next-generation sequencing. Our results suggested that 3 to 8 WNV virus genomes comprise a typical WNV plaque-forming unit. However, some plaque-forming units were highly diverse, containing more than 20 discrete input genomes. We plan to identify the virus genome numbers associated with a single-cell infection and compare them with the results in a plaque-forming unit. We are also investigating the mechanism of polyinfection, which we hypothesize occurs through the aggregation of multiple, individually packaged, virus particles. To visualize WNV virus aggregation, we are developing a "flow virometry" assay that will allow us to precisely quantify the number of virus particles in virus aggregates.

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GENOMIC CHARACTERIZATION OF DENGUE VIRUS CIRCULATION IN ETHIOPIA

Adugna Abera[1], Houriiyah Tegally[2], Geremew Tasew[1], Eduan Wilkinson[2], Abraham Ali[1], Feyisa Regasa[1], Getachew Tollera[1], Dawit Wolday[1], Dereje Beyene[3], Tulio de Olivera[2]

[1]Ethiopian Public Health Institute, Addis Ababa, Ethiopia, [2]Centre for Epidemic Response and Innovation (CERI), School of Data Science and Computational Thinking, Stellenbosch University, Stellenbosch 7600, South Africa, Cape Town, South Africa, [3]Addis Ababa University, Addis Ababa, Ethiopia

Dengue virus is becoming more prevalent in tropical and subtropical regions of the world, including Ethiopia. In Ethiopia, dengue fever cases have been reported in five regions, however, the circulating serotypes and genotypes are not well known. This study investigated the circulating serotypes and genotypes of dengue virus using phylogenetic analysis. The study also compared these patterns to previous studies conducted in African countries and in a global context. A cross-sectional study was conducted in three hospitals to collect blood samples from patients with acute febrile illnesses. The samples were screened for three arboviral pathogens (DENV, CHIKV, and ZIKV) using RT-PCR. For dengue isolates, serotyping was performed using a CDC kit. Phylogenetic analysis was conducted using sequenced data, and the results were compared with previous studies conducted in Africa and other dengue-endemic countries. Seasonality and human mobility were taken into account to predict and understand the viral introduction. In this study, two serotypes (DENV1 and DENV3) were isolated from Dire Dawa, and DENV3 was isolated from the Afar region. The DENV1 serotype belongs to genotype III of the major lineage A, while the DENV3 serotype showed two transmission clusters and belongs to genotype III and

major lineage B. This study contributes to a deeper understanding of the circulating serotype and genotype in Ethiopia, which could inform decision-makers and help plan national and public health strategies to manage the emergence of dengue.

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MOLECULAR CHARACTERIZATION OF SARS-COV-2 VARIANTS IN PATIENTS LIVING IN DIFFERENT PROVINCES OF BURKINA FASO

Emilie S Badoum, Augustin Konkobo, Abdul-Wahab D Traore, Samuel S Serme, Jean W Sawadogo, Issa Nebie, Alphonse Ouedraogo, Sodiomon B Sirima

Groupe de Recherche Action en Santé, Ouagadougou, Burkina Faso

The initial strain of SARS-CoV-2 that appeared at the end of 2019 and has since spread around the world mutates regularly. Since the start of the epidemic, the SARS-CoV-2 genome has changed and evolved as a result of the many mutations that have occurred. Like all viruses, SARS-CoV-2 mutates constantly during multiplication, copying its genetic material and sometimes making mistakes or mutations in the process. Whole-genome sequencing of SARS-CoV-2 using next-generation sequencing (NGS) has shown to be a powerful tool for studying coronavirus 2019 (COVID-19) and tracking the evolution and spread of the virus. The aim of this study was to identify the different SARS-COV-2 variants circulating in different zones in Burkina Faso. Samples were collected at health facilities located in different second level towns (Kaya, Ouahigouya and Tenkodogo) in Burkina Faso during the period of the SARS-CoV-2 pandemic. 156 SARS-CoV-2 genomes obtained from RtPCR-positive collected were analyzed. The analyses were carried out using the MinION (Oxford Nanopores Technology). Genomic sequences were assigned to phylogenetic clades using NextClade and to Pango lineages using pangolin. After analysis of the results, the SARS-CoV-2 genomes obtained in this study can be classified into 15 phylogenetic clades and 36 existing Pango lineages. Five distinct variants were identified: Omicron (46.15%), Delta (35.90%), Eta (7.69%), Iota (7.69%) and Alpha (2.56%). The Delta variant (56.25%) was most common in subjects living in rural areas. The Omicron variant was not only most common among those living in urban areas (48.39%), but also among subjects who presented as suspected cases (54.05%) of the disease. As in many other countries around the world, several variants of concern of SARS-CoV-2 virus were present during the COVID-19 pandemic in Burkina Faso. This study provides additional data on SARS-CoV-2 variants in the country.

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ELUCIDATING THE MOLECULAR EPIDEMIOLOGY OF WEST NILE VIRUS IN SOUTHERN NEVADA

Austin J. Tang[1], Will Bendik[2], Karen L. Figueroa Chilito[1], Zoee Sanchez[1], Miklo Acala[1], Katharine Major[1], Richard M. Oxborough[1], Chad L. Cross[1], Vivek Raman[2], Louisa A. Messenger[1]

[1]University of Nevada, Las Vegas, Las Vegas, NV, United States, [2]Southern Nevada Health District, Las Vegas, NV, United States

West Nile Virus (WNV) is a positive-sense single-stranded RNA virus transmitted by
Culex mosquitos (*Cx. quinquefasciatus, Cx. tarsalis, Cx. pipiens*). The disease caused by this arbovirus can be benign or associated with poor prognoses including encephalitis, meningitis, or meningoencephalitis in humans. WNV first appeared in the United States in 1999 and rapidly spread westward reaching Nevada in 2004. Subsequently, WNV has been found annually in the state, including Las Vegas in Southern Nevada. Due to recent El Niño events, Las Vegas has seen unusual amounts of precipitation. Ongoing local climate change is likely to result in increases in mosquito-human contact. Despite ample knowledge of WNV in general, little is known about its origins in Nevada. A WNV surveillance program is in place in Southern Nevada, spearheaded by the Southern Nevada Health District (SNHD). The SNHD collect mosquitoes during the peak mosquito breeding months of April through October using encephalitis vector surveillance (EVS), Gravid, and BG Sentinel traps. Between March 2023-April 2024, over 3000 *Culex* mosquitoes were collected, with 30 pools positive for WNV. While this provides information about local WNV prevalence, there is still a paucity of data regarding the source of WNV in Southern Nevada. In this study, we aimed to elucidate the genetic origins of WNV using next-generation whole-genome sequencing techniques. cDNA was synthesized from forty-two unique WNV RNA samples, derived from pooled *Culex* spp. collected from 2020-2023, identified by positive qPCR results (Ct value range: 16-31). A hemi-nested PCR approach, based on 12 published primer sets was used to amplify across the entire WNV genome. Library preparation and sequencing is ongoing using the Oxford Nanopore Technologies MinION™ platform. Bioinformatic analysis will be used to reveal the genetic diversity of WNV in Clark County and to determine whether WNV strains are endemic or are reintroduced from other geographical disease foci annually.

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ROTAVIRUS AND STRAIN DIVERSITY: DISENTANGLING THE REASSORTMENT RATES OF PAIRWISE SEGMENT COMBINATIONS

Suvanthee Gunasekera[1], Daivi Patel[1], Nicola F. Mueller[2], Pamela P. Martinez[1]

[1]University of Illinois at Urbana-Champaign, Urbana, IL, United States, [2]University of California San Francisco, San Francisco, CA, United States

Rotavirus is an RNA virus with 11 segments and is the main cause of severe gastroenteritis in infants and young children. This virus exhibits significant diversity in strains globally, with multiple strains co-circulating in the population. To better understand the population dynamics of this highly diverse pathogen, it is imperative to study the mechanisms that generate and maintain diversity at the population level. One such mechanism is reassortment, which refers to the ability of segmented viruses to exchange their segments during coinfection, thereby producing hybrid progeny. Although reassorted viruses have been previously characterized, the rate at which these reassortment events occur and are observed at the population level is an open question. In this study, we reconstructed the reassortment networks and inferred reassortment rates by implementing a Bayesian phylogenetic method. We analyzed 142 publicly available human rotavirus A whole-genome sequences from the virus variation database and the GenBank collected globally during 1974-2019. We inferred co-reassortment rates for all pairs of segments, showing that these rates vary drastically between segment pairs, with values ranging from 0.03 to 0.11 reassortment events per lineage per year. The highest rates were observed in events reassorting the antigenic segments, VP4 or VP7. In contrast, the lowest reassortment rates were seen in pairs with VP2 and VP3, which play roles in core assembly and genome replication. Notably, each segment has a wide range of co-reassortment rates with its different pairs. These findings suggest that reassortment is not only influenced by the type of segment (antigenic or non-antigenic) but also by the combination of segments in the rotavirus strain. We conclude that different reassortment rates of both antigenic and non-antigenic segments of rotavirus could lead to shifts in diversity patterns, with unknown consequences for vaccine efficacy, which is known to be very low in low- and middle-income countries.

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CLINICAL AND GENOMIC CHARACTERIZATION OF DENGUE VIRUS OUTBREAK IN MALI

John Collins[1], Fousseyni Kane[2], J. Kenneth Wickiser[1]

[1]Columbia University Mailman SPH, New York, NY, United States, [2]University Clinical Research Center (UCRC), Bamako, Mali

An outbreak of dengue fever transpired in November and December 2023, resulting in roughly 30 reported deaths and 700 cases by December 19.[1] Aedes aegypti, the mosquito from which dengue virus and other diseases are spread, has been the cause of a number of arboviral outbreaks in the West African region, and climate changes may further result in these outbreaks becoming more severe and frequent.[2] We employed the pan-viral

sequencing assay known as VirCapSeq-VERT[3] at the University Clinical Research Center (UCRC) in Bamako, Mali to identify dengue prevalence, discover potential co-infections, and recover complete genomes to better characterize the spread of dengue virus. Of 23 identified cases of dengue virus, 61% were male and the mean age of cases was 43. 83% experienced fever and 57% reported headache. Several cases displayed symptoms of gingival bleeding, anemia, jaundice, and myalgia. Roughly 65% of detected dengue virus cases were identified as serotype 3 (DENV-3), falling within the phylogenetic clade nearest genotype 3 sequences. Bayesian analysis via Monte Carlo chain method[4] further explored the temporal evolution of the genomic sequences detected by VirCapSeq-VERT, and revealed the need for better arboviral genomic surveillance in West Africa.

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DENGUE VIRUS DETECTION AND GENOMIC ANALYSIS IN A HIGH JUNGLE REGION OF NORTHERN PERU IN 2020 AND 2023

Yordi Tarazona-Castro[1], **Miguel A. Aguilar-Luis**[1], Wilmer Silva-Caso[1], Victor Zavaleta Gavidea[2], Ronald Aquino-Ortega[1], Hugh Watson[3], Eliezer Bonifacio-Vélez de Villa[1], Luis J. del Valle[4], Jorge Bazán Mayra[2], Juana del Valle-Mendoza[1]

[1]Universidad Peruana de Ciencias Aplicadas, Lima, Peru, [2]Direccion Regional de Salud Cajamarca, Cajamarca, Peru, [3]Evotec ID, Lyon, France, [4]Universitat Politècnica de Catalunya, Barcelona, Spain

In Peru, more than 50% of arbovirus cases are caused by dengue (DENV), a disease transmitted by arthropods (arbovirus). Our study assessed the prevalence and circulating DENV genotypes among febrile patients. Between June 2020 and July 2023, 4413 serum samples were collected from patients with acute febrile illness (AFE) in the province of Jaén, department of Cajamarca, located in the high jungle of northern Peru. The diagnosis of DENV was made by real-time RT-PCR and NS1 and IgM ELISAs. ILUMINA technology was used to sequence and assemble the genome. The phylogenies were constructed using maximum likelihood phylogenetic inference (ML). DENV was confirmed in 2131 cases (48.29%), where 249 (11.68%) were diagnosed by RT-PCR, 961 (45.10) by RT-PCR and Elisa NS1, 58 (2.72%) by RT-PCR and IgM ELISA, 130 (6.10) by NS1 ELISA, 146 (6.85%) by NS1 ELISA and IgM, 389 (18.25%) by IgM and 198 (9.29%) by essays. Infected patients were mostly aged 18 to 39 years (53.01%), followed by those aged 40 to 59 years (18.91%) and those aged 12 to 17 years (10.65%). Female patients (56.78%) also had a higher incidence of DENV. Among the infected population, headaches (89.32%), arthralgia (77.56%), myalgia (76.93%) and fever (76.35%) were the most common clinical symptoms. Based on phylogenetic analysis of nine complete DENV genomes, the South American lineage of DENV-1 genotype V has circulated in Peru since 2021, and lineage 5 of the cosmopolitan DENV-2 genotype has circulated since 2019. They were also found cocirculating in Jaén between 2022 and 2023. As a result of the recent co-circulation of two DENV genotypes in the high jungle of northern Peru, we report a high incidence of DENV in the high jungle. In Peru, DENV is showing a divergent pattern of distribution and diversity compared to other outbreaks in Peru and South America, so we emphasize the importance of powerful genomic surveillance to understand its distribution.

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DETECTION AND WHOLE GENOMIC CHARACTERIZATION OF TWO UNUSUAL REASSORTANT DS-1-LIKE ROTAVIRUS A STRAINS CO-CIRCULATING IN BOLIVIA IN 2023

Alejandra Gabriela Torrez Mamani, Belén Claudia Choque Pardo, Cinthia Copeticona Callejas, Sonia Guadalupe Jiménez Pacohuanca, Lucia Isabel Mendieta Elena, Sergio Gutiérrez Cortez, Carla Calderón Toledo, Volga Íñiguez Rojas

Molecular Biology and Biotechnology Institute, La Paz, Bolivia, Plurinational State of

Group-A rotavirus (RVA) is the most common cause of acute gastroenteritis (AGE) in young children. Following the introduction of the rotavirus vaccine, a significant decline in the burden of the RVA-associated disease was observed. In the last few years, the emergence and spread of unusual DS-1-like intergenogroup reassortant rotavirus strains have been reported across different countries around the world.The objective of this study was to report the detection and full genome characterization of unusual intergenogroup reassortant RVA circulating strains using an NGS-based approach. To this end, 317 fecal samples collected from hospitalized children with AGE across different regions of the country during seasonal and non-seasonal outbreaks were analyzed by ELISA and real-time PCR. A total of 136 (43%) episodes of AGE were associated with RVA, and 34 samples were further selected for genomic analysis. Viral dsRNA was extracted from feces using a QIAamp Viral RNA Mini Kit (Qiagen). The cDNA libraries constructed using the NEBNext Ultra RNA Library Prep Kit were sequenced on an ISeq100 platform (Illumina). The genotype of each of the 11 RVA genes was determined using Rotavirus A Genotyping online tool (versión 0.1). For each gene multiple alignments were carried out using MAFFT 7.0, and phylogenetic trees were constructed using neighbor-joining in MEGA-X. Fifteen samples were found to have a complete genome; eight and seven of those were categorized as belonging to equine-like G3P[8] and G12P[6], respectively. Based on the genomic analysis, RVA strains of both genotypes displayed the segment constellations of G3P[8]-I2-R2-C2-M2-A2-N1/N2-T2-E2-H2 and G12-P[6]-I2-R2-C2-M2-A2-N2-T2-E2-H2. To our knowledge, this is the first description of DS-1-like intergenogroup reassortant strains displaying two different co-circulating genotypes in Bolivia. This data supports the need for continued RVA surveillance of circulating strains in the country.

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ASSESSMENT OF SARS-COV-2 GENOMIC SURVEILLANCE IN THE DEMOCRATIC REPUBLIC OF CONGO, CHALLENGES AND PERSPECTIVES

Nelson Kashali[1], Prince Akili[1], Chloé Musuamba[1], Rilia Ola[1], Gradi Luakanda[1], Emmanuel Lokilo[1], Raphael Lumembe[2], Princesse Paku[1], Elzedek Mabika[1], Gabriel Kabamba[2], Francisca Muyembe[1], Jean Claude Makangara[2], Adrienne Amuri[1], Eddy Kinganda[2], Edith Nkwembe[1], Steve Ahuka[1], Jean-Jacques Muyembe[1], Placide Mbala[1]

[1]National Institute for Biomedical Research, Kinshasa, Congo, Democratic Republic of the, [2]Microbiology Department, Cliniques Universitaires de Kinshasa, University of Kinshasa, KINSHASA, Congo, Democratic Republic of the

December 2019, the world has been turned upside down by the emergence of SARS-CoV-2 which spread across the entire planet. The publication of the first sequence made it possible to characterize the strain and develop vaccines and therapies. In the DRC, the first case was confirmed on March 10, 2020, and the first sequence made public 2 weeks later. In the course of the Covid-19 pandemic, the emergence of variants of interest made it necessary to continue and intensify genomic surveillance. This work enabled us to discuss the role of whole-genome sequencing whole genome sequencing to support the pandemic response and describe the emergence of variants of concern/interest in SARS-CoV2. The positive samples were sent to the sequencing laboratory. We then proceeded with extraction, Amplification, the various library preparations were carried

out respectively by two kits, Midnight for the Nanopore platform and Coviseq for the Illumina platform. integrated the fasta consensus pipeline and GeVarLi for Illumina and Artic for Nanopore. Lineage assignment was performed using Pangolin and Nextclade software, genomes with over 80% coverage were submitted to Gisaid**.** Genomic monitoring has made it possible to detect the introduction and circulation of various variants of interest. Variants of interest: In 2021, we note that the Delta variant circulated throughout the year before predominating during the 3rd wave. It then gave way to the B.1.640 and Omicron variants. In 2022, the majority of variants were Omicron with its sub-variants BA.1, BA.2, BA.3, BA.4, BA.5, BE.1, BF, BN.1, BQ.1, XBB. We also note the presence of B.1.640, Beta and Delta. In 2023, the Omicron variant was predominantly detected in the samples analyzed. To date, the DRC has shared 2233 sequences on Gisaid. We cannot predict where the new variants of concern will appear, but we should count on their early detection and characterization in places where genomic genomic surveillance is advanced.

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ENHANCED IFN-Γ, BUT NOT IL-2, RESPONSE TO *MYCOBACTERIUM TUBERCULOSIS* ANTIGENS IN HIV/ LATENT TB CO-INFECTED PATIENTS ON LONG-TERM HAART

Dawit Gebreegziabiher Hagos

Mekelle University, college of health Sciences, Mekelle, Ethiopia

HIV-infected individuals with latent TB infection are at increased risk of developing active TB. HAART greatly reduces the incidence rate of TB in HIV-infected patients and reconstitutes MTB-specific immune response in the first 12 months of therapy. Evaluation of M.TB-specific functional immune responses in HIV/latent TB co-infected patients who were on HAART for at least 1.5 up to 9 years as compared to HAART-naïve patients were done. Three-hundred sixteen HIV-infected patients without active TB were screened by tuberculin skin testing for M. tuberculosis infection and peripheral blood mononuclear cells (PBMCs) were isolated from 61 HIV/ latent TB co-infected patients (30 HAART-naïve and 31 HAART-treated). IFN-γ and IL-2 ELISPOT as well as CFSE cell proliferation assays were performed after stimulation with M. tuberculosis antigens PPD and ESAT-6. The median frequency of PPD and ESAT-6 specific IFN-γ secreting cells was significantly higher in HAART-treated patients compared to HAART-naïve patients, p=0.0021 and p=0.0081 respectively. However, there was no significant difference in the median frequency of IL-2 secreting cells responding to PPD (p=0.5981) and ESAT-6 (p=0.3943) antigens between HAART-naïve and-treated groups. Both IFN-γ and IL-2 responses were independent of CD4+ T cell count regardless of the HAART status. Notably, the frequency of PPD and ESAT-6 specific IL-2 secreting cells was positively associated with CD4+ T cell proliferation while inversely correlated with duration of HAART, raising the possibility that M. tuberculosis-specific IL-2 response that promote the antigen-specific CD4+ T cell proliferation diminish with time on antiretroviral therapy in HIV/latent TB co-infected patients. This study shows an increased M. tuberculosis-specific IFN-γ, but not IL-2, response in HIV/latent TB co-infected patients with long-term HAART, consistent with only partial immune restoration. Future studies should, therefore, be done to prospectively define the rate and extent to which functional immune responses to M. tuberculosis are restored after long-term HAART.

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COMPUTATIONAL STRUCTURE-BASED DESIGN OF THE SPIKE RBD IMPROVES SARS-COV-2 VACCINES

Thayne Henderson Dickey, Zhantong Wang, Rui Ma, Wai-Kwan Tang, Sachy Orr-Gonzalez, Tarik Ouahes, Palak Patel, Holly McAleese, Brandi Richardson, Brett Eaton, Michael Murphy, Jennifer L. Kwan, Nichole D. Salinas, Baoshan Zhang, Li Ou, Qi Qiu, Lingshu Wang, Tatsiana Bylund, Wing-Pui Kong, Wei Shi, Yaroslav Tsybovsky, Lingyuan Wu, Qiong Zhou, Ridhi Chaudhary, Misook Choe, Mohammed El Anbari, Adam S. Olia, Reda Rawi, I-Ting Teng, Danyi Wang, Shuishu Wang, Michael R. Holbrook, Lynn E. Lambert, Tongqing Zhou, Peter D. Kwong, Niraj H. Tolia

National Institutes of Health, Bethesda, MD, United States

SARS-CoV-2 vaccines, almost all of which contain the spike protein, have dramatically reduced morbidity and mortality due to COVID-19. However, vaccine efficacy wanes as protective antibody titers decrease and escape variants emerge. Improved SARS-CoV-2 vaccines that elicit higher neutralizing antibody titers could counteract such decreases in titer. We have created a computational structure-based design method named SPEEDesign to identify amino acid changes that increase neutralizing antibody titers, focus the immune response to desired epitopes, and improve antigen production and stability. Nine amino acid changes to the receptor-binding domain (RBD) of the spike protein increase the neutralizing antibody titers elicited by monomeric WA1 RBD approximately 10-fold. Production yields also increase approximately 10-fold for all variants tested, including WA-1, XBB.1.5, and B.1.351. These same amino acid changes enable the production of a BA.5 RBD nanoparticle vaccine that elicits potent and broadly neutralizing antibody titers in mice. Finally, incorporation of these amino acid changes into the full-length spike protein significantly increases neutralizing antibody titers elicited in mice and monkeys. These results suggest that the efficacy of most SARS-CoV-2 vaccines, regardless of platform and strain, could be improved by incorporating the nine amino acid changes identified by SPEEDesign. Additionally, we note that the SPEEDesign method is modular and generalizable and thus could be applied to diverse pathogens and antigens.

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CORRELATION BETWEEN CLINICAL BIOMARKERS AND LUNG PATHOLOGY OVER THE COURSE OF ACUTE COVID-19

Joao Luiz Silva-Filho[1], Vanessa Herder[1], Matthew P. Gibbins[1], Monique Freire dos Reis[2], Gisely Cardoso Melo[3], Michael J. Haley[4], Carla Cristina Judice[5], Fernando Fonseca Almeida Val[3], Mayla Borba[2], Tatyana Almeida Tavella[5], Vanderson de Sousa Sampaio[6], Charalampos Attipa[1], Fiona McMonagle[1], Derek Wright[1], Marcus Vinicius Guimaraes de Lacerda[3], Fabio Trindade Maranhão Costa[5], Kevin N. Couper[4], Wuelton Marcelo Monteiro[3], Luiz Carlos de Lima Ferreira[3], Christopher Alan Moxon[1], Massimo Palmarini[1], Matthias Marti[1]

[1]University of Glasgow, Glasgow, United Kingdom, [2]University of Amazonas State, Manaus, Brazil, [3]Tropical Medicine Foundation Dr. Heitor Vieira Dourado, Manaus, Brazil, [4]University of Manchester, Manchester, United Kingdom, [5]University of Campinas, Campinas, Brazil, [6]Tropical Medicine Foundation Dr. Heitor Vieira DouradoUniversity of Glasgow, Manaus, Brazil

COVID-19 is characterized by a broad range of symptoms and disease trajectories. Understanding the correlation between clinical biomarkers and lung pathology over the course of acute COVID-19 is necessary to understand its diverse pathogenesis and inform more precise and effective treatments. Here, we present an integrated analysis of longitudinal clinical parameters, peripheral blood biomarkers, and lung pathology in COVID-19 patients from the Brazilian Amazon. We identified core clinical and peripheral blood signatures differentiating disease progression between recovered patients from severe disease and fatal cases. Signatures were heterogenous among fatal cases yet clustered into two patient groups: “early death” (< 15 days of disease until death) and “late death” (> 15

days). Progression to early death was characterized systemically and in lung histopathology by rapid, intense endothelial and myeloid activation/chemoattraction and presence of thrombi, associated with SARS-CoV-2$^+$ macrophages. In contrast, progression to late death was associated with fibrosis, apoptosis and abundant SARS-CoV-2$^+$ epithelial cells in post-mortem lung, with cytotoxicity, interferon and Th17 signatures only detectable in the peripheral blood 2 weeks into hospitalization. Progression to recovery was associated with higher lymphocyte counts, Th2 and anti-inflammatory-mediated responses. By integrating ante-mortem longitudinal systemic and spatial single-cell lung signatures, we defined an enhanced set of prognostic clinical parameters predicting disease outcome for guiding more precise and optimal treatments. Finally, this study represents a major advance in the investigation of acute respiratory infections by integrating serial clinical data and peripheral blood samples with histopathological and spatially-resolved single-cell analyses of post-mortem lung samples.

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DISENTANGLING DIFFERENCES IN DENGUE VIRUS INFECTION RISK ACROSS SEX IN A LONGITUDINAL COHORT IN KAMPHAENG PHET, THAILAND

Marco Hamins-Puertolas[1], Darunee Buddhari[2], Henrik Salje[3], Derek A.T. Cummings[4], Stefan Fernandez[2], Aaron Farmer[2], Surachai Kaewhiran[5], Direk Khampaen[5], Anon Srikiatkhachorn[2], Sopon Iamsirithaworn[5], Adam Waickman[6], Stephen J. Thomas[6], Timothy Endy[7], Alan L. Rothman[8], Isabel Rodriguez-Barraquer[1], Kathryn B. Anderson[6]

[1]University California San Francisco, San Francisco, CA, United States, [2]Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand, [3]University of Cambridge, Cambridge, United Kingdom, [4]University of Florida, Florida, FL, United States, [5]Ministry of Public Health, Nonthaburi, Thailand, [6]State University of New York Upstate Medical University, Syracuse, NY, United States, [7]Coalition for Epidemic Preparedness Innovations, Washington D.C., DC, United States, [8]University of Rhode Island, Kingston, RI, United States

Dengue virus (DENV) infection is a significant public health concern, with incidence increasing across the globe. To determine the impact of sex on infection risk we analyze data from an ongoing multigenerational longitudinal cohort that began enrollment in 2015 with 3020 participants across 494 households in Kamphaeng Phet, Thailand. Yearly serological sampling of participants yielded 12,161 intervals with 12.1% of intervals having an inferred DENV infection, allowing us to study infection risks across age and sex. At the population scale, females are as likely as males to be infected (OR 0.91; 95% CI 0.81-1.02). However, for individuals of child-bearing age (defined by the 95% of mothers who gave birth between 16-38 in this cohort) we find that sex is a significant risk factor for DENV infection, with females more likely to be infected than males in both univariate (OR 1.37; 95% CI 1.06-1.77) and multivariate logistic regression analyses (aOR 1.44; 95% CI 1.09-1.89) when accounting for prior immunity, seasonality, and household random effects. We incorporated information on if an individual had given birth in the previous year to explore whether risk can be attributed to pregnancy and associated biological changes. We found no significant impact of pregnancy on infection risk (aOR 1.18; 95% CI 0.86-1.61) when also controlling for sex, prior immunity, seasonality, and household random effects. We hypothesize that the observed differences by sex are driven by behavior, e.g., extended exposure to an environment with a susceptible newborn. Using generalized additive models we further explore these results by incorporating age, sex, and household composition to disentangle the behavioral and biological risk factors for DENV infection in the cohort. This work sheds light on the biological, social, and behavioral determinants of dengue virus infection risk and should help to guide efforts to mitigate virus transmission in endemic settings.

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VACCINE-INDUCED T CELL RESPONSES CONTROL FLAVIVIRAL CHALLENGE INFECTION WITHOUT NEUTRALIZING ANTIBODIES

Shirin Kalimuddin[1], Christine YL Tham[2], Yvonne FZ Chan[1], Shou Kit Hang[2], Kamini Kunasegaran[2], Adeline Chia[2], Candice YY Chan[1], Dorothy HL Ng[1], Jean XY Sim[1], Hwee Cheng Tan[2], Ayesa Syenina[2], Noor Zayanah Hamis[2], Valerie Chew[2], Yan Shan Leong[2], Jia Xin Yee[2], Jenny G. Low[1], Kuan Rong Chan[2], Eugenia Z. Ong[2], Antonio Bertoletti[2], Eng Eong Ooi[2]

[1]Singapore General Hospital, Singapore, Singapore, [2]Duke-NUS Medical School, Singapore, Singapore

A significant impediment to viral vaccine development has been the lack of well-defined immune correlates of protection. Increasing evidence suggests a crucial role for T cells, but the level of vaccine-induced T cells needed, and the extent to which they alone can control acute viral infection in humans remains uncertain. To address this knowledge gap, we conducted a randomized double-blind vaccination and challenge study in human volunteers, using the live-attenuated yellow fever (YF17D) and chimeric Japanese encephalitis (JE/YF17D) vaccines. Study volunteers were randomized to receive either YF17D vaccination followed by JE/YF17D challenge 28 days later, or JE/YF17D vaccination followed by YF17D challenge. Viremia, humoral and T cell responses pre- and post-vaccination, as well as pre- and post-challenge infection were longitudinally assessed in all study volunteers. Both YF17D and JE/YF17D induced T cell responses against their shared capsid and non-structural proteins, without inducing cross-neutralizing antibody responses. YF17D induced a greater magnitude of antigen-specific T cell responses compared to JE/YF17D, and vaccination with YF17D was able to reduce mean viremia levels, antibody titers and symptom rates after JE/YF17D challenge. Importantly, even without neutralizing antibodies, viral control after challenge infection was achievable to the extent of undetectable viremia and absence of seroconversion in some vaccinees. Indeed, high vaccine-induced T cell responses, specifically against the capsid protein, correlated with the level of viral control. Our findings further validate the importance of T cell immunity in controlling acute viral infection, and suggests a potential correlate of protection for flaviviral infections and vaccines.

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DELETIONS IN THE 3' UNTRANSLATED REGION COMPROMISED TRANSLATION INITIATION TO ATTENUATE A DENGUE VIRUS 3 VACCINE STRAIN

Kiven Kumar, Esteban Finol, Hwee Cheng Tan, Wy Ching Ng, Eng Eong Ooi

DUKE-NUS Medical School, Singapore, Singapore

Phases I-III clinical trials of TV003, a live attenuated dengue vaccine, have demonstrated favorable safety, immunogenicity, and efficacy profiles. TV003 consist of four DENVs, components attenuated via nucleotide deletions in the 3′ untranslated region (3'UTR). The mechanism behind the attenuation of these vaccine strains remains unknown. To address this knowledge gap, we began by focusing on the DENV-3 component of TV003, which was developed using wild-type DENV-3 Sleman/78. Two mutants were generated, both with 30 nucleotide deletions (Δ30) in dumbbell (DB) II, one additionally with 31 nucleotide deletions (Δ30/31) in DBI of the 3'UTR, alongside Sleman/78, Sleman/78Δ30 (insufficiently attenuated), and Sleman/78Δ30/31 (vaccine strain), offer a unique opportunity to discern the attenuation mechanism from other 3'UTR interactions, evident through a progressive loss of function in these DENV variants. Using Gibson assembly, we constructed infectious clones of these 3 DENV-3. Sleman/78Δ30/31 showed slower replication in Huh-7 cells and primary MoDC, with smaller plaque sizes in BHK-21 monolayers than Sleman/78 and Sleman/78Δ30. To identify binding partners of wild type and mutant DENV-3 3'UTR, we utilized total protein extract from Huh-7 cells in RNA-affinity chromatography coupled with mass spectrometry. We found enrichment of ribosomal proteins (RPs) and translation initiation factors

(TIFs) in wild-type 3'UTR, which were deficient in the Δ30 and Δ30/31 3'UTRs; Δ30/31 3'UTR bound the fewest RPs and TIFs. Consistent with the loss of binding findings, the expression kinetics of prM, NS3 and NS5 proteins of Sleman/78Δ30/31 was the slowest, compared to Sleman/78 and Sleman/78Δ30. Similarly, replacement of the DENV-3 open reading frame with enhanced green fluorescent protein (eGFP) showed lowest eGFP expression with Sleman/78Δ30/31, compared to the other two DENV-3s. Our results suggest that, beyond forming the pan-handle structure of the DENV genome, the 3'UTR through the DBI and DBII secondary structures contribute to recruiting factors involved in translation, reduced efficiency of which attenuated Sleman/78Δ30/31.

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CROSS-NEUTRALIZING ANTIBODY RESPONSES ELICITED BY THE CHIKUNGUNYA VACCINE VLA1553

Whitney C. Weber[1], Zachary J. Streblow[1], Craig N. Kreklywich[1], Michael Denton[1], Gauthami Sulgey[1], Magdalene M. Streblow[1], Dorca Marcano[2], Paola N. Flores[2], Rachel M. Rodriguez-Santiago[2], Luisa Alvarado[2], Vanessa Rivera-Amill[2], William B. Messer[3], Romana Hochreiter[4], Karin Kosulin[4], Katrin Dubischar[4], Vera Bürger[4], Daniel N. Streblow[1]

[1]Oregon Health and Science University, Beaverton, OR, United States, [2]Ponce Health Sciences University, Ponce, Puerto Rico, [3]Oregon Health and Science University, Portland, OR, United States, [4]Valneva Austria GmbH, Vienna, Austria

In November 2023, the first vaccine against chikungunya virus (CHIKV) was approved by the U.S. Food and Drug Administration (brand IXCHIQ®, referred to as VLA1553). Approval came after decades of burden of chikungunya fever in South America, Southeast Asia, and Africa. CHIKV has cocirculated with other pathogenic alphaviruses for decades and as the vaccine rolls out, questions remain regarding the cross-reactive immunity elicited by VLA1553 and potential cross-protection for populations susceptible to multiple alphaviruses. Here, we quantified cross-neutralizing antibody (nAb) responses against arthritogenic alphaviruses in 30 individuals that received VLA1553 in trials NCT04546724 (301) and NCT04838444 (303) conducted in non-endemic settings at one month, six months, and one year post-vaccination. We quantified nAbs against CHIKV strains LR2006 (ESCA), 181/25 (Asian) and 2021 isolate of Tocantins, Brazil (Brazil7124/ESCA). We found the potency of nAbs against the CHIKV strains in vaccinees was nearly identical for the CHIKV strains, with geometric mean 50% plaque reduction neutralization titers ($PRNT_{50}$) ranging ~3000-5000 at one year post-vaccination. We also quantified cross-nAb $PRNT_{50}$ against o'nyong-nyong (ONNV), Mayaro (MAYV) and Ross River (RRV) viruses which were 1156, 650, and 39, respectively. We compared the vaccinee's responses to cross-nAbs elicited by CHIKV infection in 9 individuals in the endemic setting of Puerto Rico at 8-9 years post-infection. We found no significant differences when comparing cross-nAbs between vaccinees at one year post-vaccination and participants at 8 years post-infection for CHIKV-LR2006, MAYV, and RRV, but the $PRNT_{50}$ for CHIKV-181/25, CHIKV-Brazil7124 and ONNV trended slightly significantly higher following infection. Finally, we used antigenic cartography to demonstrate vaccinee and infection sera cluster antigenically. These data imply that VLA1553 elicits a cross-nAb breadth that extends to related alphaviruses to a similar potency of CHIKV infection, which may have important cross-protective implications for individuals susceptible to alphavirus cocirculation.

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DENGUE NS1 ANTIBODIES ARE ASSOCIATED WITH CLEARANCE OF VIRAL NS1

Aileen Y. Chang[1], Michelle Premazzi[1], Evelyn Mendoza Torres[2], Peifang Sun[3], Liliana Encinales[4], Joseph Goulet[5], Jani Vihasi[6], Ying Cheng[6], Karol Suchowiecki[1], Wendy Rosales[2], Richard Amdur[7], Alexandra Porras-Ramirez[8], Alejandro Rico-Mendoza[8], Carlos Herrera-Gomez[1], Ivan Zuluaga[2], Liam Halstead[1], Scott Halstead[9], Gary Simon[1], Kevin Porter[6], Rebecca Lynch[1]

[1]George Washington University, Washington, DC, United States, [2]Universidad Libre, Barranquilla, Colombia, [3]Henry Jackson Foundation, Bethesda, MD, United States, [4]Allied Research Society Colombia, Barranquilla, Colombia, [5]Veterans Affairs, New York, NY, United States, [6]Naval Medical Research Center, Silver Spring, MD, United States, [7]Feinstein Institute for Medical Research, Manhasset, NY, United States, [8]Universidad El Bosque, Bogota, Colombia, [9]Uniformed Services University, Bethesda, MD, United States

Dengue vascular permeability syndrome, the syndrome whereby dengue infection leads to plasma leakage and shock, is the primary cause of death in severe dengue infections. The protective versus potentially pathogenic role of dengue NS1 antibodies is not well understood. The main goal of our analysis was to characterize the relationship between free NS1 concentration and NS1 antibody titers in primary and secondary dengue infection in order to better understand the presence and duration of NS1 antibody complexes in clinical dengue infections. Participants with acute dengue infection were recruited from Atlántico and Magdalena Departments in Northern Colombia from 2018 to 2020. Symptom assessment including dengue signs and symptoms, chart review and blood collection was performed. Primary versus secondary Dengue was assessed serologically. NS1 titers and anti-NS1 antibodies were measured daily. We observe that patients with secondary infection have higher antibody titers than those in primary infection, and we find a negative correlation between anti-NS1 antibody titer and NS1 protein. We demonstrate that in a subset of secondary infection, there are indeed NS1 antibody-antigen complexes at the admission day during the febrile phase that are not detectable by the recovery phase. Furthermore, dengue infection status is associated with higher circulating sialidases. The negative correlation between antibody and protein suggests that antibodies play a role in clearing this viral protein.

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CHARTING THE IMPACT OF MATERNAL ANTIBODIES AND EXPOSURES ON SAPOVIRUS IMMUNITY IN EARLY CHILDHOOD FROM A NICARAGUAN BIRTH COHORT

Sylvia I. Becker-Dreps[1], Michael Mallory[1], Fredman Gonzalez[1], Yaoska Reyes[1], Nadja A. Vielot[1], Boyd L. Yount[1], Kaitlyn Cross[1], Christian Toval-Ruiz[1], Lester Gutierrez[2], Jan Vinje[3], Ralph Baric[1], Lisa Lindesmith[1], Filemon Bucardo[1]

[1]University of North Carolina, Chapel Hill, NC, United States, [2]University of Costa Rica, San Jose, Costa Rica, [3]CDC, Atlanta, GA, United States

Sapovirus was recognized as the second leading cause of acute gastroenteritis (AGE) in children under 24 months of age in the multi-site MAL-ED cohort study. While vaccines against sapovirus may reduce AGE burden, a major challenge to their development is a lack of information about natural immunity to sapovirus. We characterized the development of humoral immunity to sapovirus over the first 3 years of life in a Nicaraguan birth cohort. We measured sapovirus-specific IgG responses in serum collected between 2017 and 2020 from mothers soon after delivery and at 6 time points in children (6 weeks to 3 years of age, n=112 dyads), using virus-like particles representing three sapovirus genotypes (GI.1, GI.2, GV.1). Sixteen (14.3%) of the 112 children experienced at least one sapovirus AGE episode, of which GI.1 was the most common genotype. Seroconversion to GI.1 and GI.2 was most common between 5 and 12 months of age, while seroconversion to GV.1 peaked at 18 to 24 months of age. Most seroconversions were not accompanied by AGE symptoms. All children who experienced sapovirus GI.1 AGE seroconverted and developed genotype-specific IgG responses. In summary, Infants are born with broad

sapovirus-specific IgG, similar to their mothers, that declines to its lowest levels in mid-infancy. After this age, genotype-specific seroconversions occur, and by 24 to 36 months of age, seropositivity patterns of children resemble that of their mothers. By tracking humoral immunity to sapovirus over the first 3 years of life, this study provides important insights for the design of future pediatric sapovirus vaccines.

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SIGNALING CIRCUITS INVOLVED IN THE SELECTION OF HIGH-AFFINITY ANTIGEN-SPECIFIC B CELLS IN THE GERMINAL CENTER

Timothy Chege Kuria, Thomas H. Winkler, Andrea Schneider
Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany

The germinal center (GC) functions as the site where somatic hypermutation, affinity maturation, and the selection of high-affinity B cells occur. Two selection models are proposed within the GC: a death-limited model and a birth-limited model. In the simplified death-limited model, low-affinity GC B cells undergo apoptosis, while high-affinity B cells do not. In the more practical birth-limited model, selection signals are conveyed through increased upregulation of metabolic factors in high-affinity cells, leading to accelerated proliferation compared to low-affinity GC B cells. Despite the selection model, high-affinity B cells consistently outperform low-affinity B cells in the GC. However, the exact signaling pathways governing this selection are not yet fully elucidated. An experimental approach involving the use of a 4-hydroxy-3-nitrophenyl (NP) antibody mouse model combined with a non-responsive NP recipient mouse model was employed. High and low-affinity B cells from donors were competitively transferred into the recipient mice. Recipient mice were sacrificed on days 6 and 9 of the GC response, and the GC response was assessed. RNA was extracted from donor B cells, followed by bulk RNA sequencing and analysis of differential gene expression to identify potential genes involved in GC selection. High-affinity B cells consistently outperformed low-affinity B cells, even when the initial ratio of high-affinity to low-affinity B cells was 1:138. Differential expression analysis of low and high-affinity GC B cells revealed a set of genes. Initially, focus was placed on a single gene with significant biological function. Through multiple subsequent experiments, it was observed that high-affinity B cells downregulated the expression of the candidate gene not only at the transcriptome level but also at the protein level. This project identified a novel gene that seems to play a role in regulating the GC selection process and the differentiation into antibody-secreting cells. Valuable insights were gained through the potential identification of a new signaling pathway within GC selection, mediated by the novel gene.

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PRIMARY ZIKA VIRUS INFECTION INCREASES HETEROTYPIC DENGUE VIRUS SERUM NEUTRALIZATION UPON SECONDARY DENV-3 INFECTION IN RHESUS MACAQUES

Jenna M. DeLuca[1], Matthias Lingemann[1], Hunter J. Ries[2], Chelsea M. Crooks[2], Giacomo Sidoti-Migliore[1], Taylor J. McGee[1], David Castañeda[3], Ellie K. Bohm[3], Julia C. Pritchard[4], Stephen S. Whitehead[5], Matthew T. Aliota[3], Thomas C. Friedrich[2], Mattia Bonsignori[6]

[1]Translational Immunobiology Unit, Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States, [2]University of Wisconsin-Madison, Department of Pathobiological Sciences, Madison, WI, United States, [3]University of Minnesota, Twin Cities, Department of Veterinary and Biomedical Sciences, Saint Paul, MN, United States, [4]University of Minnesota, Twin Cities, Department of Veterinary and Biomedical Sciences, Saint Paul, MD, United States, [5]Arbovirus Vaccine Research Section, Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States, [6]National Institutes of Health, Bethesda, MD, United States

Zika (ZIKV) and dengue (DENV) viruses co-circulate in the equatorial tropics. Due to genetic and structural similarities, antibody (Ab) cross-reactivity between ZIKV and DENV is common, and pre-existing ZIKV immunity can be protective, pathogenic, or neutral on successive DENV infections. Assessing the effect of prior infections on Ab responses in observational human studies is complicated by the heterogeneity in infection histories and the ability of both viruses to cause paucisymptomatic and asymptomatic infections. Here, we used rhesus macaques (RM) to model the effect of a prior ZIKV infection on the specificity and neutralizing activity of serum Ab elicited by subsequent DENV-3 infection. Flavivirus-naïve (n=8) or ZIKV-immune RMs (n=10) were subcutaneously inoculated with 10^4 PFU DENV-3 strain 6629, originally isolated from a human in Nicaragua in 2013. Plasma vRNA burden, serum Ab binding and neutralization titers were assessed over 90 days post-DENV-3 infection. RMs were productively infected with DENV-3 and all resolved infection by 15d post-inoculation. Prior ZIKV infection did not affect DENV-3 viral load peak or viremia duration. Primary DENV-3 infection yielded minimal, non-neutralizing ZIKV-cross-reactive binding Ab (geometric mean [GM] peak EC_{50} Ab titers = 89) whereas ZIKV-immune RMs developed high ZIKV-cross-neutralizing Ab titers (GM peak reporter viral particle neutralization titer $[RVPNT]_{50}$ = 2492), consistent with recall of pre-existing cross-reactive Ab. Prior ZIKV infection did not affect DENV-3 binding or neutralizing Ab titers. However, compared to RMs with primary DENV-3 infection, ZIKV-immune RMs developed significantly higher peak neutralizing serum Ab titers against other DENV serotypes (GM $RVPNT_{50}$ peak values: DENV-1 = 540 vs <100, DENV-2 = 1307 vs 135, and DENV-4 = 606 vs <100). In conclusion, pre-existing ZIKV immunity did not have a detrimental impact on the course of secondary DENV-3 infections. Instead, prior ZIKV immunity engaged Ab specificities that cross-neutralize heterotypic DENVs and skewed Ab recall responses toward epitopes that are present and accessible on all DENV serotypes.

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LIFESTYLE SCORES ARE ASSOCIATED WITH CELLULAR IMMUNE PROFILES IN HEALTHY TANZANIAN ADULTS

Jeremia J. Pyuza[1], Marloes M.A.R. van Dorst[2], Koen Stam[2], Linda Wammes[2], Marion König[2], Vesla Kulaya[3], Yvonne Kruize[2], Wesley Huisman[2], Nikuntufya Andongolile[4], Anastazia Ngowi[5], Elichilia R. Shao[6], Alex Mremi[1], Pancras C.W. Hogendoon[7], Sia E. Msuya[8], Simon Jochems[9], Wouter A.A. de Steenhuijsen Piters[9], Maria Yazdanbakhsh[9]

[1]Department of Pathology, Kilimanjaro Christian Medical Centre, Moshi, Tanzania, Moshi, Tanzania, United Republic of, [2]Leiden University Center for Infectious Diseases (LUCID), Leiden University Medical Center, 2333 ZA Leiden, the Netherlands, Leiden, Netherlands, [3]Kilimanjaro Clinical Research Institute (KCRI), Kilimanjaro Christian Medical Center, Moshi, Tanzania, Moshi, Tanzania, United Republic of, [4]Department of clinical laboratory, Kilimanjaro Christian Medical Centre, Moshi, Tanzania, Moshi, Tanzania, United Republic of, [5]Department of community medicine, Kilimanjaro Christian Medical Centre, Moshi, Tanzania, Moshi, Tanzania, United Republic of, [6]Department of Internal Medicine, Kilimanjaro Christian Medical Center (KCMC), Moshi Tanzania, Moshi, Tanzania, United Republic of, [7]Department of Pathology, Leiden University Medical Center, Leiden The Netherlands, Leiden, Netherlands, [8]Institute of Public Health, Kilimanjaro Christian University Medical College (KCMUCo), Moshi, Tanzania, Moshi, Tanzania, United Republic of, [9]Leiden University Center for Infectious Diseases (LUCID), Leiden University Medical Center, 2333 ZA Leiden, the Netherlands, leiden, Netherlands

Different immune profiles have been associated with responses to vaccines in high-income regions of the world. Importantly, immune system and vaccine responses can vary across geographical locations worldwide. Not only are responses different between high and low-middle-income countries (LMICs) but also between rural and urban populations within LMICs. Lifestyle factors such as housing conditions, exposures to microorganisms, parasites and diet are variables associated with rural-/urban-living. However, the relationships between lifestyle factors and immune profiles have not

been mapped in detail. Here, a lifestyle score was developed based on household assets, housing conditions and recent dietary history of an individual and its association with cellular immune profiles was studied. Immune profiling of healthy Tanzanians adults across four rural-/urban areas was performed using mass cytometry. Seventeen of *80* clusters were associated with location or lifestyle score, with eight identifiable only when using lifestyle scores. Rural residents with low lifestyle scores showed higher frequencies of $CD56^+$ NK cells, plasmablasts, atypical memory B cells, T helper 2 cells, Regulatory T cells, and activated $CD4^+$ T effector memory cells expressing CD38, HLA-DR, and CTLA-4. In contrast, those with high lifestyle scores, most of whom living in urban areas, showed a less activated state of the immune system and were enriched for naïve $CD8^+$ T cells. Using an elastic net machine learning model, we identified 'cellular immune signatures' by assessing the association between cell clusters and lifestyle scores. Assuming a link between these immune signature and vaccine responses, these signatures may inform us the cellular mechanisms underlying vaccine hyporesponsiveness, reduced autoimmunity, and allergies in low- and middle-income countries.

6301

APPLYING A ELECTROCHEMILUMINESCENCE MULTIPLEX SEROLOGIC ASSAY TO DETECT AND DIFFERENTIATE ZIKA AND DENGUE VIRUS EXPOSURES DURING LONG-TERM FOLLOW UP OF A COMMUNITY COHORT IN BRAZIL

Nivison Ruy R. Nery Jr[1], Joseph Lu[2], Cate Muenker[3], Pablo Aguilar[1], Mariam O. Fofana[3], Jaqueline S. Cruz[1], Daiana de Oliveira[1], Stephen J. Thomas[2], Federico Costa[4], Mitermayer G. Reis[1], Derek A.T. Cummings[5], Adam Waickman[2], Albert I Ko[6]

[1]Goncalo Moniz Institute, Salvador, Brazil, [2]Institute for Global Health, State University of New York, New York, NY, United States, [3]Department of Epidemiology of Microbial Diseases, Yale School of Public Health, New Haven, CT, United States, [4]Institute of Collective Health, Federal University of Bahia, Salvador, Brazil, [5]Department of Biology, University of Florida, Gainesville, FL, United States, [6]Department of Epidemiology of Microbial Diseases, Yale School of Public Health, NEW HAVEN, CT, United States

The cross-reactivity between flavivirus has been a major barrier to performing epidemiological investigations that address fundamental questions on how many people were exposed to the Zika virus (ZIKV) in the Americas and whether immunity persists for extended periods after exposure. These questions are critical for suspected cases continue to be detected in regions where population immunity is presumably high. To address this challenge, we developed an electrochemiluminescence assay and evaluated its performance for the serological detection of ZIKV and DENV infections in a cohort from Salvador, Brazil, which was followed from 2014 to 2024. We previously found that 70% of the cohort were exposed to ZIKV in 2015. In this pilot evaluation, we evaluated samples obtained before and after the epidemic for evaluation in a multiplex electrochemiluminescence assay (MesoScale Discovery, MSD) that detects ZIKV and DENV NS1-specific IgG antibodies. We determined the sensitivity and specificity of fold changes in ZIKV IgG antibodies as compared to DENV IgG antibodies. Among the 1453-member cohort, we selected 106 participants among which 58% (61) had serologic evidence of a DENV exposure prior to the ZIKV epidemic. Of the 61 and 45 participants with and without prior DENV exposure, respectively, 66% (40) and 67% (30) demonstrated MSD seroconversion to ZIKV after the epidemic. The MSD assay demonstrated 95% sensitivity and 93% specificity for detecting ZIKV infection among individuals previously exposed to DENV, and 97% sensitivity and 98% specificity among individuals without prior DENV exposure. These findings provide preliminary evidence the multiplex electrochemiluminescence assay is sensitive and specific for detecting ZIKV infection in a population with high background DENV exposure. We are extending these analyses to address whether ZIKV antibodies persist during a ten-year follow-up of our cohort and whether there is continued circulation of ZIKV. If validated, the assay may hold promise as a tool in detecting ZIKV transmission in populations where infections may be underrecognized and in screening pregnant women during prenatal care.

6302

UNDERSTANDING IMMUNITY TO MPOX AND SMALLPOX VACCINATION TO INFORM ON SEROSURVEILLANCE, DIAGNOSTIC DEVELOPMENT, AND NEXT-GENERATION VACCINES

Scott Jones, Bethany Hicks, Cathy Rowe, Bassam Hallis, **Ashley Otter**

UK Health Security Agency, Salisbury, United Kingdom

In early 2022, a cluster of Monkeypox virus (MPXV) cases were identified within the UK with no prior travel history, suggesting localised transmission of MPXV within the UK, particularly affecting gay, bisexual, & other men who have sex with men. Subsequently, a global outbreak occurred & Mpox continues to spread, with outbreaks in South-East Asia. Public health agencies worldwide have offered the Smallpox vaccination (IMVANEX/JYNNEOS) as a means to provide protection & limit the spread of MPXV. Through the development of a comprehensive set of multi-antigen ELISA & Luminex assays, we have been able to assess the serological responses to ~27 different Orthopoxvirus antigens in individuals with one, two, or three doses of Smallpox vaccination, & those with prior infection with MPXV. Furthermore, a subset of these have been tested for their neutralisation capacity against MPXV & VACV, as well as epitope mapping using a 15-mer peptide library against 33 MPXV-specific proteins. Using diverse Orthopoxvirus antigen ELISAs & Luminex assays, we observe differential trends in antigen-specific antibody dynamics after Smallpox vaccination, with variable waning by antigen. Prior MPXV infection induces similar responses to vaccination, in addition to an infection-specific response to A27. We observe trends in neutralisation against MPXV & VACV consistent with previous findings, whilst epitope mapping also shows similar yet distinctive antibody binding between both Smallpox-vaccinated & those with prior Mpox infection. Here, we show that both MPXV-infected & Smallpox-vaccinated individuals mount immune responses to a diverse yet core set of poxvirus antigens, with previous infection inducing a similar response. Neutralisation & epitope mapping varied by infection & vaccination, with core yet distinctive responses between infected & vaccinated individuals. We have since used these data together to develop next-generation serological tools for serosurveillance, and diagnostic development, & aid in future vaccine or therapeutic development to support the ongoing response to Mpox.

6303

ROBUST T CELL RESPONSES IN ADULT MICE PROVIDE INSIGHTS INTO PROTECTION AGAINST LA CROSSE VIRUS ENCEPHALITIS

Reem Alatrash, Bobby Brooke Herrera

Rutgers Global Health Institute, Rutgers University, New Brunswick, NJ, United States

La Crosse virus (LACV), a negative-sense RNA bunyavirus, is a causative agent of pediatric encephalitis, often leading to severe clinical outcomes, including fatalities. LACV encephalitis predominantly affects individuals aged 16 years or younger, indicating an age-associated vulnerability. This susceptibility is recapitulated in murine models, with weanling mice (≤3 weeks old) exhibiting LACV-induced neuropathology, while adult mice (≥6 weeks old) display resistance. Despite the severity of LACV infections, approved vaccines or therapies are lacking, and the immunological mechanisms safeguarding adult mice from severe disease remain elusive. This study aimed to characterize the cellular response in adult and weanling mice following LACV infection. Leveraging in silico analysis, we identified peptides across the LACV proteome predicted to induce T cell responses in both mice and humans. Subsequently, through the use of ELISPOT assays, flow cytometry, and intracellular cytokine staining, we assessed the quantity and functionality of $CD4^+$ and $CD8^+$ T cells in LACV-infected adult and weanling mice. Our findings demonstrated that as early as 6 days post-infection, adult mice mount significantly more robust and polyfunctional cellular responses directed against both structural and non-structural proteins of LACV compared to weanling mice. Notably, $CD4^+$ and $CD8^+$

T cells derived from both spleens and brains of LACV-infected adult mice displayed heightened magnitude and polyfunctionality, characterized by significantly increased intracellular expression of key cytokines including IFN-γ, TNF-α, IL-2, and granzyme B. Furthermore, adoptive transfer of immune splenocytes to weanling mice 1 day prior to infection conferred protective effects. These data shed light on the role of robust, polyfunctional T cell responses in conferring disease resistance in adult mice against LACV infection. A comprehensive understanding of the cellular correlates of immunity following LACV infection is essential for the development of effective vaccines aimed at protecting children from LACV-induced disease.

6304

ZIKA VIRUS DNA VACCINES INCORPORATING DISULFIDE-BOND STABILIZATION OF ENVELOPE PROTEIN DIMERS

Yuxiang Wang, Kimberly A. Dowd, Katherine E. Burgomaster, David N. Gordon, Laura A. Vanblargan, Ted C. Pierson

National Institutes of Health, Bethesda, MD, United States

The introduction of Zika virus (ZIKV) to South America in 2015 prompted the rapid advancement of vaccine candidates through preclinical and clinical studies, many of which were designed to express the two structural proteins premembrane (prM) and envelope (E). Expression of these two flavivirus proteins is sufficient for the production of noninfectious subviral particles (SVPs). SVPs represent a favorable approach to immunization due to their incorporation and display of the antiparallel E dimers that comprise the surface of an infectious virion and have been shown to be the target of neutralizing antibodies. Because protection from ZIKV infection has been correlated with an ability to neutralize mature forms of the virus, we hypothesized that locking these dimers in place via the introduction of engineered cysteine mutations may favor the induction of E dimer-specific antibodies, while limiting the elicitation of antibodies against less desirable targets. To investigate this, we designed multiple SVP vaccine constructs incorporating various combinations of cysteine mutations previously shown to promote disulfide bond formation between neighboring E proteins within a dimer. Transient transfection studies demonstrated that not all constructs were efficiently released from cells and that temperature could impact particle formation. Western Blot analysis confirmed the presence of disulfide-bonded E dimers in a subset of constructs. Neutralization assays will be performed on sera collected from mice immunized with our panel of DNA vaccine constructs to determine if the incorporation of cysteine mutations improves the antibody response in comparison to parental constructs that lack cysteine mutations.

6305

INVESTIGATING THE ROLE OF VACCINE INDUCED HUMORAL IMMUNE RESPONSES IN PROTECTION AGAINST MARBURG VIRUS AND SUDAN VIRUS DISEASES

Troy Odo

University of Hawaii, Honolulu, HI, United States

Filoviruses cause severe disease and different members of the family have led to outbreaks in humans. Currently, two vaccines have been approved to protect against Ebola virus (EBOV) infection, but there are no vaccines licensed to protect against other filoviruses. Specifically, Sudan virus (SUDV) and Marburg virus (MARV) have demonstrated the capability of causing deadly outbreaks of disease necessitating the need for additional effective filovirus vaccines. The EBOV glycoprotein (GP) shares limited homology with SUDV and MARV glycoproteins which eliminates the chance for cross-protection of EBOV specific vaccines. To address this problem, our group developed adjuvanted recombinant glycoprotein subunit vaccines for EBOV, MARV, and SUDV. Monovalent SUDV and MARV vaccines as well as bivalent formulations containing EBOV GP and either SUDV or MARV GP were tested for efficacy in cynomolgus macaques showing full protection against lethal infection with live MARV and SUDV. Our current work further investigates the role of humoral immune responses in protection against these diseases. Specifically, we analyze high avidity, neutralizing antibodies. Neutralizing antibody titers were determined using an rVSV-GFP reporter based microneutralization assay and antigen specific binding IgG concentrations and avidity was measured using a multiplex immunoassay. Two or three doses of the both SUDV vaccine formulations induced high titers of antigen binding IgG, antigen-specific virus neutralization and increasing IgG avidity which suggests continuing B-cell maturation. Interestingly, despite increasing MARV GP specific IgG concentrations and avidity, and full protection against challenge, neither MARV GP vaccine elicited high MARV neutralizing antibody titers suggesting that neutralizing antibodies may play a limited role in protection against that virus. Overall, these results reveal important information regarding the role of the humoral immune response in protection against filovirus disease which will help to direct future vaccine development efforts as well as a better understanding of natural immunity to filoviruses.

6306

FLAVIVIRUS TOOLS FOR VACCINE RESEARCH

Edgar Davidson, Lewis J. Stafford, Allison Sheetz, Christina Go, Chida Sulli, Jennifer Pfaff-Kilgore, Nathan A. Krump, Benjamin J. Doranz

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

To help characterize the immune response to flavivirus infection and vaccines we have generated tools to analyze monoclonal antibodies (MAbs) and sera. We identify the binding sites (epitopes) for MAbs (>300 to date) at amino acid-resolution, using comprehensive alanine-scan mutation libraries (each >660 single mutations) of prM/E envelope proteins for dengue virus (DENV) serotypes 1-4, and Zika virus (ZIKV). Libraries were transfected into human cells for native expression and folding, with MAb binding to individual prM/E variants quantified by high-throughput flow cytometry. The epitopes have expanded our understanding of the immune response to prM/E and individual MAb neutralizing capabilities. Epitopes were both conformational (including quaternary) and non-conformational, were spread across prM and E domains I-III, and many have been correlated with their protective abilities against DENV or ZIKV infection. Epitope locations can give insights into MAb mechanism of action, such as MAbs that bind across adjacent E proteins, preventing rearrangements necessary for infectivity. Mapping also revealed epitopes common to DENV and ZIKV, information that can help create better vaccines and therapeutics. For vaccine development and research, we have produced reporter virus particles (RVPs) for DENV 1-4, ZIKV, West Nile and Yellow Fever viruses. RVPs are produced by expressing virus structural genes - C/prM/E - in trans with full-length replicon from which these genes were removed and replaced with a luciferase reporter gene. RVPs are safe and convenient for infectivity neutralization studies, including high-throughput analyses. RVPs are antigenically highly similar to wild type virus, and their maturation state can be readily manipulated, but are capable of only one round of infectivity, detected by luminescent readout. RVPs provide reproducible neutralization data across different production lots, volumes, time frames, laboratories, and are stable over long-term storage. DENV and ZIKV RVPs are used in studies to determine neutralization titers of sera from animals and humans immunized with candidate vaccines.

6307

THE ANGIOPOIETIN-TIE-2 AXIS IN CHILDREN AND YOUNG ADULTS WITH DENGUE VIRUS INFECTION IN THE PHILIPPINES

Hridesh Mishra[1], Michelle C. Ngai[1], Valerie M. Crowley[1], Vanessa Tran[2], Maria Salome Siose Painaga[3], James Yared Gaite[3], Patrick Hamilton[4], Kevin C. Kain[1], **Michael T. Hawkes**[5]

[1]University Health Network, Toronto, ON, Canada, [2]Public Health Ontario, Toronto, ON, Canada, [3]Lebumfacil-Santa Ana Medical Center, Toledo, Philippines, [4]University of Alberta, Edmonton, BC, Canada, [5]University of British Columbia, Vancouver, BC, Canada

Dengue virus (DENV) infection is associated with plasma leakage, which may progress to shock. The Angiopoietin (Ang)-Tie-2 axis regulates endothelial permeability. We examined the clinical utility of Ang-1, Ang-2

and the Ang-2:Ang-1 ratio for prediction of progression to severe DENV in a prospective cohort study of children and young adults (age 1 to <26 years) with DENV infection presenting to an outpatient clinic in the Philippines. Ang-1, Ang-2, and Tie-2 were measured from stored plasma by multiplex Luminex® assay. Patients were followed prospectively to document the clinical course (hospitalization, length of stay, intravenous fluid resuscitation, and transfer to a higher-level facility). We included 244 patients (median age 9 years, 40% female). At presentation, 63 patients (26%) had uncomplicated dengue, 179 (73%) had dengue with warning signs, and 2 (0.82%) had severe dengue. 181 patients (74%) were hospitalized. Ang-1 levels were lower and Ang-2 higher in patients who required hospitalization. Ang-2:Ang-1 ratio > 1 was associated with a relative risk of hospitalization of 1.20 (95% 1.03-1.36, p=0.016). A higher Ang-2:Ang-1 ratio was associated with longer length of hospital stay, higher frequency of transfer to a higher-level facility, larger intravenous fluid requirement, hemoconcentration, and thrombocytopenia. Ang-2 was correlated with procalcitonin (Kendall's τ =0.17, p=0.00012), a marker of systemic inflammation, as well as sVCAM-1 (τ =0.22, p<0.0001) and Endoglin (τ =0.14, p=0.0017), markers of endothelial activation. In conclusion, altered Ang-2:Ang-1 ratio can be detected early in the course of DENV infection and predicts clinically meaningful events (hospitalization, length of stay, and fluid resuscitation).

6308

IDENTIFICATION OF THE FLAVIVIRUS CONSERVED E-L295 RESIDUE AS A TARGET FOR THE RATIONAL DESIGN OF CANDIDATE WEST NILE LIVE-ATTENUATED VACCINES

Emily K. Mantlo[1], Bailey E. Maloney[2], So Lee Park[3], Adrienne E. Pohl[3], Natalia Costa-Ball[3], Claire Y.-H. Huang[2], Alan D.T. Barrett[4], Yan-Jang S. Huang[1]

[1]Department of Microbiology and Immunology, State University of New York Upstate Medical University, Syracuse, NY, United States, [2]Division of Vector-Borne Diseases, Centers for Disease Control and Prevention, Ft. Collins, CO, United States, [3]Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS, United States, [4]Sealy Institute for Vaccine Sciences, University of Texas Medical Branch, Galveston, TX, United States

West Nile virus (WNV) is a flavivirus endemic in the United States. There is no licensed WN vaccine. Development of a candidate WN live-attenuated vaccine (LAV) that can elicit protective immunity after a single immunization is a research priority. In the 21st century, LAVs are expected to encode multigenic mutations underlying a defined molecular basis of attenuation. The envelope (E) protein governs viral entry and is a major target for neutralizing antibodies, making it a critical component for WN LAV development. Each E protein monomer has three domains (EDI, EDII, and EDIII). Two neighboring E monomers form the dimer parallel to the virion. As a class II fusion protein, the conformational change of the E protein from dimer to trimer induces the membrane fusion for releasing viral genome into the cytoplasm. Formation of the E protein trimer is irreversible due to the relative movement between EDI and EDIII that creates a stable structure. EDI and EDIII are connected by a single EDI-EDIII linker. Five of 11 residues within the EDI-EDIII linker are conserved among flaviviruses, indicating a common mechanism for the EDI-EDIII interdomain movement. Mutations of the flavivirus conserved residues in the EDI-EDIII linker exhibit attenuating effects for yellow fever and dengue -2 viruses. However, no study has systematically examined the utility of these mutations for the rational design of candidate LAVs. As a proof-of-concept, we demonstrate that the WNV E-L295 residue in the EDI-EDIII linker is a potential target for attenuating mutation. Eight alternative amino acid substitutions of the E-L295 residue were retained in the consensus sequence of WNV mutants rescued from a cDNA infectious clone of the NY99 strain. The E-L295S mutant exhibited infectivity exceeding 6 log10PFU/ml in transfected Vero cells and yet fully attenuated WNV-NY99ic without compromising the neutralizing antibody response in 4-week-old outbred Swiss mice. Further, the genome-PFU ratio suggests that the E-L295S mutation did not significantly compromise virion assembly. Our results indicate that the E-L295 residue is a target for the rational design of candidate WN LAVs.

6309

IL-1β MEDIATES POWASSAN VIRUS INFECTION AND ESTABLISHMENT AT THE SKIN INTERFACE

Dakota N. Paine, Saravanan Thangamani

SUNY Upstate Medical University, Syracuse, NY, United States

Powassan virus Lineage I (POWV) and Lineage II (DTV) are tick-borne flaviviruses endemic to the United States and Canada that can cause severe encephalitis and meningitis and are primarily transmitted by *Ixodes scapularis and Ixodes cookei* ticks. During blood feeding, a variety of pharmacologically active proteins and other molecules are transmitted via salivary gland secretion that acts in ways that disrupt the homeostasis of the bite-site microenvironment. The mechanisms of blood feeding and tick salivary factors have been demonstrated to facilitate virus transmission. However, a significant gap exists in our current knowledge of how the immunomodulation at the tick-host-virus interface enhances POWV transmission, establishment, and dissemination to the target organs. We have demonstrated the activation of IL-1β during POWV-infected tick feeding at the transmission interface. Here, we describe the activation and secretion of IL-1β via the NLRP3 inflammasome pathway during POWV infection. We show that infection with POWV in THP-1^{PMA} cells results in the secretion of IL-1β into the supernatant, in line with LPS positive control. However, the mechanism of this secretion during POWV infection is poorly understood. We aim to utilize NLRP3-KO-THP1 cells alongside CASPdef-THP1 cells to assess the lack of NLRP3 and Caspase-1 on the cleavage/secretion mechanisms. Next, we will infect these cells with POWV with and without *Ixodes scapularis* salivary gland extract (SGE) to assess the role of salivary gland factors in activating IL-1β via the inflammasome pathway. Additionally, to fully validate our findings on a relevant model, we will utilize an *ex vivo* human skin model that utilizes whole dermal tissue to assess the impacts of tick-borne virus infection. Our proposed studies will lead to a better understanding of immunomodulation at the tick-virus-host interface during POWV transmission.

6310

SUSCEPTIBILITY AND TRANSMISSION POTENTIAL OF ECTOTHERMS AND HOUSE SPARROWS TO JAPANESE ENCEPHALITIS VIRUS

Audrey Walker, Airn Hartwig, Angela Bosco-Lauth, Richard Bowen

Colorado State University, Fort Collins, CO, United States

Japanese encephalitis virus (JEV) is a vector-borne flavivirus that is known to be maintained in an enzootic lifecycle between mosquitoes, pigs and wading birds. An estimated 68,000 human cases are reported annually, and symptoms in humans can range from a mild fever to severe neurological complications. Arboviral diseases are spreading to new areas at alarming rates secondary to increases in global trade and travel, climate change and migration of both animal reservoirs and vectors. Although JEV is currently only endemic in Asia, concerns for the spread of JEV into new areas are rising as indicated by a recent outbreak on the mainland of Australia. This outbreak is causing significant public health impacts by inflicting illness in humans and creating notable economic losses to the pig industry. Given that other closely related arboviruses such as West Nile virus have spread to the U.S. over the past several decades, JEV holds high potential to become established in the U.S. However, little is known about what animal reservoirs, particularly wildlife inhabiting mosquito-dense locales, could contribute to JEV ecology in the U.S. Here we report that ball pythons, garter snakes, and house sparrows are susceptible to JEV genotypes I and III, but not to JEV genotypes II and IV. Frogs exhibited susceptibility to JEV genotype I. Meanwhile, toads, alligators and green anoles were not susceptible to any of the four genotypes of JEV. Our results expand upon the knowledge base of susceptible species and provide evidence that domestic wildlife species could play a role in the introduction or maintenance of JEV within the U.S.

6311

EVALUATION OF 41 BIOMARKERS FOR PREDICTION OF MORE SEVERE DENGUE OUTCOMES

Nguyen L. Vuong[1], Huynh T. L. Duyen[1], Nguyen M. Tuan[2], James Whitehorn[3], Magdalena S. Lange[4], Ronald B. Geskus[1], Sophie Yacoub[1], Matthias Strobl[4]

[1]Oxford University Clinical Research Unit, Ho Chi Minh City, Vietnam, [2]Children's Hospital No. 1, Ho Chi Minh City, Vietnam, [3]Guy's and St Thomas' NHS Foundation Trust, London, United Kingdom, [4]Roche Diagnostics GmbH, Penzberg, Germany

Dengue continues to be a major public health burden globally. While most dengue cases resolve after one week, 1-5% of hospitalized patients develop severe manifestations during the critical phase (day 4-6 from fever onset), allowing a window of opportunity to identify patients at risk of progression. This nested case-control study assessed 41 biomarkers using stored blood samples from 2 previous studies performed between 2010 and 2015 in Vietnam. One study enrolled children and the other enrolled adults, all presenting within 3 days after fever onset. The biomarkers were selected from the vascular, immunological, and inflammatory pathways in dengue. Six biomarkers were analysed using the Elecsys system, and 35 were analysed using a Luminex panel. Clinical endpoints were severe dengue (based on the WHO 2009 classification) for children and moderate-to-severe plasma leakage for adults. We used logistic regression to analyse the relation of each biomarker with outcome separately, and used multivariable lasso logistic regression to select which biomarkers best predict the outcome. We found that for most biomarkers elevated values in children associated with severe dengue (the most notable were ESM-1, syndecan-1, osteopontin, ferritin, GDF-15, and angiopoietin-2), whereas for 10 markers, typically vWFA-2 and PDGFCC, the association was reversed. In adults, for almost all biomarkers elevated values associated with moderate-to-severe plasma leakage, with the most notable being IL-6, GDF-15, angiopoietin-2, procalcitonin, and IL-8. From the lasso models, 17 biomarkers were identified as promising for children, with ESM-1, syndecan-1, IL-1ra, procalcitonin, FDGFCC, and sFLT-1 having the strongest association with severe dengue. For adults, four biomarkers were identified as promising, including procalcitonin, IL-6, IL-1ra, and GDF-15. These suggested biomarkers may play a role for future biomarker based prognostic tests for dengue disease progression.

6312

NEUTROPHIL MEDIATORS LINKED TO TIGHT JUNCTION DISRUPTION AND INCREASED INTESTINAL PERMEABILITY IN SEVERE DENGUE

Andrew Teo[1], Po Ying Chia[2], Tsin Wen Yeo[1]

[1]Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore, Singapore, [2]National Centre for Infectious Diseases, Singapore, Singapore

In both pediatric and adult dengue cases, there is a correlation between disease severity and increased intestinal permeability. Notably, in dengue mouse models, infection activates monocytes and tissue macrophages that result in viral dissemination and heightened neutrophil infiltration within the gastrointestinal tract. While the breakdown of tight junctions due to neutrophil mediators has been observed in inflammatory bowel conditions, this phenomenon has not yet been reported in the context of dengue. Plasma samples from 97 adult dengue patients; 39 dengue fever (DF), 45 dengue with warning signs (DWS) and 13 severe dengue (SD) were collected in the febrile, critical and recovery phases. Samples were assayed for markers of intestinal injury; Trefoil factor 3 (TFF3), microbial translocation; lipopolysaccharide binding protein (LBP) and CD14, tight junction integrity; zona occludens-1 (ZO1) and claudin-5; and neutrophil mediators; matrix metalloprotease-8 (MMP8), myeloperoxidase, and elastase. Thirty healthy controls were included. In the febrile and critical phases, all measured proteins were elevated in dengue samples compared to controls and proteins returned to baseline at recovery phases. In the febrile phase, SD subjects had higher ZO1, myeloperoxidase and elastase levels compared to DWS and DF. In the critical phase, TFF3, CD14 and myeloperoxidase were increased in SD vs DWS and DF. In the febrile phase, LBP was significantly associated with ZO1 (r=0.43, P<0.001) and CD14 was associated with claudin-5 (r=0.41, P<0.001), indicating intestinal injury and microbial translocation. Additionally, in the febrile phase, ZO1 was associated with MMP8 (r=0.67 P<0.01), myeloperoxidase (r=0.23, P=0.03) and elastase (r=0.38, P<0.01), and claudin-5 with myeloperoxidase (r=0.01. P<0.01) and elastase (r=0.22, P=0.03), suggesting neutrophil mediators to disrupt tight junctions in the gut. In adults with dengue, increased intestinal permeability and microbial translocation in severe disease were associated with disruption of the tight junctions mediated by neutrophil mediators.

6313

DIFFERENTIAL EFFECT OF MOSQUITO SALIVA FROM DISTINCT SPECIES ON HUMAN DERMAL ENDOTHELIAL CELL FUNCTION *IN VITRO* AND WEST NILE VIRUS PATHOGENESIS *IN VIVO*

Imke Visser[1], Eleanor M. Marshall[1], Charlotte Linthout[2], Constantianus J.M. Koenraadt[2], Melanie Rissmann[1], Marion P.G. Koopmans[1], Barry Rockx[1]

[1]Department of Viroscience, Erasmus MC, Rotterdam, Netherlands, [2]Laboratory of Entomology, Wageningen University, Wageningen, Netherlands

During probing and feeding, an infected mosquito injects both virus and saliva into the skin of the host. The presence of mosquito saliva in the skin increases arbovirus pathogenesis in the bitten host, however the exact mechanism behind this remains to be determined. It is hypothesized that disease enhancement is dependent on the function of the dermal endothelium, where an increased permeability aids in the influx of virus-susceptible cells to the bite site and therefore more cells for the virus to replicate in. The effect of mosquito saliva on the human dermal endothelium has been studied primarily for *Aedes aegypti*. Here, we investigate and compare the effects of saliva from *Culex* and *Aedes* species on the human dermal endothelial cell function *in vitro*. Furthermore, we investigate the effect of *Culex* saliva on West Nile virus (WNV) pathogenesis in a mouse model. We found that salivary gland extract from anthropophilic mosquito species (*Aedes* and *Cx. pipiens molestus*) induce permeability of the human dermal endothelium, while an ornithophilic mosquito species (*Cx. pip. pipiens*) does not. We identified that this effect is due to the presence of protease(s) in *Cx. pipiens molestus* saliva. In addition, we show that the presence of *Cx.* saliva at the WNV inoculation site *in vivo* leads to an increased mortality rate, more consistent weight loss and slightly higher viremia compared to inoculation of WNV alone. Moving forward, identification and characterization of novel salivary proteins from distinct mosquito species will advance the development of intervention methods to combat potential transmission risks and disease severity of emerging mosquito-borne pathogens.

6314

DETECTION OF WEST NILE VIRUS IN FORMALIN-FIXED, PARAFFIN-EMBEDDED TISSUES FROM FATAL CASES BY USING RT-PCR AND *IN SITU* HYBRIDIZATION: INSIGHTS INTO PATHOGENESIS

Lindsey Estetter, Julian A. Villalba, Julu Bhatnagar

Centers for Disease Control and Prevention, Atlanta, GA, United States

West Nile Virus (WNV) is a flavivirus transmitted through infected birds by mosquitos to humans, and the leading cause of mosquito-borne disease in the continental United States. Although most people do not develop symptoms or develop only minor symptoms, about 1 in 150 people infected with WNV develop serious central nervous system (CNS) illness, such as encephalitis or meningitis, and 1 in 10 with serious illness die. The risk of serious illness increases for individuals over the age of 60 and those with comorbidities such as cancer, hypertension, kidney disease, and organ transplant associated risk factors. Due to the low incidence of serious illness and death, little information is available on the pathogenesis in humans. The

goal of our study was to compare the results of RT-PCR assay targeting the NS1 gene to the results of other tissue-based assays for the detection of WNV in formalin-fixed, paraffin-embedded (FFPE) autopsy tissues, and to better understand the tissue tropism and pathogenesis of the virus through *in situ* hybridization (ISH). RNAscope ISH probes were designed and a WNV ISH assay was developed. FFPE autopsy tissues from the CNS from 29 cases positive for WNV by RT-PCR were tested by immunohistochemistry (IHC) and ISH. WNV genomic/replicative RNA was detected by ISH in 24/29 (83%) cases and viral antigens were detected by IHC in 14/29 (48%) cases. The median age of infected individuals was 64 years old, and the median duration of illness was 19 days. Fifteen of 29 (52%) cases had at least one known comorbidity. WNV RNA was localized in neurons and glial cells of various parts of the CNS, including cerebellum, cerebral cortex, medulla, pons, thalamus, and spinal cord. Tissue-based molecular assays expand diagnostic opportunities, particularly when conventional specimens are unavailable, and provide deeper insights into viral tissue tropism, sites of replication, and pathogenesis.

6315

EXPLORING THE ROLE OF HOST GLYCOSAMINOGLYCANS ON FLAVIVIRUS NS1-MEDIATED ENDOTHELIAL DYSFUNCTION

E. Vanessa Jimenez Posada[1], Julianna L. Follmar[2], Francielle T. Gomes de Sousa[1], Scott Espich[1], Nicholas Lo[1], Robert Beatty[1], Scott B. Biering[1], Kamil Godula[2], Eva Harris[1]

[1]Division of Infectious Diseases and Vaccinology, School of Public Health, University of California, Berkeley, Berkeley, CA, United States, [2]Department of Chemistry and Biochemistry and Glycobiology Research and Training Center, University of California, San Diego, CA, United States

The mosquito-borne flaviviruses cause diverse disease presentations, often involving endothelial barrier dysfunction that is driven by vasoactive cytokines and viral factors, such as nonstructural protein 1 (NS1). NS1 induces tissue-specific endothelial dysfunction and leakage by disrupting the endothelial glycocalyx layer (EGL) and intercellular junctions upon binding and internalization into endothelial cells (ECs). Glycan/NS1 interactions are a key determinant of NS1 binding to ECs. Heparan sulfate (HS) is a ubiquitous glycan component of the EGL of ECs and is present in diverse forms with variability in extent and linkages of sulfation in addition to chain length. While HS is known to be critical for NS1 cell binding, it is unclear what species of HS mediate NS1 interactions with ECs and what species are present on ECs from distinct tissues. To address this gap in knowledge, we used a glycosaminoglycan (GAG) array to determine what species of HS bound dengue virus (DENV) NS1. We found that NS1 bound well to diverse HS linkages, including 2-O-S, 3-O-S, and 6-O-S, and that the strength of DENV NS1 binding correlated with the extent of sulfation. We then tested the capacity of synthetic GAGs with these HS linkages to bind to DENV NS1 and protect against NS1-mediated endothelial hyperpermeability in human pulmonary microvascular endothelial cells (HPMEC). Using an ELISA, we found that NS1 bound strongly to certain synthetic GAGs, confirming the GAG array data. Interestingly, we found that only GAGs containing 3-O-S, and to a lesser extent 6-O-S, blocked NS1 from inducing endothelial hyperpermeability. Further, we found that a 3-O-S binding peptide abrogated the capacity of DENV NS1 to bind to ECs and trigger endothelial hyperpermeability, in contrast to a control peptide. We then confirmed the expression of multiple 3-O-S sulfotransferases on the surface of HPMEC, suggesting their expression in lung ECs, and are currently knocking out individual genes via CRISPR-Cas9. Together, these data indicate that while NS1 can bind to multiple HS species, only specific species of HS, like 3-O-S, may be important for NS1-mediated endothelial dysfunction.

6316

HETEROLOGOUS PROTECTION OF RECENT O'NYONG-NYONG VIRUS STRAIN UVRI0804 BY AN INACTIVATED CHIKUNGUNYA VIRUS VACCINE

Whitney Weber[1], Zachary J. Streblow[1], Michael Denton[1], Takeshi Andoh[1], Craig Kreklywich[1], Hans-Peter Raue[1], Gauthami Sulgey[1], Magdalene Streblow[1], Mark Heise[2], Mark K. Slifka[1], **Daniel N. Streblow**[1]

[1]Oregon Health & Science University, Beaverton, OR, United States, [2]University of Carolina at Chapel Hill, Chapel Hill, NC, United States

O'nyong-nyong virus (ONNV) is a mosquito-transmitted alphavirus identified in Uganda in 1959. The virus has potential for enzootic and urban transmission cycles, and in humans, ONNV infection manifests as fever, rash, and joint/muscle pain lasting months. There are currently no specific vaccines or antiviral treatments for ONNV. Since highly passaged alphaviruses often lose pathogenic features, we constructed an infectious clone for ONNV-UVRI0804 ($ONNV_{0804}$), a 2017 isolate from a febrile patient in Uganda (Ledermann, 2022). The recovered recombinant virus was passaged in mosquito cells and sequenced to ensure genome integrity. Viral replication for $ONNV_{0804}$ was compared to the highly passaged strain $ONNV_{UgMP30}$, and $ONNV_{UgMP30}$ replicates to higher levels in fibroblasts and Vero cells, but performed similarly in C6/36 cells. We performed a head-to-head comparison in both C57BL/6 mice and AG129 interferon-deficient mice. In both types of mice, $ONNV_{0804}$ dramatically outperformed $ONNV_{UgMP30}$. Specifically, in AG129 mice, $ONNV_{0804}$ caused a quicker onset of disease (footpad swelling/weight loss) and much higher viremia at 3 dpi. In WT mice, $ONNV_{0804}$ caused footpad swelling beginning at 5dpi, and the virus demonstrated much broader tissue distribution and higher vRNA loads at both 5 and 43 dpi relative to $ONNV_{UgMP30}$. This finding indicates that ONNV can persist in joint and muscle tissues for long periods of time, which has been associated with chronic arthritogenic disease. Mice were vaccinated with HydroVax-CHIKV using prime-only and prime/boost approaches. Neutralizing antibody titers against $ONNV_{0804}$ and CHIKV were slightly higher in the prime/boost group. At 4 weeks post-vaccination, animals were challenged with $ONNV_{0804}$ and only control animals developed viremia. Both vaccine groups had increased survival and were protected against weight loss. Significant footpad swelling occurred in the control and prime-only groups but not in the animals receiving the prime-boost CHIKV vaccine. These data imply that vaccination against CHIKV can protect against ONNV infection and disease even for a contemporary, highly pathogenic strain.

6317

GENETIC ANCESTRY-ASSOCIATED DIFFERENCES IN DENGUE VIRUS INFECTION OF PRIMARY HUMAN SKIN CELLS

Michelle M. Martí, Priscila M. S. Castanha, Jocelyn M. Taddonio, Jeremy J. Martinson, Simon M. Barratt-Boyes

University of Pittsburgh, Pittsburgh, PA, United States

Host genetic ancestry is a risk factor for severe dengue; however, the lack of translatable models has hindered studying this relationship. We acquire discarded human skin specimens from elective cosmetic surgeries to use for *ex vivo* skin explants or digestion into single cell suspensions to study dengue virus (DENV)-host interactions. Genetic ancestry is determined for all donors through a panel of 128 ancestry informative markers specific for European ancestry (EA) and African ancestry (AA). Skin explants were inoculated with DENV-2 (strain 16681) and analyzed with confocal microscopy at 24 hours post-infection (hpi). This revealed a striking correlation between infection in the epidermal and dermal layers with an increase in proportion of EA. EA donors also had a three-fold increase in recruitment of CD163+ macrophages to the site of infection and a two-fold increase in infection of those cells. AA donors had significantly higher levels of interferon-α while EA donors had a marked inflammatory response with significantly higher levels of interleukin-1β. To identify mechanistically what is responsible for these observed differences, we separated epidermal and

dermal layers of human skin and digested them into single cell suspensions. Cells were infected with DENV-2 and analyzed by flow cytometry at 24hpi. Preliminary data indicate that epidermal cells, most notably keratinocytes, from EA donors have significantly higher levels of infection than AA donors. Contrary to *ex vivo* explant data, there was no significant ancestry-associated difference in infection of isolated dermal cells. These initial findings suggest that epidermal keratinocytes are potentially the driving force behind ancestral differences observed in intact tissue. This work provides biologic evidence of ancestry-associated differences in cutaneous responses to DENV. Identification of cells or innate proteins responsible for protection will provide potential targets for therapeutic development against severe disease.

6318

ARBOVIRUS DISEASE PATHOGENESIS IN OBESE AND TYPE-II DIABETIC-LIKE MICE

Natalia Ingrid O. Silva[1], Sasha R. Azar[2], Vidyleison N. Camargos[1], Rafael K. Campos[3], Rumei Yun[3], Jiehua Zhou[1], Alice F. Versiani[1], Shannan L. Rossi[3], Nikos Vasilakis[1]

[1]University of Texas Medical Branch, Department of Pathology, Galveston, TX, United States, [2]Center for Tissue Engineering, Department of Surgery, Houston Methodist Research Institute, Houston, TX, United States, [3]University of Texas Medical Branch, Department of Microbiology and Immunology, Galveston, TX, United States

Mosquito-borne viruses are important public health threats, and Mayaro virus (MAYV) is an arbovirus with epidemic potential. Also, underlying chronic diseases like diabetes, cardiovascular disease, and cancer, affect more than 40 million individuals who live in arbovirus-endemic areas. Clinical data indicate that arbovirus infections in diabetic patients lead to more severe outcomes and higher mortality compared to the non-diabetic. We hypothesized that individuals with preexisting diabetes could have increased MAYV replication, resulting in severe disease outcomes and mosquito transmission alterations. We employed murine model of insulin resistance/obesity as proxy for Type II diabetes mellitus (T2DM). Leptin receptor mutant $LEPR^{db/db}$, $LEPR^{db/WT}$, and wild type (WT) C57BL/6J mice were pretreated with IFNAR blocking antibodies to render them permissive to MAYV infection via infected mosquito bite. Acute viremia, viral load, pathogenesis, and immune responses were quantified. The model demonstrated a predictable pattern of viremia with titers starting to increase at 2 days post infection (dpi) (7.6, 5.2 and 4.77 $\log_{10}$ ffu/mL, respectively), and with highest titers (9.2 and 8.5$\log_{10}$ ffu/mL) at 4 dpi. No significant differences in viremia were observed between T2DM genotypes on any day. MAYV was detected in all tissues analyzed, with highest viral loads detected in liver and spleen of WT (8.7 - 8.9 $\log_{10}$ffu/mL), and $LEPR^{db/db}$ (8.82 - 9.33 $\log_{10}$ ffu/mL) mice, and spleen of $LEPR^{db/WT}$ (9.1 $\log_{10}$ ffu/mL). Although higher titers were detected in $LEPR^{db/db}$ mice tissues, no significant differences in viral load were observed compared to $LEPR^{db/WT}$ or WT controls. Histopathological analysis showed necrotic foci in some $LEPR^{db/db}$ livers of infected animals, which was not detected in the controls. In summary, MAYV infection of $LEPR^{db/db}$, $LEPR^{db/WT}$, and WT C57BL/6J yielded no genotype dependent difference in serum viremia, and no significant association of peak viremia with tissue viral burden suggesting that these observations were driven by host-intrinsic factors (e.g. cytokines, clotting factors) as opposed to direct viral action.

6319

PRM AND E SEQUENCE VARIATION ALTERS THE STRUCTURE ENSEMBLE OF ZIKV TO INFLUENCE ANTIBODY EPITOPE ACCESSIBILITY

Marisa E. McGrath[1], Elizabeth Bardwil-Lugones[1], Jessica L. Guyette[1], Kimberly A. Dowd[1], Katherine E. Burgomaster[1], David N. Gordon[1], Patrick Dolan[2], Theodore C. Pierson[1]

[1]National Institutes of Health, Vaccine Research Center, Bethesda, MD, United States, [2]National Institutes of Health, Laboratory of Viral Diseases, Bethesda, MD, United States

Zika virus (ZIKV) is a mosquito-borne flavivirus discovered in 1947 in Uganda. ZIKV has been responsible for multiple outbreaks in humans, including the 2015 epidemic of the Americas. Flavivirus virions are characterized by the presence of 180 envelope (E) structural proteins on the surface, arranged as antiparallel dimers, that represent the major target of neutralizing antibodies. Virions also incorporate an equal number of a second structural protein, premembrane (prM), that is cleaved by the host protease furin during viral egress. In the mature form of the virus, 90 E homodimers lie flat on the viral membrane, with short membrane-bound M peptides located beneath the E protein herringbone lattice. This complex and dense arrangement of E proteins results in limited accessibility of many surfaces to binding by neutralizing antibodies. Antibodies may access cryptic epitopes that are variably accessible amongst the ensemble of states sampled by flaviviruses. To investigate the effect of amino acid variation on the accessibility of cryptic E protein epitopes, we produced a library of ZIKV reporter virus particles using structural proteins of 174 genetically diverse strains that possess naturally occurring variation in the structural proteins prM and E. We performed neutralization studies using E protein-specific monoclonal antibodies that poorly neutralized a reference strain H/PF/2013 to identify strains that display unexpected neutralization sensitivity. These studies revealed distinct clusters of genetically related strains that facilitate the rapid identification of single amino acid substitutions in prM and E proteins that influence epitope accessibility of distal sites on the E protein. These studies will provide a high-resolution understanding of how amino acid variation contributes to the antigenic structure of ZIKV and will inform antigen design and vaccine development.

6320

BEYOND THE ROOST: EXPLORING THE IMPACTS OF A MODIFIED DIET ON MERS-COV INFECTION IN THE JAMAICAN FRUIT BAT (*ARTIBEUS JAMAICENSIS*)

Maggie Priore, Kalani M. Williams, Emma Harris, Shijun Zhan, Juliette Lewis, Allison Vilander, Phillida Charley, Rebekah C. Kading, Tony Schountz, Anna Fagre

Colorado State University, Fort Collins, CO, United States

Bats harbor a wide range of high-consequence zoonotic viruses, yet many ecological and biological mechanisms underlying the risk of spillover remain understudied. Several field studies have shown that the loss of food sources, often caused by climate change and anthropogenic disturbance, alters bat behavior and potentially increases the risk of viral spillover. Middle East Respiratory Syndrome coronavirus (MERS-CoV) is a zoonotic bat-borne virus endemic to the Arabian Peninsula, where evidence of spillover to dromedary camels and humans has been recorded. To interrogate physiologic processes that influence viral shedding and cross-species transmission, we sought to determine how an altered diet, mimicking natural periods of low-quality forage, impacts infection dynamics and immune responses in bats. Our preliminary work examining immune responses of Jamaican fruit bats (*Artibeus jamaicensis,* Aj's) following immunization with a virus-like particle expressing Nipah virus glycoprotein suggest that bats fed a diet restricted in protein content develop a more robust neutralizing antibody response than those provided standard diet. To determine whether similar immunologic patterns hold true in bats experimentally inoculated with MERS-CoV, we altered the diet composition of Aj's infected with MERS-CoV. Aj's were fed a protein-restricted diet, with one group inoculated with MERS-CoV (n=12) and a second group mock-

inoculated with sterile PBS (n=12). A third group of uninfected bats (n=3) were cohoused with experimentally infected bats (n=3) to assess potential bat-to-bat transmission. Results will be presented that compare protein-restricted Aj's to those fed their standard diet to compare viral shedding rates and serological, immunological, and histopathological analyses between the diet groups.

6321

INVESTIGATION OF VIRUS-HOST INTERACTIONS IN SEVERE FEVER WITH THROMBOCYTOPENIA SYNDROME VIRUS INFECTION USING A TRANSCRIPTOMICS APPROACH

Lei Wen[1], Hin Chu[1], Shuofeng Yuan[1], Feifei Yin[2], **Jasper Chan**[1]
[1]The University of Hong Kong, Hong Kong, Hong Kong, [2]Hainan Medical University, Haikou, China

Severe fever with thrombocytopenia syndrome virus (SFTSV) is an emerging tick-borne bunyavirus that can cause severe disease in human with a mortality rate of more than 10%. Local transmission of SFTSV has been reported in a number of Asian countries, including China, Japan, South Korea, Vietnam, and Thailand. Symptomatic SFTSV infection usually presents as an acute febrile illness with high fever, thrombocytopenia, and hemorrhage. To provide novel insights into the virus-host interactions in SFTSV infection, we investigated the transcriptomics profile of SFTSV-infected Huh-7 (human hepatoma) cells using RNA-Seq. Our transcriptomics analysis identified 164 (33 down- and 131 up-regulated) differentially expressed genes (DEGs) between mock and SFTSV infection groups at 8 hours post-infection (hpi). Compared to 8 hpi, the expression profile at 48 hpi was largely altered with more than 2900 DEGs. Among these DEGs, 113 out of 164 (68.9%) DEGs at 8 hpi were also differentially expressed at 48 hpi. Gene ontology and pathway enrichment analyses showed that most of the perturbations were related to the host immune response, particularly those related to the cytokine and chemokine response. Protein-protein interaction network of DEGs identified three clusters. In the largest cluster (cluster 1), there were 80 nodes and 647 edges in Cluster 1 with average node degree 16.2, suggesting that the DEGs in this cluster were highly connected. The DEGs in Cluster 1 are mainly involved in neutrophil apoptotic process, neutrophil chemotaxis, and T-helper 17 cell differentiation. The top 5 hub genes with high connectivity in the network regarded as key regulators in cluster 1 were IL-6, IL-1B, CXCL-8, ICAM-1 and PTGS-2. In summary, our study characterized the SFTSV-induced host transcriptomics perturbations and may facilitate the identification of host factors as potential antiviral targets for SFTSV infection.

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MYELOID CELL REPLICATION PHENOTYPES UNDERLIE EPIZOOTIC POTENTIAL OF ALPHAVIRUSES

Jennifer Lynn Hyde
University of Washington, Seattle, WA, United States

Alphaviruses are globally distributed +ssRNA viruses which are transmitted by mosquito vectors. Viral determinants which impact the transmission and pathogenesis of numerous alphaviruses have been identified, including amino acid coding changes in the viral attachment protein (E2). However, the role of viral RNA secondary structure in viral emergence is still poorly defined. We have developed a computational approach to compare RNA structures across the alphavirus genus in order to identify RNA structure signatures associated with specific viral properties (e.g. RNA structures unique to epizootic viruses). Using this approach we have identified several regions in the Venezuelan equine encephalitis virus and Sindbis virus genomes that are predicted to contain RNA structures relevant for emergence and pathogenesis of these viruses. Surprisingly, despite encoding distinct viral RNA structures, pathogenic/epizootic strains of these different viral species were all observed to replicate differentially in myeloid cells, suggesting that myeloid cell replication fitness may be a hallmark of viral emergence for alphaviruses. Changes in myeloid cell replication fitness were also associated with changes in pathogenesis in a small animal model. Using molecular approaches, we have identified several RNA binding proteins which differentially interact with these viral RNA structures, indicating that distinct molecular mechanisms underlie this shared replication phenotype across different alphaviruses. Presently we are investigating the mechanism by which altered myeloid cell replication contributes to transmission and pathogenesis in other relevant species.

6323

THERAPEUTIC EFFICACY OF ARTEMETHER LUMEFANTRINE PLUS SINGLE DOSE PRIMAQUINE FOR THE TREATMENT OF UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA IN IRRIGATED AGRO INDUSTRIAL METAHARA SUGAR FACTORY, CENTRAL ETHIOPIA

Henok Hailgiorgis Mekonnen
Ethiopian Public Health Institute (EPHI), Addis Ababa, Ethiopia

Artemether-lumefantrine (AL) plus a single dose of primaquine is recommended as the first-line treatment of uncomplicated *P*lasmodium *falciparum* malaria in Ethiopia. WHO recommends regular monitoring of the therapeutic efficacy of frontline drugs. Anti malaria drug resistance is now a threat to malaria control programs. This study was conducted to assess the therapeutic efficacy of AL with a single dose of primaquine for the treatment of uncomplicated *P. falciparum* malaria in Central Ethiopia. A one arm prospective study was conducted at the Metahara Sugar Factory site from December 2022 to February 2023 following the WHO protocol. Eighty-seven patients were enrolled and each patient was treated with a standard, 6 doses of AL given twice daily for 3 days under partial supervision, Moreover, each patient was given a single dose of primaquine on Day 0. Clinical and parasitological responses were assessed during the 28 day follow-up period. Outcomes of treatment were defined according to the standard WHO classification. Recurrent parasitemia was genotyped. The outcome of the present study revealed that PCR uncorrected and corrected cure rates at day 28 were 97.7% (95% CI: 91.8-99.8%, and 98.8% (95%CI: 91.0-99.4%), respectively. The high parasite and gametocyte clearance rate (100%) was recorded on day 3. Fever was resolved in all patients on day 2. Hemoglobin level was significantly improved on day 28 compared to both day 0 and day 14. There was no evidence of severe adverse events during the study period. The results of this study revealed that AL with single does PQ treatment recommended by the National Malaria Elimination Program is highly efficacious with a high parasite clearance rate and fast resolution of fever in the study setting. Regular monitoring of AL plus PQ efficacy, including molecular markers of drug resistance studies is suggested in this and another malarious area of the country.

6324

LOW LEVEL OF ANTIMALARIAL DRUG RESISTANCE IN 2014-15: INTEGRATION OF PRIMAQUINE INTO INDIA'S ANTIMALARIAL DRUG POLICY 2013

Shrikant Nema[1], Nazia Ali[2], Kristan A. Schneider[3], Sri Krishna[2], Anil Kumar Verma[2], Aparup Das[2], Praveen Kumar Bharti[1]
[1]ICMR-National Institute of Malaria Research, New Delhi, India, [2]ICMR-National Institute of Research in Tribal Health, Jabalpur, India, [3]University of Applied Sciences Mittweida,, Germany, Germany

The surveillance and containment of antimalarial drug resistance play a pivotal role in the efforts of countries striving to eliminate malaria. It is imperative to monitor the evolution of resistance through studies conducted before and after changes in treatment policies. Between 2014 and 2015, a thorough study collected 939 P. falciparum-positive blood samples from ten sites in India, organized into four clusters. Sequencing amplified PCR products identified point mutations in genes linked to drug resistance: Pfdhfr, Pfdhps, Pfmdr1, and Pfk13. Triple Pfdhfr mutants were exclusive to northeast India near the Myanmar border, contrasting with the central region dominated by wildtype. Pfdhps wildtypes prevailed nationwide, lacking double mutants. Pfmdr1 wildtype dominated, except in Northwest India with nonsynonymous double mutations. Pfk13 exhibited synonymous mutations, primarily in Central India. Low drug resistance pressure and

geographic cluster heterogeneity were indicated by linkage disequilibrium and principal component analysis. India displayed low drug resistance levels during the transition from CQ to SP to ACTs, surpassing global endemic countries. India's unique treatment policy included gametocidal primaquine (PQ), potentially slowing resistance spread. The study underscores India's comparatively low drug resistance, possibly due to gametocidal and schizonticidal drug use, limiting parasite transmission. Conducted nationwide from 2014-2015, the study establishes a baseline for monitoring and understanding ACT resistance emergence and dissemination in India. Highest resistance occurred in Northeast India near Myanmar, a region prone to resistance. Primaquine's broad use, with gametocidal and schizonticidal properties, likely crucially sustains low resistance, averting strong selection pressures.

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MOLECULAR SURVEILLANCE OF *PLASMODIUM FALCIPARUM* DRUG RESISTANCE MARKERS IN GHANA

Mona-Liza E. Sakyi[1], Osumanu Ahmed[1], Ignatus N. Dorvi[1], Joyce M. Ngoi[1], Kukua Thompson[1], Samirah Saiid[1], Isaiah Debrah[1], Enock K. Amoako[1], Collins M. Morang'a[1], Sonia Goncalves[2], Alexandria Harrott[2], Cristina Ariani[2], Yaw Aniweh[1], Gordon A. Awandare[1], Dominic P. Kwiatkowski[2], William L. Hamilton[2], Lucas N. Amenga-Etego[1]

[1]West Africa Centre for Cell Biology of Infectious Pathogens, Accra, Ghana, [2]Wellcome Sanger Institute, Hinxton, United Kingdom

Antimalarial drug resistance looms as a threat to malaria control. Artemisinin-based combination therapy (ACT) remains the standard treatment for uncomplicated malaria. However, the emergence of artemisinin partial resistance and increasing tolerance of *Plsodium falciparum* to ACT partner drugs in Africa cast a shadow on the efficacy of ACTs. Therefore, necessitating genomic surveillance of *P. falciparum* to monitor known antimalarial drug resistance markers in *Pfcrt, Pfmdr1, Pfkelch13, Pfdhfr, and Pfdhps* genes and to look for novel markers of resistance. We demonstrated the utility of amplicon sequencing of longitudinal cross-sectional samples collected from clinical sources in an ongoing study across Ghana for surveillance using Oxford Nanopore Technologies (ONT). We sequenced 285 samples and analyzed the presence of haplotypes in known antimalarial drug resistance genes for *P. falciparum.* We found no evidence of mutations in the *kelch13* gene known to mediate artemisinin partial resistance. The *Pfmdr1*-N86Y and YI84F mutations associated with multi-drug resistance were identified at a prevalence of 1.4% and 73.3% respectively. The combination of PfcrtK76T, found at a prevalence of 7.0% with Pfmdr1 N86Y is characterized to mediate LUM resistance. Our study revealed a high prevalence (83.9%) of triple mutation (IRNI) in *Pfdhfr*, across all sites. A quadruple mutant (IRNL) known to confer high grade SP treatment failure was observed at a low prevalence of 2.1% in six isolates. The predominant *Pfdhps* haplotypes were single mutants (SGKAA) and double mutant (AGKAA) occurring in 55.4% and 34.6% respectively. Analysis of combined haplotypes of *Pfdhfr-Pfdhps* revealed 23 unique haplotypes, with the quadruple mutant SGKAA-IRNI (47.2%) and quintuple mutant AGKAA-IRNI (29.9%) being the most prevalent. These findings highlight the absence of artemisinin resistance in Ghana but, raises concern about high prevalence of markers of ACT partner drug and sulfadoxine-pyrimethamine (SP) resistance, which are key interventions for malaria prevention during pregnancy and seasonal malaria chemoprevention among young children.

6326

INVESTIGATING THE PRESENCE OF FALSIFIED AND POOR QUALITY FIXED-DOSE COMBINATION ARTEMETHER-LUMEFANTRINE PHARMACEUTICAL DOSAGE FORMS IN KUMASI, GHANA

Simon Nyarko[1], Kwabena Ofori-Kwakye[1], Raphael Johnson[1], Noble Kwuntworbe[1], Denis Dekugmen Yar[2]

[1]Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, [2]Akenten Appiah-Menka University of Skills Training and Entrepreneurial Development, Mampong, Ghana

Artemether-Lumefantrine (AL) is a highly effective and commonly used Artemisinin-based Combination Therapy (ACT) for treating uncomplicated malaria caused by *Plasmodium falciparum*, including drug-resistant strains. However, ineffective regulatory systems in resource-limited settings can lead to the infiltration of poor-quality and counterfeit anti-malarial medicines into the pharmaceutical supply chain, causing treatment failures, prolonged illness, and disease progression. The objective of the study was to assess the quality of selected brands of fixed-dose combination (FDC) AL tablets and suspensions marketed in Kumasi, Ghana. A total of fourteen brands of FDC AL medicines, comprising eight tablets and six suspensions were purchased from various retail pharmacy outlets in Kumasi, Ghana. All samples were subjected to thorough visual inspection as a quick means of checking quality through meticulous observation of the packaging or dosage form. The quality parameters of the tablets were determined using uniformity of weight, hardness, friability, and disintegration tests. Suspensions were assessed based on pH and compared with the British Pharmacopeia (BP) standard. The samples were then analyzed for drug content (assay) using Reverse-Phase High Performance Liquid Chromatography. All the tablet samples conformed to BP specification limits for uniformity of weight, hardness friability, and disintegration time. The drug assay analysis demonstrated that all the tablets met the BP specifications. The results of the pH studies showed that out of the six brands of suspension investigated, five (83.3%) were compliant with the official specification for pH, while one (16.7%) failed the requirement. Unlike the tablet brands, drug content analysis of the six suspensions showed that two (33.3%) were substandard. The artemether and lumefantrine content in these failed suspensions were variable (artemether: 81.31% - 116.76%; lumefantrine: 80.35% - 99.71%). The presence of substandard drugs underscores the necessity for robust pharmacovigilance and surveillance systems to eliminate counterfeit and substandard drugs.

6327

RECRUDESCENCE OF *PLASMODIUM FALCIPARUM* AFTER QUININE THERAPY: A CASE REPORT

Jenni Shannin Gaw Alip, Ralph Aniceto

Divine Word Hospital, Tacloban, Philippines

The recurring bouts of malaria caused by the parasites' persistence in red blood cells are the fundamental cause of recrudescence. This can be attributed to a variety of factors, including inadequate drug exposure, drug resistance, suboptimal dosage, noncompliance, or subpar medications. This is the case of a returning traveler to the Philippines, a forester from the Republic of Congo, where malaria *Plasmodium falciparum* is endemic. Presenting with fever prior to and upon return to Philippines. He was previously treated with IV quinine once a day for 3 days, multiple times since 2010 until 2023, without previous malarial smears or polymerase chain reaction. The patient presented with febrile episodes; the physical examination was unremarkable; however, the patient smear revealed malaria P. falciparum trophozoites. The patient was admitted and treated with artemether-lumefantrine 20/120 mg tablets for 3 days, followed by primaquine 15 mg/tablet on the 4th day, and was discharged. He was prescribed mefloquine 250 mg/tab, 1 tab weekly, as prophylaxis. Since then until present date, patient remains to be asymptomatic despite being in an endemic area. Recurrent malarial exposure, in the absence of malarial prophylaxis, and with inadequate treatment, the risk for recrudescent malaria increases.

6328

FIRST EVALUATION OF MOLECULAR AND PATHOGEN GENOMIC IMPACT ON *PLASMODIUM FALCIPARUM* POPULATION FOLLOWING SEASONAL MASS DRUG ADMINISTRATION WITH DIHYDROARTEMISININ-PIPERAQUINE IN A HIGH TRANSMISSION HIGHLY SEASONAL SETTING IN WEST AFRICA

David McGregor

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

The World Health Organisation conditionally recommends the use of mass drug administration (MDA) for burden reduction in moderate to high transmission settings. *P. falciparum* is endemic to the Bijagos Archipelago, a remote collection of islands off the coast of West Africa. In November 2019, Soga Island had the highest estimated peak malaria season prevalence with 48% (95%CI: 39.8-56.3%) by qPCR. The MATAMAL trial (NCT04844905) delivered six rounds of MDA with dihydroartemisinin-piperaquine (DP) across the archipelago in 2021 and 2022. Nested within MATAMAL, this study delivered DP MDA to Soga in August, September, and October 2022. MDA coverage was over 80% for each round. One month after the final MDA round the estimated prevalence was 5.6% (95% CI: 3.3-8.8%). Nine and 12 months following the final MDA round the estimated prevalence was respectively 1.7% (August 2023, 95% CI: 0.54-3.8%) and 2.6% (December 2023, 95% CI: 1.1-5.1%). Therapeutic efficacy studies for DP and the first-line anti-malarial agent artemether-lumefantrine (AL) were also conducted across the MATAMAL trial site. The impact of multiple rounds of DP MDA on drug resistance of the *P. falciparum* parasite population is a significant knowledge gap and a potential significant threat to malaria control and elimination. I recruited 111 patients presenting with uncomplicated *P. falciparum* infection diagnosed by rapid diagnostic test and treated with AL. *P. falciparum* parasitemia 28 days since commencing AL was 81% (20/105) when assessed by PCR. The impact of MDA on the parasite phylogenomic structure and transmission using identity by descent (IBD) and multiplicity of infection (MOI) was investigated. The prevalence of molecular markers of drug resistance between MDA rounds was also examined. For the first time, this study investigates close monitoring of the efficacy of first-line therapy with molecular approaches during and shortly after MDA. This study provides an approach which may be invaluable to rapidly identify an emergent threat of parasite drug resistance in the context of future scale up of mass preventive chemotherapy strategies across endemic areas.

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PHENOTYPIC ASSESSMENT OF MOLECULAR MARKERS ASSOCIATED WITH SULFADOXINE-PYRIMETHAMINE RESISTANCE IN SENEGAL

Katelyn Vendrely Brenneman[1], Mamy Yaye Die Ndiaye[2], Wesley Wong[1], Stephen Schaffner[3], Imran Ullah[1], Abdoulaye Tine[2], Mouhamad Sy[2], Tolla Ndiaye[2], Amy Gaye[2], Mame Fama Ndiaye[2], Mariama Toure[2], Nogaye Gadiaga[2], Aita Sene[2], Awa Bineta Deme[2], Baba Dieye[2], Mamadou Samb Yade[2], Khadim Diongue[2], Younouss Diedhiou[2], Fatou Ba Fall[4], Doudou Sene[4], Medoune Ndiop[4], Ibrahima Diallo[4], Mame Cheikh Seck[2], Aida Sadikh Badiane[2], Jules François Gomis[2], Mouhamadou Ndiaye[2], Mamadou Alpha Diallo[2], Ibrahima Mbaye Ndiaye[2], Bronwyn MacInnis[3], Dyann F. Wirth[1], Daouda Ndiaye[2], Sarah Volkman[1]

[1]Harvard TH Chan School of Public Health, Boston, MA, United States, [2]International Research & Training Center in Applied Genomics and Health Surveillance, Dakar, Senegal, [3]The Broad Institute, Cambridge, MA, United States, [4]Senegal National Malaria Control Program, Dakar, Senegal

Sulfadoxine-pyrimethamine (SP) is used for chemoprevention in Senegal for intermittent preventive treatment in pregnancy (since 2004) and seasonal malaria chemoprevention (since 2013). SP resistance occurs via the accumulation of *Pfdhfr* and *Pfdhps* mutations. Using whole genome sequence data from samples collected (2000 - 2022) from malaria patients at health facilities across Senegal, we observed near fixation of *Pfdhfr* triple mutant (N51I, C59R, S108N) and fluctuation in *Pfdhps* A437G and S436A mutation frequencies over time. It is unclear how these mutations influence drug resistance and fitness phenotypes in natural isolates. To test this, we classified natural parasite isolates into groups based on their *Pfdhps* ('double'; S436A, A437G) and *Pfdhfr* ('triple') alleles. Parasites were culture-adapted and phenotyped for antimalarial drug susceptibility (EC_{50}) and fitness. Preliminary data show that all parasites with the *Pfdhfr* triple allele were significantly more resistant to pyrimethamine (PYR) compared to *Pfdhfr* wildtype parasites (p<0.0001-0.0445). Moreover, we observed a range of phenotypes among PYR resistant parasites according to their *Pfdhps* status: the *Pfdhfr* triple + *Pfdhps* double mutant parasite was significantly more PYR resistant than the *Pfdhfr* triple in combination with either *Pfdhps* single mutation S436A (p=0.0126) or A437G (p=0.0001-0.05). We found *Pfdhfr* triple parasites were significantly more resistant to PYR + 100uM sulfadoxine compared to *Pfdhfr* wildtype parasites (p<0.0001), but there was no statistical difference between *Pfdhfr* triple parasites. Pairwise competitive growth assays revealed the *Pfdhfr* triple + *Pfdhps* double mutant parasite as the most competitively fit of all parasites tested; interestingly, this parasite was also the most PYR resistant. Assessment of these mutations in an isogenic Senegalese background will help determine the causal role of *Pfdhps* mutations in drug resistance and fitness, predict the evolutionary trajectory of SP resistance in Senegalese parasites, and provide molecular markers for ongoing surveillance to monitor and guide the use of SP-based interventions.

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KNOWLEDGE OF ANTIMALARIALS BY PATIENT LEAVING HEALTH FACILITIES IN THE DEMOCRATIC REPUBLIC OF CONGO

Therese Maleluka Mpiempie Ngamasata, Tarcisse Kapene Kilara, Mireille Amba Ngale, Laurène Mesia, Percy Velela, Raissa Songo, Emmanuella Muhima, Gauthier Mesia Kahunu, Pierre Michel Nsengi Ntamabyaliro

University of Kinshasa, Kinshasa, Congo, Democratic Republic of the

Malaria remains one of the leading causes of death in the DRC, particularly among children under five, with thousands of deaths each year. Despite the availability of safe and effective medicines, the DRC accounts for over 12% of global malaria deaths. One possible factor contributing to this situation is the inappropriate use of antimalarials by patients, which may be due to a lack of understanding of the correct dosage. As part of a national survey on rational use of antimalarials, we assessed patients' knowledge of prescribed antimalarials in 33 health facilities in all 11 former provinces of the DRC. Methods.This was a descriptive cross-sectional study conducted in 2018. All patients leaving the pharmacies of the selected 33 health facilities were invited to participate by answering a questionnaire on the course of dispensing and knowledge of the antimalarials they had just received. Data were analysed using SPSS version 25 software. Results.A total of 845 participants were interviewed; 68.6% knew the name of the antimalarials they had just received. However, 74% did not know the number of intakes per day and 61% did not know the dose. 72% did not know the duration of treatment. 90% had not been informed about possible side effects. 60% reported that dispensers did not specify storage conditions and 94% did not know how to store antimalarial drugs. Only a small percentage of participants knew all the relevant information about their antimalarial drug. ConclusionMalaria patients are discharged from hospital without receiving relevant information about their medication. This lack of information may lead to inappropriate use of medicines, which could have a negative impact on the fight against this deadly disease. It is important to develop effective interventions to address this situation.

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EFFICACY AND SAFETY OF ARTEMETHER-LUMEFANTRINE FOR THE TREATMENT OF UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA AMONG CHILDREN UNDER FIVE IN BENIN, 2022

Irene Cavros[1], Augustin Kpemasse[2], Alexis Sacca Yarou Maye[2], Ramani Saliou[3], Sakariahou Kpanou[2], William Houndjo[2], Julien Aïssan[2], Cyriaque Dossou Affoukou[2], Davy Yamonmi[3], Marcel Affoukou[3], Alla Zlochevska[3], Mamadou A. Diallo[4], Awa B. Deme[4], Bassirou Ngom[4], Aita Sene[4], Djiby Sow[4], Daouda Ndiaye[4], Patrick Condo[5], Virgile Gnanguenon[6], Ahmed Saadani Hassani[7], Aurore Ogouyemi Hounto[2]

[1]President's Malaria Initiative, Malaria Branch, U.S. Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]National Malaria Control Program, Benin Ministry of Health, Cotonou, Benin, [3]Benin Ministry of Health, Cotonou, Benin, [4]International Research and Training Center on Applied Genomics for Health Surveillance, Dakar, Senegal, [5]U.S. President's Malaria Initiative, U.S. Agency for International Development, Kampala, Uganda, [6]U.S. President's Malaria Initiative, U.S. Agency for International Development, Cotonou, Benin, [7]U.S. President's Malaria Initiative, U.S. Centers for Disease Control and Prevention, Dakar, Senegal

In Benin, artemether-lumefantrine (AL) is the first-line treatment for uncomplicated *Plasmodium falciparum* malaria. In accordance with World Health Organization recommendations to routinely test antimalarial efficacy, a study was conducted between June and December 2022 in three sentinel sites: Bohicon in the south, Allada in the south-central department, and Parakou in the north. Participants (children 6 to 59 months old) were monitored for 28 days to assess their clinical and parasitological responses. Molecular correction was performed using PCR fragment-length analysis of *msp1* and *msp2* genes and the poly alpha microsatellite to differentiate recrudescence from new infection. Molecular markers of resistance were assessed using targeted amplicon deep sequencing.

In Allada and Parakou, 115 patients received AL per site. The Bohicon arm had 70 participants. Uncorrected 28-day Kaplan-Meier efficacy was 94% (95% confidence interval (CI):89-100) in Bohicon, 95.6% (92-99) in Allada, and 89.6% (84-95) in Parakou. PCR-corrected efficacy was 98.5% (96-100) in Bohicon, 97.4% (94-100) in Allada, and 94.8% (91-99) in Parakou. The proportion of patients with parasitemia on day three of follow-up was 3% in Bohicon, 1% in Allada, and 2% in Parakou.

A total of 284 non-failure samples and 30 late treatment failure samples were sequenced for *pfk13, pfmdr1, pfcrt, pfdhfr,* and *pfdhps* genes. No *pfk13* mutations were found. Low rates of *pfmdr1* N86Y (3.7-8.8%), moderate to high rates of Y184F (41-74%), and low rates of the *pfcrt* K76T (2-38%) allele suggest a circulating parasite population that may be predisposed to reduced lumefantrine susceptibility. The *pfdhps* A437G mutation was found in 98% of Bohicon samples, 100% of Allada samples, and 94% of Parakou samples. The *pfdhps* haplotype of interest VAGKGS, which has been observed to be moving westward across the Sahel region, was detected in one sample in Allada.

Results from this study indicate that both components of AL, the current first-line treatment for malaria in Benin, remain effective.

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MONITORING INTERMITTENT PREVENTIVE TREATMENT ON PREGNANT WOMEN EFFICACY THROUGH ANTENATALS CLINICS IN SENEGAL

Marie Pierre Diouf[1], Mary Aigbiremo Oboh[2], Isaac Akhénaton Manga[3], Safietou Kande[1], Aminata Cole Lo[1], Amadou Seck[1], Fatou Faal[4], Babacar Faye[3], Paul Miligan[5], Corinne Merle[6], Alfred Amambua-Ngwa[4], Jean-Louis Abdourahim Ndiaye[1]

[1]UFR Santé, Université Iba Der Thiam, Thies, Senegal, [2]Rochester Institute of Technology, Rochester, New York, NY, United States, [3]Service Parasitologie Mycologie, Cheikh Anta Diop University, Dakar, Senegal, [4]Medical Research Council Unit, London School of Hygiene & Tropical Medicine, Serekunda, Gambia, [5]London School of Hygiene & Tropical Medicine, London, United Kingdom, [6]World Health Organization Tropical Disease Research, Geneva, Switzerland

Intermittent Preventive Treatment in pregnant woman (IPTp) and Seasonal Malaria Chemoprevention (SMC) are two strategies recommended for the prevention of malaria among the most vulnerable groups. These strategies using Sulfadoxine Pyrimethamine (SP) have been implemented in Senegal and have coexisted in some areas for six years. This could increase the pressure on the parasite and lead to resistance. It is therefore necessary to assess this resistance by monitoring molecular markers. Monitoring SP resistance among pregnant women attending the antenatal clinics (ANC) could be a cost-effective approach. This study was conducted during the high malaria transmission period of 2019 among women attending ANC in Senegal to determine the prevalence of SP resistance markers. After consent obtained, Rapid Diagnostic Test performed, three dry blood spots on Whatmann paper were collected. All samples were analysed by real-time PCR (VarATS gene testing) to determine parasite carriage. Positive samples were genotyped by High Resolution Melting for mutations in the dihydrofolate reductase and dihydropteroate synthase genes. A total of 1050 pregnant women were included and the parasite prevalence was 57.14%. The prevalence of the I164L mutation was 11.67% and there was no association with gravidity (10.81% in primigravida; 11.95% in multigravida; p=0.40) or SP intake (12.92% in SP- group; 10.64% in SP+; p=0.44). The prevalence of A581G was 12.16% in primigravida and 16.15 % in multigravida (p=0.20). This mutation was no associated with SP intake (p=0.50). In primigravida before the first dose of SP, the prevalence was 14.29% for I164L and 15.58 % for A581G. Among those who had taken at least one dose of SP, the prevalence was 7.04 % for the I164L and 8.45% for A581G. The differences were not statistically significant. The quintuple mutation was not found then SP is still effective for IPTp in Senegal, however, regular surveillance of molecular markers of resistance in pregnant women is necessary for informed decision-making. This monitoring could be carried out in pregnant women who come to ANC, providing an alternative to cross-sectional surveys.

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MOLECULAR SURVEILLANCE OF ARTEMISININ-RESISTANT *PLASMODIUM FALCIPARUM* PARASITES IN MINING AREAS OF THE RORAIMA INDIGENOUS TERRITORY IN BRAZIL

Jacqueline de Aguiar-Barros[1], Fabiana Granja[2], Rebecca de Abreu-Fernandes[3], Lucas Tavares de Queiroz[3], Daniel da Silva e Silva[2], Arthur Camurça Cité[4], Natália Ketrin Almeida-de-Oliveira Mocelin[3], Cláudio Tadeu Daniel-Ribeiro[3], **Maria de Fatima Ferreira-Da-Cruz**[3]

[1]Malaria Control Center, Epidemiological Surveillance Department, General Health Surveillance Coordination, SESAU-RR, Boa Vista, Brazil, [2]Biodiversity Research Centre, Federal University of Roraima, Boa Vista, Brazil, [3]Fundação Oswaldo Cruz, Rio de Janeiro, Brazil, [4]Research Support Center in Roraima (NAPRR) of the National Institute for Amazonian Research, Boa Vista, Brazil

Multidrug Artemisin-resistant (ART-R) *Plasmodium falciparum* parasites represent a challenge for malaria elimination worldwide. Molecular monitoring in the Kelch domain region (*pfk13*) gene allows tracking mutations in parasite resistance to artemisinin. The increase of illegal miners in Roraima Yanomami indigenous land (IYL) could favor ART-R parasites. Thus, the study aimed to investigate ART-R in patients from illegal gold mining in the YIL of Roraima, Brazil. A questionnaire was applied and blood was collected from 48 patients diagnosed with *P. falciparum* (*Pf*) or mixed malaria (*Pf +P. vivax*). The DNA was extracted and the *pfk13* gene was amplified by PCR. The amplicons were subjected to DNA Sangers sequenced; the entire amplified fragment was analyzed. Among patients, 96% (46) were from illegal mining areas of the YIL. All parasite samples carried the wild-type genotypes / ART-sensitive phenotypes. These data reinforce the continued use of ACTs in Roraima as well as the maintenance of systematic monitoring for early detection of parasite populations resistant

to ART, mainly in areas exposed to the individual's influx from mining areas, such as the YIL. This is especially true when the achievement of falciparum malaria elimination in Brazil is planned and expected by 2030.

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IN VITRO SENSITIVITY TO ANTIMALARIALS AND GENETIC MARKERS OF RESISTANCE OF KENYAN *PLASMODIUM FALCIPARUM*

Ben Chitama[1], Shinya Miyazaki[1], Peterson Gitonga Mathenge[2], Osamu Kaneko[1]

[1]Nagasaki Institute Of Tropical Medicine, Nagasaki, Japan, [2]Dana Farber Cancer Institute, Department Of Cancer Immunology And Virology, Harvard Medical School, Boston, MA, United States

Resistance to antimalarial drugs poses a challenge to malaria eradication. This study aimed to analyze both the in vitro sensitivity and genetic markers of resistance of 30 Kenyan clones of *Plasmodium falciparum* collected from Lake Victoria area in 2014-2015 to eight antimalarial drugs. Only five clones were resistant to chloroquine with a resistance-associated CRT triple mutation haplotype(I_{74}-E_{75}-T_{76}), indicating the resistance is lessening. We found a novel mutation I859 in MDR1 potentially associated with increasing the half maximal inhibitory concentration (IC_{50}) of chloroquine resistant clone and is now under further investigation. All clones were resistant to pyrimethamine, and showed resistance-associated DHFR haplotypes (29 I_{51}-R_{59}-N_{108}, 1 I_{51}-N_{108}-L_{164}, and 1 I_{51}-R_{59}-N_{108}-L_{164}). All showed DHPS E_{540} mutation associated with sulfadoxine resistance, indicating the quadruple mutant of DHFR(I_{51}-R_{59}-N_{108})-DHPS(E_{540}) against Fandidar® are dominant. Five clones were resistant to mefloquine, and no association was observed with any known genetic marker, including copy number variation of the mdr1 gene locus. One clone was resistant to lumefantrine, but we found no association with MDR1 F184 mutation. The remaining clones were susceptible to dihydroartemisinin, piperaquine, amodiaquine, and quinine; no mutations associated with resistance were observed in k13.

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QUALITY OF MALARIA TREATMENT AND COUNSELING FOR CHILDREN YOUNGER THAN FIVE YEARS IN OUTPATIENT DEPARTMENTS IN TANZANIA, 2020-2023

Patrick Gulinja[1], Francis Levira[1], Abdon Rwabilimbo[1], Binto Manyama[1], Frank Chacky[1], Sarah-Blythe Ballard[2], Albert Ikonje[1], Lulu Msangi[1], Naomi Serbantez[1], Chonge Kitojo[1], Kanuth Dimoso[1], Mwaka Kakolwa[1], Happiness Nyanda[1], Samwel Lazaro[1], Dunstan Bishanga[1]

[1]Ifakara Health Institute/Pmi Shinda (Defeat) Malaria Project, Dar es Salaam, Tanzania, United Republic of, [2]3.U.S. President's Malaria Initiative, US Centers for Disease Control and Prevention,, Dar es Salaam, Tanzania, United Republic of

High quality malaria case management reduces malaria morbidity and mortality and prevents the emergence of antimalarial resistance. To assess malaria case management quality in outpatient departments (OPD) in Tanzania, we analyzed OPD Malaria Service and Data Quality Improvement (MSDQI) supportive supervision (SS) data for children younger than five years. MSDQI OPD data were collected using standard SS visit checklists. Quality of treatment and counseling were assessed using parasitological confirmation of malaria diagnosis, adherence to weight-based dosing and dosing frequency, satisfactory treatment adherence counseling, client demonstration of understanding of prescription, and health care worker (HCW) solicitation of caregiver questions. Exit interviews were conducted for caregivers who completed treatment (observed and not observed during SS) to measure HCW service quality. From 2020–2023, 5,030 SS visits were conducted for children under five years old with suspected malaria. Malaria tests were ordered for 4,755 (94.5%), not ordered for 127 (2.5%), and 148 (2.9%) had missing testing information. Of children tested for malaria, 4,630 (97.4%) had test results with 2,414 (52.1%) testing positive. Of those testing positive, 2,343 (97.1%) were prescribed artemisinin-based combination therapy (ACT). Of 2,616 with negative or missing tests, 152 (5.8%) received ACTs. Of the 2,495 children given ACTs, the dose was correct for 98.8%, dosing frequency correct for 98.7%, treatment administration counseling sufficient for 97.8%, treatment understanding confirmed for 72.2%, and questions solicited for 59.0%. Exit interviews were conducted for 4,479 caregivers; 85.0% could explain how to give medicine at home and 82.9% reported receiving instructions on when to return. The quality of malaria testing and treatment among children under five is high. While patients demonstrated a good understanding and recall of anticipatory guidance, data suggest some providers might not sufficiently engage their patients in two-way communication. Interventions designed to address this might improve the quality and outcome of services.

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ASSESSING ANTIMALARIAL *EX-VIVO* DRUG EFFICACY IN WEST AND CENTRAL AFRICA FROM IMPORTED *PLASMODIUM FALCIPARUM* MALARIA CASES IN FRANCE BETWEEN 2016 AND 2023: A GENOTYPE-PHENOTYPE ASSOCIATION STUDY

Jason Rosado[1], *Abebe A. Fola[2], Sandrine Cojean[3], Veronique Sarrasin[3], Bruno Pradines[4], Romain Coppée[5], Justine Bailly[1], Rebecca Crudale[2], French National Reference Center for Imported Malaria group[6], Sandrine Houzé[7], Jeffrey A. Bailey[2], Jérôme Clain[1]*

[1]Université Paris Cité, Institut de Recherche pour le Développement, Paris, France, [2]Department of Pathology and Laboratory Medicine, Center for Computational Molecular Biology, Brown University, Providence, RI, United States, [3]Centre National de Référence du Paludisme, Assistance Publique-Hôpitaux de Paris, Hôpital Bichat-Claude-Bernard, Paris, France, [4]Centre national de référence du Paludisme, Institut hospitalo-universitaire Méditerranée Infection, Marseille, France, [5]Université Rouen Normandie, Rouen, France, [7]Université Paris Cité, Institut de Recherche pour le Développement, Centre National de Référence du Paludisme, Assistance Publique-Hôpitaux de Paris, Hôpital Bichat-Claude-Bernard, Paris, France

Malaria continues to pose a persistent global health threat. Artemisinin-based combination therapies (ACTs) are pivotal, yet emerging resistance threats their efficacy in several regions. Monitoring drug resistance, especially in Africa, is crucial due to limited therapeutic alternatives. We retrospectively analyzed 805 *Plasmodium falciparum* isolates (2016-2023) from travellers returning to France from mainly West and Central Africa by assessing the *ex-vivo* susceptibility of six antimalarials (chloroquine, amodiaquine, lumefantrine, piperaquine, mefloquine, dihydroartemisinine; growth inhibition assays) and sequencing key resistance markers across *P. falciparum* genome by molecular inversion probes (MIPs). Over 90% of isolates carried the *pfdhfr* IRN mutations (codons 51-59-108), over half harboured *pfdhps* S436A and A437G mutations, whereas *pfdhps* A613S prevalence was at 15%. *Pfmdr1* exhibited the NFD haplotype (codons 86-184-1246) in 54.8% of isolates. *Pfcrt* mutations were present in 20-30%, and *pfkelch13* mutations occurred in 0.3% (Y493H, I543T and A675V). IC_{50} values revealed reduced susceptibility to mefloquine (49% of isolates with IC_{50}>30nM), chloroquine (10% with IC_{50}>100nM), and amodiaquine (3% with IC_{50}>80nM). Less than 1% showed high IC_{50} for dihydroartemisinin, piperaquine, and lumefantrine. Genotype-phenotype analysis emphasized *pfcrt* and *pfmdr1* associations, with haplotypes influencing *ex-vivo* susceptibility to several drugs. *Pfcrt* K76T-mutant haplotypes IETSENTI and IETSENII (codons 74-75-76-220-271-326-356-371) were associated with decreased susceptibility to chloroquine and amodiaquine and increased susceptibility to dihydroartemisinin, lumefantrine and piperaquine. *Pfmdr1* NFD haplotype correlated with reduced susceptibility to dihydroartemisinin, lumefantrine, mefloquine, and piperaquine. Our longitudinal study shows a low prevalence of artemisinin resistance markers and of *ex-vivo* resistance to the main partner drugs in contemporary isolates from West and Central African countries. This suggests that ACTs are not immediately threatened in these regions of Africa.

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IRRESISTIBLE β-CARBOLINE DERIVATIVE ACTIVE AGAINST PROLIFERATING AND QUIESCENT RING STAGES OF ARTEMISININ-RESISTANT *PLASMODIUM FALCIPARUM*

Reagan S. Haney[1], Leticia S. Do Amaral[1], Emilio F. Merino[1], Emily K. Bremers[1], Joshua H. Butler[1], Victoria Mendiola[1], Jopaul Mathew[2], Dennis Kyle[1], Maxim Totrov[3], Paul R. Carlier[4], **Belen Cassera**[1]

[1]University of Georgia, Athens, GA, United States, [2]Virginia Tech, Blacksburg, VA, United States, [3]Molsoft LLC, San Diego, CA, United States, [4]University of Illinois Chicago, Chicago, IL, United States

Malaria remains a serious parasitic disease in the world, with over 240 million new infections and over 600,000 deaths each year, mostly caused by *Plasmodium falciparum*. Due to the rising resistance to current antimalarial drugs, there is an urgent need to develop new chemotherapeutic agents that engage new targets in the malaria parasite. Antimalarial drugs targeting the *P. falciparum* ring stage are highly attractive as they can prevent the development of the trophozoite and schizont stages that are sequestered by the cytoadherence of infected erythrocytes to the endothelial cells of deep vascular beds in vital organs. The ring stage also precedes gametocytogenesis, the intraerythrocytic sexual development stage required for transmission of the parasite to the *Anopheles* mosquito vector, thus reducing or blocking transmission of the disease. It is known that exposure to dihydroartemisinin (DHA) induces a quiescent state in the Plasmodium *falciparum* ring stage. Quiescence is a survival mechanism of *Plasmodium* following drug treatment. This phenomenon increases the risk of clinical failure following artemisinin-based combination therapies by slowing parasite clearance and allowing the selection of parasites resistant to partner drugs. We have discovered a novel β-carboline class of antimalarials that has demonstrated an inability to select resistant parasites in vitro, kills both the proliferating and the DHA-quiescent ring stages of sensitive and DHA-resistant strains of *P. falciparum*, and has a promising oral PK profile. In addition to extensive medicinal chemistry, we are characterizing the mechanism of action of this novel antimalarial class using an array of approaches, including chemoproteomics and fluorescent and electron microscopy. Our studies revealed that this β-carboline class displays a fast-killing profile and that it may act through a novel mechanism of action affecting hemoglobin uptake and digestion.

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ARTEMISININ RESISTANCE MUTATIONS IN *PFCORONIN* IMPEDE HEMOGLOBIN UPTAKE

Imran Ullah[1], Madeline A. Farringer[1], Anna Y. Burkhard[1], Erica Hathaway[2], Malhar Khushu[1], Bailey C. Willett[1], Sara H. Shin[1], Aabha I. Sharma[1], Kairon L. Shao[1], Morgan C. Martin[1], Jeffrey D. Dvorin[3], Daniel L. Hartl[1], Sarah K. Volkman[1], Selina Bopp[1], Sabrina Absalon*[2], Dyann F. Wirth*[1]

[1]Harvard T.H. Chan School of Public Health, Boston, MA, United States, [2]Indiana University, Indianapolis, IN, United States, [3]Harvard Medical School, Boston, MA, United States

Mutations in *Pfcoronin* (*Pfcrn*), including R100K/E107V, drive *in vitro* evolved artemisinin (ART) resistance in Senegalese *Plasmodium falciparum* isolates. However, the mechanisms underlying *Pfcrn*-mediated ART resistance remain unknown. Immunofluorescence microscopy in late-stage asexual parasites showed no difference in *Pf*CRN localization between mutant and wildtype (WT) parasites. Ultrastructure-expansion microscopy (U-ExM) was then used to examine ring-stage parasites, the stage at which ART resistance occurs. In WT rings, *Pf*CRN localized to the parasite plasma membrane, the digestive vacuole (DV) membrane, and a newly identified preDV compartment. By contrast, in mutant ring stage parasites, *Pf*CRN was not observed at the PPM, DV, or preDV, and mutant *Pf*CRN protein expression was 30% lower compared to WT *Pf*CRN, as quantified by western blot. The preDV compartment, visible in WT parasites as an invagination in early rings, and a fully-contained compartment in older rings, was aberrant in *Pfcrn* mutant parasites. In U-ExM images, a preDV was visible in all WT parasites, but 76% of *Pfcrn* mutant parasites lacked a preDV. When present, preDVs in mutants were 21% smaller than in WT parasites. These observations led us to hypothesize that *Pfcrn* mutations might impair hemoglobin uptake, possibly via abnormal development of the preDV. Endocytosis assays showed a significant reduction ($p < 0.0001$) in uptake of hemoglobin contents (45% decrease) in mutant *Pfcrn* parasites, compared to WT, similar to a 36% decrease observed in *Pfk13-C580Y* parasites. These data suggest that mutations in *Pfk13* and *Pfcrn* both confer reduced hemoglobin uptake in early rings and may similarly mediate ART resistance by reducing heme-dependent ART activation. However, *Pf*CRN localization differs from that of *Pf*K13, which is localized at the cytostomal collar, implying that *Pf*CRN and *Pf*K13 might facilitate hemoglobin uptake through different pathways. Ongoing studies will determine whether *Pf*K13- and *Pf*CRN-coated structures share a common pathway for hemoglobin uptake or if multiple pathways are involved.

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INTERVENTION STRATEGIES FOR ENHANCING PRIMAQUINE ADHERENCE ON *PLASMODIUM VIVAX* MALARIA: RESULTS FROM A CLUSTER RANDOMIZED CONTROLLED TRIAL IN MYANMAR

Kyawt Mon Win[1], Pyae Linn Aung[1], Wang Nguitragool[1], Liwang Cui[2], Jetsumon Sattabongkot[1], Saranath Lawpoolsri[1]

[1]Mahidol University, Bangkok, Thailand, [2]University of South Florida, Tampa, FL, United States

Plasmodium vivax malaria remains a concern for malaria elimination. The completion of a 14-day primaquine (PQ) regimen is essential for the radical cure of *P. vivax* malaria by clearing liver hypnozoites. Adherence to treatment is an important issue in malaria management. This study was carried out from August 2022 to March 2023 to develop an intervention for enhancing patients' adherence to PQ treatment and evaluate the effectiveness of this intervention in a malaria-endemic area in Myanmar. Based on a previous qualitative study, an intervention package emphasizing the family-orientated, directly observed treatment (family DOT), was developed. This package included training provided to Integrated Community Malaria Volunteers (ICMVs) on the importance of radical cure, managing adverse effects, and the role of family members in administering family DOT. Trained ICMV gave on-site training to family members of *P. vivax* patients on the first day of diagnosis and standardized pamphlets were distributed to reinforce key messages on primaquine treatment adherence. Patients diagnosed with *P. vivax* were administered the prescribed drugs under supervision of trained family members. A cluster randomized controlled trial was conducted to evaluate this intervention in 10 study villages from Waingmaw township, Kachin State, Myanmar. The proportion of treatment adherence was 98.8% and 77.6% in the intervention and control groups, respectively. Parasite recurrences were assessed in both groups on days 14, 28, and 42. Although the overall malaria trend did not sharply decline, the incidence in the intervention group showed reduction over time. In malaria-endemic areas with limited human resources, interventions to improve treatment adherence should be initiated to enhance the radical cure of *P. vivax*. The National Malaria Control Program should prioritize the research areas addressing malaria recurrences, cost-effectiveness of implementing family-based DOT, and treatment adherence in the specific context of Myanmar.

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PREVALENCE OF DRUG RESISTANCE MOLECULAR MARKERS IN *PLASMODIUM VIVAX* CLINICAL ISOLATES FROM SOUTHERN PAKISTAN

Shane Zehra, Najia Ghanchi, Bushra Qureshi, Zainab Rafeeq, M.Asim Beg

Aga Khan University, Karachi, Pakistan

Despite the implementation of various malaria control strategies, malaria continues to persist as an endemic disease in Pakistan. *Plasmodium vivax* and *P. falciparum* are common species and P. vivax is prevalent across

Sindh and Baluchistan region. Due to the rise in Chloroquine resistance until 2007, the National Malaria Control Programme (MCP) guidelines in 2008 recommended Chloroquine along with sulphadoxine pyrimethamine (SP) as the first-line treatment for *P. falciparum* infection. However, rise in resistance to sulphadoxine pyrimethamine in *P. vivax* has been observed due to mutations in *pvdhfr* and *pvdhps* genes. In this study we present the frequency of drug resistance-associated mutations in *pvdhfr* and *pvdhps* genes in clinical isolates of *P. vivax* from Southern Pakistan.From 2015-2018, a total of 650 samples were collected from clinical laboratories of Aga Khan University Hospital and its outreach laboratories located across Pakistan. Blood samples of patients with malaria positive microscopy results were amplified by nested PCR using *pvdhfr* and *pvdhps* specific primers and amplified products were purified and sequenced. The results were further analyzed using Mega 6 software in comparison to Wild Type reference strains. In *pvdhfr*, non-synonymous mutations were observed at codons N50I (6.154%), F57L (1.23%), S58R (44%), S93H (0.76%), D105N (8.92%), S117N (53.38%) and G469A (4.4%) while synonymous mutation was observed at codon 69Y. One hundred and eighty-seven (28.7%) were wild type strains. Mutations in *pvdhps* gene were observed at codons A383G (0.92%), G419C (0.65%), D459A (2.92%), R491K (0.65%) and six hundred and twenty-two (95.7%) were wild type.We report sulfadoxine-pyrimethamine (SP) resistance in clinical isolates of P. vivax originating from Southern regions of Pakistan, providing valuable insights into the prevalence of drug-resistant alleles in these malaria-endemic areas. The heightened occurrence of mutations in pvdhfr genes is particularly alarming for healthcare professionals, signaling a potential challenge in combating SP resistance within the population.

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OPTIMIZING NOVEL CLASS OF ANTIMALARIAL DRUG AND PYRONARIDINE COMBINATION TO GUIDE CLINICAL DOSING AND PREVENT DRUG RESISTANCE

Laurent Dembele[1], Mohamed Maiga[1], Fanta Sogore[1], Ousmaila Diakite[1], Fatoumata Ousmane Maiga[1], Thomas Spangenberg[2], Claudia Demarta[2], Sebastian Wicha[3], Abdoulaye Djimde[1]

[1]MRTC-USTTB, Bamako, Mali, [2]Merck, Lausanne, Switzerland, [3]University of Hamburg, Hamburg, Germany

The emergence and fast spread of drug resistance calls for the development of effective antimalarial combinations. However, the selection and combination of drug dose regimens involve a complex set of considerations, and there are various complications and limits to be considered such as pharmacokinetic and pharmacodynamic interactions. To ensure adequate translation of data from bench to bedside we sought to use immediate *ex vivo Plasmodium falciparum* field isolates employed for the *in vitro* testing of mono and combination effects of novel class of antimalarial drug and pyronaridine. These data are then fed into our model to generate an interaction map that later is used to simulate meaningful clinical dose ratios. First, we demonstrated that the pharmacometric model of parasite growth and killing under monotherapy as well as combination therapy provided a well-defined description of parasite kinetics *ex vivo* against susceptible and novel class of antimalarial drug-resistant parasites. Then, the model was used for clinical trial simulations translating the *ex vivo* data into human doses for the combination of novel class of antimalarial drug and pyronaridine. While monotherapy of pyronaridine was found to provide suboptimal killing rates, even at the highest studied dose, the combination of a lower single dose of novel class of antimalarial drug and pyronaridine provided a killing rate of 90% in more than 99% of the simulated conditions until 96 h. We have established a rapid, 3R-compliant *in vitro* method allying field isolate data and modelling to help guiding clinical drug development to set meaningful doses for antimalarials in clinical development.

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PREVALENCE OF HAPLOTYPES ASSOCIATED WITH RESISTANCE OF *PLASMODIUM FALCIPARUM* TO DIHYDROARTEMISININ-PIPERAQUINE, SULFADOXINE-PYRIMETHAMINE AND AMODIAQUINE DURING SEASONAL MALARIA CHEMO-PREVENTION CAMPAIGNS AMONG CHILDREN AGED 6-15 YEARS IN MALI

Karim Traore[1], Ali Thera[1], Guillaume Bonnot[2], Drissa Coulibaly[1], Abdoulaye K. Kone[1], Fayçal Maiga[1], Anne-Lise Bienvenu[2], Stephane Picot[2], Mahamadou Ali Thera[1]

[1]Malaria Research and Training Center, Bamako, Mali, [2]Univ Lyon, Malaria Research Unit, UMR 5246 CNRS-INSA-CPE-University Lyon1, Lyon, France

The World Health Organization recently recommended extending the age of seasonal malaria chemoprevention (SMC) and the use of other molecules effective against malaria. One of the factors that could threaten the effectiveness of this strategy is resistance to the drugs used. Following a study to evaluate the tolerance and effectiveness of Dihydroartemisinin-Piperaquine (DP) used in SMC, we measured the prevalence of haplotypes associated with the resistance of *Plasmodium falciparum* to DP, to Sulfadoxine-Pyrimethamine (SP) and Amodiaquine (AQ). From September 2020 to August 2021, we carried out a randomized controlled trial in Bandiagara, Mali including 345 children aged 6 to 15 years. Participants were assigned to three treatment regimens (DP vs SP-AQ vs Albendazole) for 4 consecutive months during malaria transmission season. Sanger-Sequencing of the *dhfr, dhps* and *crt* genes was performed on 90 *P. falciparum* PCR-positive samples randomly selected on study days 1, 7, 31, 61, 91, 180. The molecular prevalence of *P. falciparum* varied from 38.9% (35/90) on day1 to 7.8% (7/89) on days 61, 91 and 180. The *CVIET* prevalence (*C72S, V73, M74I, N75E, K76T*) of the crt gene was 12.5% on day 1 and 28.6% on day 61. The prevalence of *dhfr IRN* haplotypes (*N51I C59R S108N*) varied from 78.5% to 50% respectively on day1 and day 91. The wild NCS allele was dominant at day31 (56.5%). The *dhps* haplotype *SGKAA* was dominant (50% at day1 and 57% at day61) followed by *AGKAA* (35.7–40%) respectively from day1-91 (*S436A G437A K540E A581G A613S).* A reduction in the parasite load was observed from day61. The prevalence of *dhps* and *crt* mutant haplotypes was low compared to *dhfr* and remained stable during the SMC campaigns.

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EFFICACY AND SAFETY OF ARTESUNATE-AMODIAQUINE (ASAQ) AND ARTEMETHER-LUMEFANTRINE (AL) FOR THE TREATMENT OF UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA IN TWO COUNTIES IN LIBERIA, 2022-2023

Victor Koko[1], Laura Skrip[2], Odell Kumeh[1], Trokon Washington[1], Mamadou Diallo[3], Jessica Kafuko[4], Uwem Inyang[4], Veronika Laird[5], Jethro Zawolo[6], Birhanu Getahun[6], Wahdae-mai Harmon-Gray[2], Mateusz Plucinski[7], **Jonathan S. Schultz**[5]

[1]National Malaria Control Program, Ministry of Health, Monrovia, Liberia, [2]University of Liberia, Monrovia, Liberia, [3]U.S. President's Malaria Initiative, U.S. Centers for Disease Control and Prevention, Monrovia, Liberia, [4]U.S. President's Malaria Initiative, USAID, Monrovia, Liberia, [5]Malaria Branch, U.S. Centers for Disease Control and Prevention, Atlanta, GA, United States, [6]JHPIEGO, Monrovia, Liberia, [7]U.S. President's Malaria Initiative, Malaria Branch, U.S. Centers for Disease Control and Prevention, Atlanta, GA, United States

Artemether-lumefantrine (AL) is currently the first line treatment for uncomplicated *Plasmodium falciparum* (*Pf*) malaria in Liberia. In order to ensure antimalarial treatments continue working, the WHO recommends routinely testing the efficacy of antimalarials using therapeutic efficacy studies (TES) every two years. As a result of the TES conducted in 2018, artesunate-amodiaquine (ASAQ) was withdrawn as a first-line therapeutic option in Liberia. The 2018 TES reported PCR-corrected adequate parasitological and clinical response (APCR) rates of 90.2% in Bensonville and 92.7% in Saclepea for ASAQ, and 100% in Kakata and Sinje for AL.

Given other countries in West Africa continue to report high efficacy of ASAQ, and per WHO recommendations to routinely conduct TESs, the therapeutic efficacy of ASAQ and AL was evaluated using the standard WHO protocol in Saclepea and Sinje, Liberia from August 2022 to July 2023. Eligible children aged 6 months to 5 years old with uncomplicated *Pf* malaria infection (2,000 - 200,000 asexual parasites/μL of blood) were recruited, treated with either ASAQ or AL at each site, and monitored clinically and parasitologically for 28 days. A total of 1,639 children were screened for eligibility and 305 were enrolled. Among enrolled children, 153 were treated with ASAQ (77 in Sacleapea and 76 in Sinje) and 152 were treated with AL (78 in Sacleapea and 74 in Sinje). Of the enrolled children, 299 (98%) completed the 28 days of follow up. No adverse events were reported for either ASAQ or AL during the study period. The proportion of participants with parasitemia on day 3 of follow up was 1.3% (2/153) for ASAQ, none were detected among those treated with AL. Both fall below the WHO threshold of 10%. The uncorrected APCR at day 28 was 100% (95% CI: 96.2%-100%) in Saclepea and 90.1% (95% CI: 82.6%-95.8%) in Sinje for ASAQ, and 100% (95% CI: 96.2%-100%) in Saclepea and 93.2% (95% CI: 85.6%-97.5%) in Sinje for AL. The day 3 slide positivity and uncorrected efficacy results greater than the 90% WHO threshold suggest that AL and ASAQ are likely still effective treatments for uncomplicated *Pf* malaria infections in Liberia.

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WIDESPREAD *PFHRP2/3* DELETIONS AND HRP2-BASED FALSE-NEGATIVE RESULTS IN SOUTHERN ETHIOPIA

Bacha Mekonen Tafa[1], Lemu Golassa[2], Sisay Dugassa[2], Sindew Mekasha Feleke[1]

[1]Ethiopian Public Health Institute, Addis Ababa, Ethiopia, [2]Addis Ababa University, Akililu Lemma Institute of Pathobiology, Addis Ababa, Ethiopia

Rapid diagnostic tests (RDTs) are vital for malaria case management in peripheral healthcare systems, with Histidine-rich protein-2 (HRP2) antigen detection RDTs being widely used for *Plasmodium falciparum* diagnosis. However, the emergence of *P. falciparum* strains with deleted hrp2/3 genes causing false-negative results raises concerns. This study assessed HRP2-detecting RDTs' diagnostic performance and *pfhrp2/3* deletions prevalence among symptomatic patients in southern Ethiopia. A cross-sectional study (July-September 2022) enrolled febrile patients with microscopically confirmed P. falciparum infections. Blood samples underwent microscopy, RDT (SD BiolineTM Malaria Pf/Pv Test), and molecular analysis via nested PCR for *hrp2/3* gene deletions confirmation. Among 279 PCR-confirmed *P. falciparum* cases, 89.2% had successful *msp-2* amplification, revealing common *pfhrp2/3* deletions in all health centers (57.8% prevalence). Deletions in *hrp2 exon 2*, *hrp3 exon 2*, and double deletions (*hrp2/3*) accounted for 27.3%, 30.5%, and 13.2% of cases, respectively, with variations across study sites. The SD Bioline PfHRP2-RDT test sensitivity was 76.5% compared to PCR. The study confirmed widespread *pfhrp2/3* deletions exceeding the WHO-recommended threshold (> 5%), impacting malaria control and elimination efforts in Ethiopia and beyond. The adoption of non-HRP2-based RDTs is crucial to mitigate false-negative results' consequences.

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PREVALENCE OF AND CHALLENGES IN DIAGNOSING SUBCLINICAL *PLASMODIUM FALCIPARUM* INFECTIONS: IMPLICATIONS FOR MALARIA CONTROL AND ELIMINATION IN GHANA

Abdul-Hakim Mutala[1], Kingsley Badu[1], Cristian Koepfoli[2], Stephen Opoku Afriyie[3], Abraham Badu-Tawiah[4]

[1]Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, [2]University of Notre Dame, Notre Dame, IN, United States, [3]Texas Tech University, Lubbock, TX, United States, [4]The Ohio State University, Columbus, OH, United States

Many national malaria elimination programmes (NMEP) are intensifying campaigns for malaria control and elimination. The high prevalence of subclinical infections may hamper control efforts. The detection of subclinical and low-density infection is crucial in monitoring progress towards malaria control. This study sought to determine the prevalence of subclinical infections in three districts of Ghana, the proportion that could be detected by a novel rapid diagnostic test (RDT) developed by Rapigen, and the occurrence of *hrp2/hrp3* deletions which may impede diagnosis by HRP2-based RDTs. A community-based cross-sectional study was conducted in Nkwanta South, Sekyere South and Ga South districts in Ghana. A total of 1134 whole blood samples were screened by rapid diagnostic test (RDT), expert microscopy, and *var*ATS qPCR. 304 *Plasmodium falciparum* positive samples were typed for *hrp2/hrp3* deletions by digital PCR (dPCR). Malaria prevalence was 57.1% by qPCR, 40.9% by RDT, and 8.4% by microscopy. 33.8% (219/647) of infections were sub-patent. Compared to qPCR, the sensitivity of RDT was 65.7%, and specificity of 91.9% and thus substantially higher than microscopy (sensitivity 14.4%, specificity 99.4%). The prevalence was highest in children aged 5-15 years (68.2%), followed by adults >15 years (51.2%) and children < 5 years (45.3%). Prevalence also differed across the three districts, ranging from 44.0% (183/416) in Sekyere South, 55.8% (143/253) in Ga South, to 68.8% (321/466) in Nkwanta South. No *hrp2* deletions were observed, and one sample (1/304) carried *hrp3* deletion. The high prevalence of subclinical malaria infections is likely to be a potential reservoir in sustaining malaria transmission. HRP2-based RDTs detected two thirds of the subclinical infections. Thus, community test and treat programs using highly sensitive RDTs could be a valuable strategy to reduce the reservoir and accelerate progress towards malaria control and elimination in Ghana.

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REMOSCOPE: A LABEL-FREE IMAGING CYTOMETER FOR MALARIA DIAGNOSTICS

Paul M. Lebel[1], Chris Charlton[1], Ilakkiyan Jeyakumar[1], Michelle W.L. Khoo[1], Aditi Saxena[1], Axel Jacobsen[1], James Emorut[2], Emily Huynh[3], William Wu[4], Greg Courville[1], Pei-Chuan Fu[5], Robert Puccinelli[6], Peter Olwoch[2], Grant Dorsey[7], Philip J. Rosenthal[7], Rafael Gomez-Sjoberg[1], Joseph DeRisi[1]

[1]Chan Zuckerberg Biohub, San Francisco, CA, United States, [2]Infectious Disease Research Collaboration, Tororo, Uganda, [3]University of Pennsylvania, Philadelphia, PA, United States, [4]Chan Zuckerberg Biohub (current: Tezerakt LLC at Google), San Francisco, CA, United States, [5]Chan Zuckerberg Biohub SF (current: Liminal Insights), Emeryville, CA, United States, [6]University of California, Berkeley, Berkeley, CA, United States, [7]University of California, San Francisco, San Francisco, CA, United States

Malaria diagnostic testing in high transmission settings is a challenge to global healthcare systems. Here we present remoscope, an automated imaging cytometer that scans fresh, unstained whole blood using a custom neural network on low-cost hardware. By screening up to two million red blood cells, remoscope performs quantitative stage-specific detection of *Plasmodium falciparum* in 1-12 minutes without sample fixation, staining, or slide scanning. Flow is used to achieve high cellular throughput, with blood confined to a 5 μm monolayer in low-cost disposable flow chambers. We tested remoscope performance *in vitro* by titration of cultured parasites into uninfected whole blood at concentrations of 710,000-21.7 parasites/μL. Counts generated by the remoscope demonstrated a linear response across the entire range. The potential for using remoscope in drug susceptibility assays and evaluation of clinical treatment efficacy was tested by measuring the half-maximal effective concentration (EC50) of chloroquine in a cultured W2 *P. falciparum* strain, which resulted in an EC50 value of 224 nM, vs 191 nM for flow cytometry. We next studied remoscope's diagnostic accuracy in a cohort of 500 individuals in eastern Uganda, comprising 629 unique clinic visits. Parallel measurements of parasitemia were performed using remoscope, qPCR targeting the multicopy conserved *var* gene acidic terminal sequence, and traditional Giemsa microscopy of thick blood smears. Remoscope's limit of detection with respect to qPCR was 156 parasites/μL. At this threshold, the system had a sensitivity of 86%, specificity of 95%, Positive Predictive Value (PPV) of 87%, and a Negative Predictive Value (NPV) of 94% compared to qPCR. Remoscope's speed and ease of use address practical challenges in malaria diagnostic settings

around the world. The system can also inform development of recognition models for the diagnosis of other infectious or non-communicable blood disorders.

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COMPARATIVE STUDIES OF MALARIA PARASITE NONINVASIVE AND INVASIVE DIAGNOSTIC TESTS AMONG PREGNANT WOMEN IN NIGERIA

Chinyelu Angela Ekwunife[1], Kester O. Obieze[2], Christine I. Eneanya[1], Bernice A. Ogolo[2], Ginika L. Onwuachusi[1], Izunna S. Okwelogu[1], Chinwe A. Uzuke[1]

[1]Nnamdi Azikiwe University, Awka, Nigeria, [2]Nnamdi Azikiwe University Teaching Hospital Trauma Center, Oba, Nigeria

The gold standard for malaria parasite diagnostic methods requires blood collection. This may be associated with pain, the risk of transmitting blood-borne pathogens and poor compliance when repeated sampling is needed. Pregnant women who live in the tropics who regularly attend antenatal clinics for routine checks are at risk. Hence, the potential use of non-invasive methods (saliva and urine specimens) as alternative sources of malaria parasite (DNA) in the diagnosis of malaria infection using real-time polymerase Chain Reaction (qPCR) was studied. The participants (628 pregnant women) were randomly selected patients at the Ante Natal Care (ANC) Department in Nnamdi Azikiwe University Teaching Hospital, Nigeria. The biomarkers (18S rRNA) were captured and concentrated from the samples using the magnetic bead-based. DNA recovered from these samples was evaluated by qPCR. The prevalence of malaria based on saliva specimens was 430 (68.5%). Out of this, 373 (86.7%) were true positive (TP) and 57 (13.3%) false positive (FP). The prevalence based on urine specimens was 411 (65.4%), where 365 (88.8%) were true positive (TP) and 46 (11.2%) false positive (FP). A significant association was observed between the saliva, urine qPCR and blood microscopy ($P = 0.0002$, $P < 0.05$). The malaria microscopic prevalence was 60.0%. Urine qPCR was found to have a higher Kappa coefficient agreement (0.80308) with microscopy than saliva qPCR (0.76094). When the qPCR method was compared to thick blood film-microscopy as reference standard, saliva qPCR had sensitivity of 98.94%, specificity of 77.29%, positive predictive value (PPV) of 86.73%, and negative predictive value (NPV) of 97.98%; while urine qPCR had sensitivity of 96.82%, specificity of 81.67%, PPV of 88.79%, and NPV of 94.48%. This further confirmed that qPCR of saliva and urine is a promising non-invasive approach for malaria diagnosis as their sensitivity is comparable to that of blood microscopy. It is essential to continue research to facilitate the development of a tool that will aid the control and elimination of malaria. More research that will focus on pregnant women in Nigeria is needed**.**

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COMPARISON OF PREVALENCE ESTIMATES OF *PFHRP2* AND *PFHRP3* DELETIONS IN *PLASMODIUM FALCIPARUM* DETERMINED BY CONVENTIONAL PCR AND MULTIPLEX QPCR AND IMPLICATIONS FOR SURVEILLANCE AND MONITORING

Michelle L. Gatton[1], David Smith[2], Cielo Pasay[2], Karen Anderson[2], Selam Mihreteab[3], Hugo O. Valdivia[4], Juan F. Sanchez[4], Khalid B. Beshir[5], Jane Cunningham[6], **Qin Cheng**[7]

[1]Queensland University of Technology, Brisbane, Australia, [2]QIMR Berghofer Medical Research Institute, Brisbane, Australia, [3]Ministry of Health, Asmara, Eritrea, [4]U.S. Naval Medical Research Unit SOUTH, Lima, Peru, [5]London School of Hygiene & Tropical Medicine, London, United Kingdom, [6]World Health Organization, Geneva, Switzerland, [7]Australian Defence Force Malaria and Infectious Disease Institute, Brisbane, Australia

Accuracy of malaria rapid diagnostic tests is threatened by *Plasmodium falciparum* with *pfhrp2/3* deletions. This study compares gene deletion prevalence determined by multiplex qPCR and conventional PCR (cPCR) using existing samples with clonality previously determined by microsatellite genotyping. Multiplex qPCR was performed to estimate prevalence of *pfhrp2/3* deletions in three sets of previously collected patient samples from Eritrea and Peru. The qPCR was validated by multiplex digital PCR. Sample classification was compared with cPCR, and ROC analysis used to determine the optimal ΔCq threshold that aligned results of the two assays. qPCR classified 75% (637/849) of samples as single, and 212 as mixed-*pfhrp2/3* genotypes, with a positive association between clonality and proportion of mixed-*pfhrp2/3* genotype samples. Sample classification agreement between cPCR and qPCR was 75.1% (95% CI 68.6-80.7%) and 47.8% (95% CI 38.9-56.9%) for monoclonal and polyclonal infections. qPCR prevalence estimates of *pfhrp2/3* deletions showed almost perfect (κ=0.804; 95% CI 0.714-0.895) and substantial agreement (κ=0.717; 95% CI 0.562-0.872) with cPCR for Peru and 2016 Eritrean samples, respectively. For 2019 Eritrean samples the prevalence of double *pfhrp2/3* deletions was approximately two-fold higher using qPCR. The optimal threshold for matching assay results was ΔCq=3. In conclusion, multiplex qPCR and cPCR produce comparable estimates of gene deletion prevalence when monoclonal infections dominate, but qPCR provides higher estimates where multiclonal infections are common.

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ACUTE UNDIFFERENTIATED FEBRILE ILLNESSES SURVEILLANCE IN TWO MILITARY HEALTH FACILITIES IN ABUJA, NIGERIA

Hanan El Mohammady[1], Rania Nada[1], Isabelle Nakhla[1], Mary Adenuga[2], Inalegwu Ochai[2], Feyisayo Jegede[2], Ojor Ayemoba[3]

[1]U.S. Naval Medical Research Unit – EURAFCENT, Cairo, Egypt, [2]Defence Reference Laboratory (DRL), Abuja, Nigeria, [3]Nigerian Ministry of Defence Health Implementation Programme (MODHIP), Abuja, Nigeria

The distribution of etiologies causing acute undifferentiated febrile illnesses (AUF) in Nigeria has limited study due to the high malaria burden. We investigated the prevalence of etiologies causing AUF using malaria microscopy, PfHRP2 rapid diagnostic test (RDT), and qPCR targeting 16 pathogens. From February 2023 to February 2024, 1,118 adult AUF patients were enrolled at two sites in Abuja, Nigeria: The Defence Headquarters Medical Center (DHQMC), and the 063 Nigerian Air Force Hospital (NAFH). The DHQMC enrolled 557 adults, with a median age of 28 years, and 45% females, while the NAFH enrolled 561 cases, with a median age of 30 years, and 54% females. Muscle pains, joint weakness and headache were common in >90% of enrollees in both sites, whereas chills/rigors were more prevalent at the DHQMC (96%) than at the NAFH (61%). Malaria prevalence was 35% and 39% by microscopy with parasitemia density ranged from 40 to 492,000 parasite/µl and from 222 to 432,000 parasite/µl in DHQMC and NAFH, respectively. RDT detected 37% and 41% of cases at the DHQMC and NAFH, respectively. Microscopy positive/PfHRP2 RDT negative cases were less than 3.5% at both sites. Malaria qPCR performed on 965 samples showed higher positive detection rates than microscopy and RDT with 52% at the DHQMC and 57% at the NAFH. Malaria detection rates in enrolled cases varied significantly according to the season, reaching a maximum of 100% during the wet season, and a minimum of 0% in the dry season. Malaria data obtained with the three detection techniques showed a 78% concordance and indicated that 58% of cases tested positive for malaria with at least one technique. The qPCR panel showed that *Salmonella* was detected in 3% and 0.4% of samples at the DHQMC and the NAFH, respectively, whereas Epstein–Barr virus was only detected at the DHQMC. Our data confirm the high burden of malaria in Nigeria. The detection of AUF cases during malaria low season, along with the high negative qPCR detection rates for AUF pathogens, suggest a need for more advanced molecular tools that provide broader information about febrile disease epidemiology and etiology.

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PLASMODIUM FALCIPARUM HISTIDINE RICH PROTEIN 2/3 DELETIONS AND REPEAT MOTIFS IN INDIA: CHALLENGES IN RAPID DIAGNOSTIC TESTS - BASED MALARIA DIAGNOSIS

Praveen Kumar Bharti[1], Shrikant Nema[1], Sri Krishna[2], Anil Kumar Verma[2]

[1]ICMR-National Institute of Malaria Research, New Delhi, India, [2]ICMR-National Institute of Research in Tribal Health, Jabalpur, India

Rapid diagnostic tests (RDTs) for malaria use the histidine-rich protein 2 (PfHRP2) for the detection of *Plasmodium falciparum,* with cross-reactivity extending to PfHRP3, a structural homolog. In this study, we have examined the gene deletions and sequence variation in the *Pfhrp2* and *Pfhrp3* gene in *P. falciparum* isolates from Chhattisgarh state, India, and correlated these variations with RDT reactivity. A total of 264 *P. falciparum* positive samples were PCR amplified for *Pfhrp2* and *Pfhrp3* genes and subsequently sequenced. Amino acid sequences were analyzed for repeat variations and their association with RDT reactivity. Among the samples, *Pfhrp2* and *Pfhrp3* showed 3.8% and 14% of gene deletion respectively. Nucleotide sequences for the *Pfhrp2* gene were successfully obtained from 101 and 95 sequences were acquired from *Pfhrp3*. *Pfhrp2* exhibited 15 distinct repeat motifs, and *Pfhrp3* showed 10. Notably, no correlation was found between variations in the size of *Pfhrp2* repeat types 2 and 7 and the performance of a commercial RDT at low parasite densities. The study suggests that gene deletions and sequence diversity in *Pfhrp2* and *Pfhrp3* genes in the Chhattisgarh state are unlikely to adversely affect the effectiveness of currently used PfHRP2 baaed RDTs. However, a larger-scale study encompassing other endemic states in India is recommended over time for a comprehensive understanding.

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UNCOVERING TREATMENT GAPS: A CLOSER LOOK AT MALARIA CASE MANAGEMENT IN A DISTRICT REFERRAL HOSPITAL IN GHANA, 2023

Joel Jeffrey Idun-Acquah[1], Afia Frimpomaa Asare Marfo[2]

[1]National Malaria Elimination Programme, Accra, Ghana, [2]Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

Malaria remains a significant global public health concern, notably in the WHO African region, where it continues to rank among the leading causes of mortality and morbidity. Effective case management plays a pivotal role in malaria control efforts, yet concerns persist regarding poor adherence to established guidelines on management. We assessed adherence to malaria case management guidelines to inform interventions aimed at strengthening case management practices. A cross-sectional study was conducted, involving patient records of 350 suspected malaria cases systematically sampled from the Outpatients' Department of Tema General Hospital in Ghana. Data extracted were signs and symptoms, malaria testing, and treatment. The proportion of cases prescribed antimalarials and compliance with the Test, Treat, and Track policy (T3) were determined. Chi-squared tests and logistic regression were conducted for potential associations. Among the 350 cases reviewed, 91% (318/350) underwent testing by either microscopy or malaria Rapid Diagnostic Test. More than half (53%, 186/350) received antimalarial prescriptions, with 47% (87/186) receiving prescriptions prior to receipt of test results. About 26% (50/192) of suspected cases testing negative received antimalarials. Prescription of antimalarials was significantly associated with age (p=0.007), prescriber category (p=0.006), and requests for laboratory investigations (p<0.001). Treatment adherence to guidelines was observed in 60% (211/350) of cases, with significant variations among prescribers (p=0.001). Only 17% (60/350) of patients were scheduled for follow-up; 37% (22/60) attended the follow-up sessions. Although testing rate for suspected malaria cases was notably high, adherence to malaria case management guidelines suggested room for improvement, with lack of comprehensive tracking of cases. Implementing measures to increase adherence and track cases is needed to optimize malaria management outcomes.

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COST-EFFECTIVENESS COMPARISON OF MALARIA DIAGNOSIS SCENARIOS WITH SYSMEX XN-31 IN A NON-ENDEMIC AREA.

Stephane Picot

Malaria Research Unit, Universite de Lyon, UMR 5246 CNRS-INSA-CPE, Lyon, France

Malaria diagnosis in non-endemic areas relies frequently on screening with RDT or molecular method followed by microscopic confirmation. The majority of laboratories in non-endemic areas receive a low number of suspected malaria cases with a low positivity rate. This epidemiological context underpins the need for innovative diagnosis methods. In the context of limited resources for medical care, the cost-effectiveness of diagnosis workflow is a major argument for decision makers and final buyers. The objective was to measure in monetary and non-monetary values the cost-effectiveness of the most current standards of care compared to scenarios with Sysmex XN-31 haematology analyser. A decision tree allowing visualization of the different diagnosis pathways was built. The structure of the study population was based on the real cohort of patients attending health care at Lyon university hospital for suspected malaria in 2023. The costs of intervention and controls were calculated by the addition of the direct + indirect costs (working time including training, quality proficiency and operational execution).An incremental cost-effectiveness ratio (ICER) was used to compare the difference between the costs and health outcomes of intervention and controls. The intervention (XN-31+microscopy) was the most effective scenario. Microscopy was weakly dominated by the intervention, and both LAMP+microscopy and RDT+microscopy were strongly dominated. This cost-effectiveness analysis was needed to clarify the real price of malaria diagnosis according to the different scenarios available. Considering both the workload of each method and their respective diagnosis accuracy, it appears that the association of microscopy to XN-31 is the best scenario from a monetary and non-monetary perspective.

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IDENTIFYING SUBGROUPS WITH DECREASED PERFORMANCE CHARACTERISTICS OF MALARIA RAPID DIAGNOSTIC TESTS

Geoffrey Guenther[1], Alick Sixpence[2], Charles Mangani[3], Alfred Matengeni[4], Don P. Mathanga[4], Miriam K. Laufer[5], Karl B. Seydel[6], Lauren M. Cohee[7], Clarissa Valim[2]

[1]Boston Children's Hospital, Boston, MA, United States, [2]Boston University School of Public Health, Boston, MA, United States, [3]Kamuzu University of Health Sciences, Blantyre, Malawi, [4]MAC Communicable Diseases Alert Centre, Blantyre, Malawi, [5]University of Maryland School of Medicine, Baltimore, MD, United States, [6]Michigan State University College of Osteopathic Medicine, East Lansing, MI, United States, [7]Liverpool School of Tropical Medicine, Liverpool, United Kingdom

The point-of-care malaria rapid diagnostic test (MRDT) is a crucial and cost-effective diagnostic tool, allowing prompt treatment to decrease the high burden of malaria morbidity and transmission. In a 2011 systematic review of real-world MRDT performance in uncomplicated malaria compared to microscopy or polymerase chain reaction (PCR), the average sensitivity and specificity of the most common tests was 95% or greater. However, recent data on MRDT performance show a more nuanced and complicated picture, suggesting that the sensitivity and specificity of the tests can vary significantly with age and some other characteristics. Under the aegis of the Malawi International Center of Excellence in Malaria Research (ICEMR), we evaluated the performance characteristics of MRDT in population subgroups when compared to quantitative PCR based on 1324 symptomatic health center visits conducted between 2019 and 2021, representing 510 participants (average of 2.6 visits/participant). 46% of

malaria PCR tests and 36% of MRDTs were positive; 85% of +PCR tests also had a +MRDT. As expected, the likelihood of a +MRDT was higher during the rainy (60%) compared to the dry (54%) season (p=0.04). Those with +MRDTs were more likely to report fever and headache (74% vs 52% for fever, 59% vs 38% for headache, p<0.05 for both). Those with +MRDTs were more likely to report vomiting (26% vs 8%, p<0.05), but less likely to report diarrhea (4% vs 10%, p<0.05) or cough (16% vs 39%, p<0.05). There were 174 false negative (24%) and 50 false positive (9%) MRDT results. Overall, the sensitivity of MRDTs was 67% and the specificity was 91%. MRDT sensitivity was the lowest among subjects aged < 2 years (46%), and the highest among those 5-15 years (83%). Parasite densities in false -MRDTs were generally <100 per microliter and varied significantly with age in both false -MRDTs and true +MRDTs. The widest range of parasite densities in false negative results were in those 5-15 years. Parasite density of true +MRDT results decreased with age. In our study, MRDTs performed less well than expected in certain populations, which may inform deployment and development of these important tools.

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FALSE NEGATIVE MALARIA RAPID DIAGNOSTIC TESTS ON A LACTATE DEHYDROGENASE-BASED KIT AMID INCREASING *PLASMODIUM OVALE* PREVALENCE IN KENYA

Raphael Okoth[1], Catherine Muriuki[1], Alfred Odindo[1], Benjamin Opot[1], Dennis Juma[1], Jackline Juma[1], Agnes Cheruiyot[1], Redemptah Yeda[1], Edwin Mwakio[1], Maurine Mwalo[1], Risper Maisiba[1], Farid Abdi[1], Timothy Egbo[2], Hoseah M. Akala[1]

[1]Department of Emerging and Infectious Diseases (DEID), Walter Reed Army Institute of Research-Africa (WRAIR-Africa), Kenya Medical Research Institute (KEMRI) / Walter Reed Project, Kisumu, Kenya, [2]Walter Reed Army Institute of Research-Africa (WRAIR-Africa), Kisumu, Kenya

Malaria rapid diagnostic tests (mRDTs) are widely used tests in resource limited settings to guide prompt malaria case management. The *P. falciparum* specific histidine-rich protein-based (*Pf*HRP2) mRDTs are commonly used and occasionally in combination with the *Plasmodium spp* lactate dehydrogenase (*p*LDH) specific band to detect infection containing non-*falciparum* malaria. Besides ongoing efforts to evaluate HRP2/3 deletions causing false negative *P. falciparum* diagnoses, it is increasingly important to establish the accuracy of pan-*p*LDH band given the increasing frequency of non-*falciparum* infections for effective case management. Here, we characterize polymorphisms causing false negative diagnosis of non-*falciparum* infections using combo mRDTs. In 2023-2024, 105 blood samples from febrile individuals at a health facility were tested for presence of malaria using combo mRDTs followed by microscopy and PCR to confirm malaria presence and determine species composition. Genotyping of polymorphisms within the *p*LDH gene of associated species is ongoing. Out of 105 samples tested using HRP2 and pan-pLDH bands of the mRDT, 84 were confirmed positive for malaria, whereas 6 samples (5.71%) failed to react to these mRDT bands but were positive through microscopy. Molecular analyses revealed that 94/105 samples were positive for malaria by PCR including those that had failed to react to both test bands. Assessment of species composition showed that *P. falciparum* (*Pf*) was the most prevalent, found in 80 samples (85.10%), followed by *P. ovale wallikeri* (Pow) 5, (5.32%), *P. malariae* (*Pm*) 2, (2.13%) and *Pf-P. ovale curtisi* mixed infections 2, (2.13%). Notably, the six infections that were unreactive by both the HRP2 and pan-*p*LDH bands comprised 4 *P. ovale wallikeri* and 2 *P. malariae* infections. A review of clinical data confirmed that these patients presented with classical symptoms of malaria in absence of *P. falciparum*. These findings underscore the urgency for enhanced surveillance and diagnostic accuracy, suggesting a reevaluation of the shift towards pan-pLDH based diagnostics in areas where non-falciparum species are prevalent.

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UNUSUAL PRESENTATION OF MALARIA IN A PEDIATRIC PATIENT DELAYING DIAGNOSIS

Shankar Kafle, Shovana Karki, Anil Dev Pant, Abhimannu Jha, Ram Chandra Adhikari

Tribhuvan University Teaching Hospital / Maharajgunj Medical College, Kathmandu, Nepal

A six months old female child from Terai region of Nepal presented with fever, vomiting, and bloody stool for three months. She was referred to Tribhuvan University Teaching Hospital, Kathmandu. Despite various investigations and treatments, including high-dose antibiotics for Pyrexia of unknown origin (PUO), the patient remained unwell and her health condition was deteriorating and got admitted to the PICU. Laboratory findings revealed pancytopenia with polychromasia. It was already 14 days of her PICU when a Peripheral blood examination revealed the presence of malarial parasites, suggesting specifically *Plasmodium vivax*. Subsequent Rapid kit test also support the diagnosis. Prompt treatment with Tab. Chloroquine and Tab. Primaquine resulted in complete recovery.

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A PHASE 1B STUDY TO CHARACTERIZE THE SAFETY AND PHARMACOKINETIC/PHARMACODYNAMIC RELATIONSHIP OF MMV367 (GSK701) IN ADULT PARTICIPANTS EXPERIMENTALLY INFECTED WITH BLOOD- STAGE *PLASMODIUM FALCIPARUM*

Bridget E. Barber[1], Robin Denhardt Eriksson[2], Etienne Guirou[2], Julia Flynn[2], Nischal Sahai[3], Chris Muller[3], Indika Leelasena[3], Ivan Lim[3], Carla Evangelista[3], Sue Mathison[3], Stacey Llewellyn[1], Rebecca Webster[1], Adam J. Potter[1], Michael W. Marx[4], Francisco-Javier Gamo[5], Rachel A. Gibson[6], Raman Sharma[7], Laura M. Sanz[5], Anthony Cahn[6], Benoit Bestgen[2], Stephan Chalon[2]

[1]QIMR Berghofer Medical Research Institute, Brisbane, Australia, [2]MMV Medicines for Malaria Venture, Geneva, Switzerland, [3]University of Sunshine Coast, Brisbane, Australia, [4]ICON Clinical Research Germany GmbH, Langen, Germany, [5]Global Health Medicines R&D, GSK, Tres Cantos, Spain, [6]Global Health Medicines R&D, GSK, Stevenage, United Kingdom, [7]Clinical Pharmacology Modelling and Simulation, GSK, Stevenage, United Kingdom

The rapid spread of antimalarial drug resistance presents a major threat to malaria control and necessitates the development of new antimalarial compounds with novel mode of action. MMV367 is a first-in-class, fast acting, orally bioavailable antimalarial co-developed by MMV (sponsor) and GSK with potent activity against *Plasmodium falciparum*, including resistant strains. Safety, tolerability, and pharmacokinetics were recently evaluated in a first-in-human study (NCT05507970) with results supporting progression of MMV367 as a potential new treatment for uncomplicated malaria. We therefore undertook a Phase 1b volunteer infection study to characterise the pharmacokinetic/pharmacodynamic (PK/PD) relationship of MMV367 in healthy adults experimentally infected with blood-stage *P. falciparum* (NCT05979207). Twelve participants were enrolled across 2 cohorts of 6 participants. Participants were inoculated with *P. falciparum* infected erythrocytes and treated on day 8 with different single oral doses of MMV367, including subtherapeutic doses to observe recrudescence. In cohort 1, participants were dosed with 20 mg (n=3), 90 mg (n=2) or 1500 mg (n=1). Parasite clearance was rapid in all participants, with a median (95% CI) observed clearance half-life of 3.04 (2.78 - 3.37), 3.97 (3.58 - 4.46) and 3.11 (2.72 - 3.64) hours for the 20 mg, 90 mg, and 1500 mg doses, respectively. Parasite recrudescence occurred in only 1 participant (treated with 20 mg), requiring rescue treatment on day 22. In cohort 2, participants were dosed with 3 mg (n=3), 5 mg (n=2), or 10 mg (n=1). All 6 participants received rescue treatment with artemether-lumefantrine by day 4 post administration of MMV367 and recrudescences allowed identification of the PK/PD relationship. The 12 participants experienced a total of 211 adverse events (median 19, range 6 - 38). The majority were mild to moderate and

consistent with clinical symptoms of malaria. There were no serious adverse events. In summary, this study supports the further clinical development of MMV367 in patients as a single dose treatment of uncomplicated malaria.

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DOSE-OPTIMIZATION OF THE FIXED-DOSE TRIPLE COMBINATION ANTIMALARIAL THERAPY ARTEMETHER-LUMEFANTRINE-AMODIAQUINE

Joel Tarning

Mahidol Oxford Tropical Medicine Research Unit, Bangkok, Thailand

Artemisinin-based combination therapy (ACT) is the first-line therapy for uncomplicated *falciparum* malaria. Triple-ACTs (TACTs) have emerged as a treatment option to combat declining efficacy of ACTs and to prevent the development of drug resistance. In this study, we aimed to develop an optimal fixed-dose regimen of artemether-lumefantrine-amodiaquine through population pharmacokinetic modelling and simulation. Three published pharmacokinetic models were used to simulate concentration-time profiles of competing dose regimens. Two publicly available data bases were used to generate virtual patients with biologically plausible weight-age combinations; NHANES from the US CDC (n = 53,833) and the SMAC network (n = 26,051). The pharmacometric model for each drug was applied to simulate a total of 1,000 patients for each bodyweight and dosing regimen. All pharmacometric simulations were performed in NONMEM. Simulated concentration-time profiles were compared between standard and optimal dosing. To minimize the extent of modifications needed to combine two existing treatments, the current dosing ratio of artemether-lumefantrine (20/120 mg) was retained, with the addition of 40 mg amodiaquine. This drug-to-drug-ratio was kept throughout the dosing bands in order to simplify manufacturing, implementation, and further development of a fixed-dose co-formulated product. The standard 4 dosing bands were transformed to 5 dosing bands to achieve more equivalent exposure in all weight groups and to reduce fluctuations in peak concentrations. The proposed optimal dosing was constructed to achieve equivalent drug exposure to artemether-lumefantrine-amodiaquine in small children compared to adults, while safeguarding that no patients received higher peak concentrations of amodiaquine compared to current dose recommendations. In conclusion, an optimal fixed-dose TACT regimen was developed to maximise the chance of cure by providing a somewhat increased mg/kg dose of artemether-lumefantrine in small children and large adults, while minimising the risk of adverse events associated with high doses of amodiaquine.

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PRE-REFERRAL RECTAL ARTESUNATE IN CHILDREN WITH SEVERE MALARIA: ANY BENEFIT?

Ayorinde Adehin[1], Marie A. Onyamboko[2], Caterina Fanello[1], Nicholas PJ. Day[1], Nicholas J. White[1], **Richard M. Hoglund**[1], Joel Tarning[1]

[1]Mahidol Oxford Tropical Medicine Research Unit, Bangkok, Thailand, [2]Kinshasa School of Public Health, University of Kinshasa, Kinshasa, Congo, Democratic Republic of the

Malaria still poses a major health risk in Sub-Saharan Africa and untreated *Plasmodium falciparum* infections can progress to severe malaria and death, especially in young children. The first line treatment of severe malaria is parenteral artesunate, but this treatment requires adequate healthcare facilities to be able to administered in an effective and safe manner. In rural malaria-endemic areas, the access to appropriate care could involve prolonged transportation. Therefore, pre-referral rectal artesunate could be a life-saving option to keep the patient stable before adequate facilities can be reached. In the present study, observed data from intravenously and rectally administered artesunate in children with malaria were analyzed to determine the pharmacokinetic properties and the bioavailability of rectal artesunate. Concentration-time measurements of artesunate and dihydroartemisinin were collected from a clinical trial conducted in the Democratic Republic of Congo. The study was designed as cross-over trial in which the child randomly received rectal or intravenous artesunate at enrollment and the alternative regimen 12 hours later. All patients received intravenous quinine at the same time for safety reasons. Pharmacokinetic data was analysed using NONMEM. Artesunate and dihydroartemisinin was successfully described by a two-compartment disposition models with one transit compartment describing the absorption of the rectal formulation. The estimated absolute bioavailability of the rectal formulation was low with a high variability between patients. The final model was linked to an existing pharmacodynamic model, describing parasite density, and used to simulate the clinical impact of the rectal formulation. According to these simulations, even with low bioavailability, the rectal formulation resulted in enough concentrations of artesunate and its metabolite, to eliminate malaria parasites while transporting the patient to an appropriate health care facility. In conclusion, these findings add to existing knowledge and strongly encourage the use of rectal artesunate as a pre-referral treatment.

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THE EFFECT OF SINGLE LOW-DOSE PRIMAQUINE TREATMENT FOR UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA ON HEMOGLOBIN LEVELS IN ETHIOPIA: A LONGITUDINAL COHORT STUDY

Kassahun Habtamu[1], Hallelujah Getachew[2], Ashenafi Abossie[3], Assalif Demissew[4], Arega Tsegaye[5], Teshome Degefa[5], Xiaoming Wang[6], Ming-Chieh Lee[6], Guofa Zhou[6], Solomon Kibret[7], Christopher L. King[8], James W. Kazura[9], Beyene Petros[1], Delenasaw Yewhalaw[5], Guiyun Yan[6]

[1]Addis Ababa University, Addis Ababa, Ethiopia, [2]Arbaminch College of Health Sciences, Arbaminch, Ethiopia, [3]Arba Minch University, Arbaminch, Ethiopia, [4]Ambo University, Ambo, Ethiopia, [5]Jimma University, Jimma, Ethiopia, [6]University of California at Irvine, Irvine, CA, United States, [7]West Valley Mosquito and Vector Control District, Irvine, CA, United States, [8]Case Western Reserve Universit, Cleveland, OH, United States, [9]Case Western Reserve University, Cleveland, OH, United States

To interrupt residual malaria transmission and achieve successful elimination of *Plasmodium falciparum* in low-transmission settings, the World Health Organization recommends the administration of a single dose of 0.25 mg/kg (or 15 mg/kg for adults) primaquine (PQ) combined with artemisinin-based combination therapy (ACT) without glucose-6-phosphate dehydrogenase (G6PD) testing. However, due to the fear of hemolysis in patients with G6PD deficiency (G6PDd), PQ use is not widely practiced. Hence, this study aimed to assess the safety of a single low dose of PQ administered to patients with G6PD deficiency. A longitudinal cohort study was conducted with patients treated for uncomplicated *P. falciparum* malaria with either single-dose PQ (0.25 mg/kg) (SLD-PQ) + ACT or ACT alone. Microscopy-confirmed uncomplicated *P. falciparum* malaria patients visiting health facilities in Arjo Didessa, Southwest Ethiopia, were enrolled in the study from September 2019 to November 2022. Patients with uncomplicated *P. falciparum* malaria were followed up for 28 days through clinical and laboratory diagnosis for G6PD levels and hemoglobin (Hb) concentrations. Hb data were taken on days (D) 0, 7, 14, 21, and 28 following treatments with SLD-PQ + ACT or ACT alone. A total of 249 patients with uncomplicated *P. falciparum* malaria were enrolled in this study. Of these, 83 (33.3%) patients received ACT alone, and 166 (66.7%) received ACT combined with SLD-PQ treatment. The median age of the patients was 20 years. G6PD deficiency was found in 17 (6.8%) patients, 14 males and 3 females. There were 6 (7.2%) and 11 (6.6%) phenotypic G6PD-deficient patients in the ACT alone and ACT + SLD-PQ arms, respectively. No difference in mean Hb levels ($P = 0.157$) was observed in patients treated with ACT + SLD-PQ (mean Hb = 0.45 g/dL; 95% CI = 0.39 – 0.52) post-treatment compared to patients treated with ACT alone (mean Hb = 0.30 g/ dL; 95% CI = 0.14 – 0.47). Our findings showed that single low-dose primaquine (SLD-PQ) treatment for uncomplicated *P. falciparum* malaria is safe and does not increase the risk of hemolysis in G6PD deficient patients.

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REPEAT IVERMECTIN MASS DRUG ADMINISTRATIONS FOR MALARIA CONTROL II: PRIMARY OUTCOME

Fabrice Some[1], Anthony Some[1], Emmanuel Sougue[1], Ollo Da[1], Nikiema Frederic[1], Cheick O. Ouedraogo[2], Tereza Magalhaes[3], Lyndsey I. Gray[4], Will Finical[4], Greg Pugh[4], Paula Lado[4], Jenna Randall[4], McKenzie Colt[2], Martina Wade[2], Kacey Richards[2], Hannah Sproch[2], Elizabeth Zhang[2], Julia Ellman[2], Ijeamaka Ichebe[2], Conner L. Jackson[5], Mengli Xiao[5], Emma J. Wu[5], Hannaha C. Slater[6], Teun Bousema[7], Brian D. Foy[5], Roch K. Dabire[1], **Sunil Parikh**[2]

[1]Institut de Recherche en Sciences de la Sante, Bobo-Dioulasso, Burkina Faso, [2]Yale School of Public Health, New Haven, CT, United States, [3]Texas A&M University, College Station, TX, United States, [4]Colorado State University, Fort Collins, CO, United States, [5]University of Colorado, Aurora, CO, United States, [6]PATH, Seattle, WA, United States, [7]Radboud Institute for Health Sciences, Nijmegan, Netherlands

Ivermectin (IVM) can kill malaria vectors that blood feed on IVM-treated people and thus may be an effective malaria vector control tool. We conducted a parallel design, double-blind, placebo-controlled cluster-randomized control trial in Burkina Faso over two consecutive years to test the safety of repeated, high-dose IVM mass drug administrations (MDA) and their efficacy for reducing incidence among children when distributed during seasonal malaria chemoprevention (SMC). Fourteen villages (clusters) were randomized 1:1. Exclusion criteria for IVM MDA included those <90cm, pregnant, or taking SMC. Participants received 3 consecutive 300 µg/kg IVM or placebo doses monthly during the rainy season. The primary outcome was incidence in children ≤10 years as assessed by weekly active case detection. Adverse events (AEs) were monitored in all participants. In the IVM and control arms, 4091 and 3525 participants including 1403 and 1262 cohort children were followed in 2019 and 2020, respectively. All clusters received new dual-ITN Interceptor® G2 in October 2019. The weekly malaria incidence rate per 100 person-weeks in the IVM and control arm was 1.78 and 1.84, respectively (IRR = 0.96; P = 0.87). The risk of AEs among MDA-eligible participants in the IVM arm was lower than among those in the control arm (risk ratio = 0.63; P = 0.0049). Membrane feeding data with treated human sera demonstrated the strong mosquitocidal activity of the drug. Blood fed *Anopheles gambiae* s.l. mosquitoes captured in IVM clusters the week after MDA in 2019 had decreased survival relative those captured from control clusters (P < 0.0001), but there was no difference in these survival rates among mosquitoes captured three weeks after MDA. EIR did not differ between arms (IVM = 0.010, control = 0.011). Repeated high dose IVM MDA was safe when integrated with SMC, but did not reduce incidence among children relative to placebo MDA, despite evidence that mosquito survivorship in the first year was reduced in the IVM arm at least a week following MDA. Confounding factors included unexpectedly low incidence over the trial and dual-chemistry LLIN distribution in the middle of the study period.

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ASSESSMENT OF THE ANTIPLASMODIAL EFFECTS < TOXICITY STUDY OF ENDOPHYTES FUNGI EXTRACT ISOLATED FROM *ALSTONIA BOONEI* DE WILD

Paola Cynthia Demeni Emoh[1], Noella Molisa Efange[2], Arnaud Sas Soha[3], Jean Robert Klotoé[3], Maximilienne Ascension1 Nyegue[1], Lawrence Ayong[4], Gisèle Aurelie Foko Dadji[5]

[1]University of Yaoundé 1, Yaoundé, Cameroon, [2]University of Buea, Buea, Cameroon, [3]Applied Microbiology and Pharmacology of natural substances, Research Laboratory in Applied Biology, Polytechnic School of Abomey-Calavi, University of Abomey-Calavi, Abomey-Calavi, Benin, [4]Centre Pasteur du Cameroun, Yaoundé, Cameroon, [5]Higher Teacher Training College, University of Yaounde I, Yaoundé, Cameroon

Despite all strategies implemented to eradicate malaria, it remains one of the main causes of death worldwide. This is particularly related to the expansion of multidrug-resistant strains of *Plasmodium* species of which *Plasmodium falciparum* is the most prevalent. This study aimed to assess the antiplasmodial < toxicity profiles of extracts from endophytic fungi of *Alstonia boonei*. Endophytic fungi from different parts of *A. boonei* were isolated using in Potato Dextrose Agar (PDA), Sabouraud Dextrose Agar (SDA) < Czapek Dox Agar (CDA) media. The isolates were identified < grouped morphologically and molecularly. Each fungus was grown in a liquid medium < the ethyl acetate extracts prepared were evaluated for antiplasmodial activity < for both larval using *Artemia salina* larvae < acute toxicity using rat as models. Following the isolation, 73 endophytic fungi in total, with 28 in PDA, 26 in SDA and 19 in CZA. Thirty-four (44) morphologically distinct < 35 molecularly different endophytic fungi with molecular weights ranging from 500 to 750 base pairs were obtained. Each of the 35 endophytic fungi were shown to belong to either of the following genera: *Fusarium spp, Trichoderma spp, Acremonium spp, Alternia spp, Lasiodiplodia spp, Chaetomium spp, Xylaria spp < Emericellopsis spp* with 04 non-sporulating fungi. Extracts from the 35 fungi showed IC_{50}s (inhibitory concentration 50%) ranging from 3.86 to 32.69 µg/mL against *P. falciparum* 3D7 < 5.34 to 76.28 µg/mL against *P. falciparum* Dd2 laboratory strains. The acute < larval toxicity tests showed that endophytic fungi extracts were not toxic on larvae < on rats. They therefore deserve to be exploited further as sources of potential antimalarial agents.

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IDENTIFICATION OF INHIBITORS OF MOSQUITO STAGES OF *PLASMODIUM FALCIPARUM* DEVELOPMENT USING AN *IN VITRO* CULTURE SYSTEM

Federico Appetecchia[1], Rinvee A. Tasneem[1], Flaminia Catteruccia[2]

[1]Harvard T. H. Chan School of Public Health, Boston, MA, United States, [2]Harvard T. H. Chan School of Public Health/Howard Hughes Medical Institute, Boston, MA, United States

We recently generated a new strategy for malaria control based on killing *Plasmodium falciparum* parasites in the mosquito vector using inhibitors delivered via treated surfaces such as bed nets. The discovery of novel inhibitors that can be incorporated onto mosquito nets, however, has so far been hindered by the inability to perform high-throughput screens for compounds that kill the sporogonic stages of parasite development. While efficient *in vitro* systems of sporogonic stages have been generated for rodent malaria parasites, studies are still lagging for human parasites, despite some progress. Here, we show the development of an *in vitro* culture system for *P. falciparum* mosquito stages that allows the reliable production of ookinetes and oocysts with improved yields and that can be used for high-throughput screens. We used the previously described transgenic reporter line NF54-HGL, which expresses a fusion of the green fluorescent and firefly luciferase proteins, to culture parasites *in vitro*. Ookinete and oocyst formation were achieved in the presence of insect cells and matrigel in optimized media conditions. After cell number optimization, we assessed this system in a 96-well plate format to test the inhibitory activity of several compounds with known mechanism of action against ookinete and oocyst development. Parasite viability was evaluated through live cell imaging for ookinetes, while a simplified bioluminescence-based assay was employed for day 7 oocysts and then validated also through live cell imaging. Novel drugs were identified with potent multistage activity or stage specificity activity at 1 uM, including the *Pf*PI4K inhibitor KDU692 (ookinete -specific activity), the *Pf*DHODH inhibitor DMS265 (oocyst -specific activity), and the putative *Pf*CARL inhibitor GNF179 (multi-stage activity). Current studies are focused on extending the drug screening to libraries with novel chemotypes and on the development of a high-throughput live cell imaging system.

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ACCELERATING ANTIMALARIAL DRUG DISCOVERY WITH A HIGH-THROUGHPUT SCREENING FOR FAST-KILLING COMPOUNDS

Takaya Sakura[1], Ryuta Ishii[2], Eri Yoshida[1], Kiyoshi Kita[3], Teruhisa Kato[2], Daniel Ken Inaoka[4]

[1]Department of Molecular Infection Dynamics, Institute of Tropical Medicine (NEKKEN), Nagasaki University, Nagasaki, Japan, [2]Laboratory for Drug

Discovery and Disease Research, Shionogi & Co., Ltd., Osaka, Japan, [3]School of Tropical Medicine and Global Health, Nagasaki University, Nagasaki, Japan, [4]Department of Infection Biochemistry, Institute of Tropical Medicine (NEKKEN), Nagasaki University, Nagasaki, Japan

The significance of fast-killing properties has become increasingly crucial for the advancement of next-generation lead compounds in antimalarial drug discovery, as the expedited action of these compounds has the potential to alleviate resistance concerns and enhance patient compliance with antimalarial medications, thereby addressing prominent issues associated with artemisinin-based combination therapies (ACTs). The present study introduces a high-throughput screening (HTS) approach using 1536-well plates, employing *Plasmodium falciparum* lactate dehydrogenase (PfLDH) combined with nitroreductase (NTR) and turn-on fluorescent probes to evaluate the inhibition of the growth of the asexual blood stage of malaria parasites. We applied this method to assess the parasite reduction ratio (PRR) and successfully screened a large number of compounds in a 384-plate format over a short period, simplifying the time-consuming conventional method. Our high-throughput PRR enables the early identification of fast-killing hits during the screening stages, while also facilitating the continuous monitoring of such properties in newly synthesized compounds through structure-activity relationship (SAR) studies. This approach accelerates the development of innovative fast-killing antimalarial drugs within the framework of phenotypic drug discovery campaigns.

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HIGH THROUGHPUT SCREENING IDENTIFIES COMPOUNDS WITH NANOMOLAR ANTIPLASMODIAL ACTIVITY AGAINST THE ASEXUAL STAGE PARASITES

Japheth Kibet Ng'etich[1], Normalita Eka Pravitasari[2], Takeshi Ishikawa[3], Ayato Sato[4], Shinjiro Hamano[5], Noriyuki Nishida[6], Takaya Sakura[7], Daniel Inaoka[8]

[1]Department of Molecular Microbiology and Immunology, Nagasaki University, Japan., Nagasaki, Japan, [2]School of Tropical Medicine and Global Health, Nagasaki University., Nagasaki, Japan, [3]Department of Chemistry, Biotechnology, and Chemical Engineering, Graduate School of Science and Engineering, Kagoshima Institute., Kagoshima, Japan, [4]Institute of Transformative Biomolecules (ITbM), Nagoya University., Nagoya, Japan, [5]Department of Parasitology, Institute of Tropical Medicine (NEKKEN) Nagasaki University, Nagasaki, Japan, [6]Institute of Biomedical Sciences, Nagasaki University., Nagasaki, Japan, [7]Department of Molecular Infection Dynamics, Shionogi Global Infectious Diseases Division, Institute of Tropical Medicine (NEKKEN), Nagasaki University., Nagasaki, Japan, [8]Department of Infection Biochemistry, Institute of Tropical Medicine (NEKKEN), Nagasaki University., Nagasaki, Japan

The emergence of artemisinin-resistant malaria parasite highlights the need for new drugs with novel mechanisms of action against Plasmodium infection. To identify such new compounds, we conducted a high-throughput screening campaign (HTS) using a robust fluorescent technique at a single-dose concentration of compounds included in the Nagoya Chemical Library. Primary HTS identified 1,365 hits (among 36,160 compounds) at a cut-off inhibition of 68.65%, established by the empirical rule of standard deviations (3×SD). A subset of 896 compounds was prioritized for dose-response assays following elimination owing to cytotoxicity against mammalian cells and the requirement to achieve a 1% hit rate for structure disclosure. This subset was subsequently tested for inhibitors of the mitochondrial electron transport chain (MtETC), which is the most validated target, using the 3D7-yDHODH transgenic parasite strain alongside the wild-type 3D7. We have successfully identified 23 compounds showing complete parasite inhibition at nanomolar ranges, even at the lowest tested dose (12 nM) against 3D7, and seven compounds targeting MtETC showing > 100-fold shift in EC50 (3D7 vs. 3D7-yDHODH strains). A total of 435 compounds exhibiting ≤ 6.5 µM EC50 have been prioritized for further assays against a panel of Dd2 parasites harboring mutations in well-known drug target genes as an attempt to distinguish hit compounds with unknown or similar mechanisms of action. The MtETC-targeting compounds will be subjected to kinetic studies against the recombinant PfDHODH enzyme for further validation, and compounds that elicit unknown mechanisms of action will be used to raise resistant mutants, followed by whole-genome sequencing for target elucidation.

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NOVEL DRUG DISCOVERY FOR *PLASMODIUM FALCIPARUM* MALARIA

Tanbir Najrana[1], Hannah Wei Wu[1], Alan Ardito[1], Brajesh Singh[1], Yannic Johnson[1], Nazrin Rustamzade[1], Michael Chan[1], Camille Hebert[1], Emilia Ferraro[1], Anthony Cruz[1], Sunthorn Pond-Tor[1], Rose Guilfoyle[1], Audrey Kertscher[1], Edwin W. Mwakio[2], Douglas O. Ochora[2], Farid A. Salim[2], Ashley Simkovitch[3], Matthew Araujo[3], Irina Oleinikov[3], Ashley Bunnell[4], Dipak K. Raj[5], John Michael Ong'echa[2], Hoseah M. Akala[2], Adel Nefzi[4], Andrew Oleinikov[3], **Jonathan D. Kurtis**[1]

[1]Brown University, Providence, RI, United States, [2]KEMRI, Kisumu, Kenya, [3]Florida Atlantic University, Boca Raton, FL, United States, [4]Florida International University, Miami, FL, United States, [5]University of South Florida, Tampa, FL, United States

Malaria attributable mortality is increasing and the spread of parasites resistant to the artemisinin family of compounds threatens to exacerbate this trend, thus underscoring the urgent need to identify novel anti-malarial drugs. We propose that *P. falciparum* PfGARP is a high value druggable target based on: 1) its surface expression on iRBCs, 2) its absence of amino acid homology with host proteins, 3) its absence of significant sequence variation, 4) the requirement for PfGARP for *in vivo* survival, and 5) the ability of antibody binding to PfGARP to rapidly kill parasites without engaging immune effector functions. To develop a drug based on PfGARP binding, we screened the 30 million compound CTS-FIU library to identify compounds that inhibit binding of anti-PfGARP to rPfGARP protein. We reason that compounds which bind to the same region of PfGARP targeted by parasite-lethal anti-PfGARP antibodies will be enriched for effective, PfGARP targeting anti-malarials. We identified 2 sub-libraries containing compounds which bind to PfGARP. Using our positional scanning method, we deconvoluted one sub-library (>45k compounds based on a bis-cyclic guanidine-*S*-butyl scaffold) to 7 compounds which kill 3D7 strain *P. falciparum* parasites with IC_{50} ~ 25-50nM. We focused on one of these highly active compounds, #19, and demonstrated it had similar killing activity against several drug resistant parasite strains (Dd2, CamWT_C580Y). Compound #19 had an IC_{50} >50uM for cytotoxicity as measured in several human cell types including PBMCs, lung epithelial (A549), macrophage (THP-1) as well as bacteria (*E. coli*) giving it a selectivity index >1,000. Compound #19 is freely soluble in aqueous vehicles and, when delivered SQ, has a half-life of 17 hrs in mice. When dosed SQ at 5mg/kg, compound #19 rapidly cleared parasites in the *P. falciparum*/NSG model. We are evaluating the *in vitro* efficacy of compound #19 in freshly isolated parasites from our field sites and evaluating mutagenic/genotoxic potential (Ames, micronucleus), cardiac toxicity (hERG), and vehicle formulations to advance compound #19 as a parenteral treatment for severe falciparum malaria.

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DIHYDROARTEMISININ-PIPERAQUINE AS AN ALTERNATIVE TO SULFADOXINE-PYRIMETHAMINE FOR INTERMITTENT PREVENTIVE TREATMENT IN PREGNANCY: A META-ANALYSIS OF MATERNAL, BIRTH, AND INFANT OUTCOMES

Michelle E. Roh[1], Julie Gutman[2], Mywayiwawo Madanitsa[3], Abel Kakuru[4], Hellen C. Barsosio[5], Simon Kariuki[5], John Lusingu[6], Frank Mosha[7], Richard Kajubi[4], Moses R. Kamya[8], Don Mathanga[9], Jobiba Chinkhumba[10], Miriam K. Laufer[11], Eulambius Mlugu[12], Appolinary A.R. Kamuhabwa[13], Eleni Akillu[14], Omary Minzi[13], Roland Nnaemeka Okoro[15], Ado Danazumi Geidam[16], John David Ohieku[15], Jenny Hill[17], Meghna Desai[2], Prasanna Jagannathan[18], Grant Dorsey[19], Feiko O. ter Kuile[17]

[1]Institute for Global Health Sciences, University of California, San Francisco, San Francisco, CA, United States, [2]Malaria Branch, Division of Parasitic

Diseases and Malaria, Centers for Disease Control and Prevention (CDC), Atlanta, GA, United States, [3]School of Global and Public Health, Kamuzu University of Health Sciences, Blantyre, Malawi, [4]Infectious Diseases Research Collaboration, Kampala, Uganda, [5]Kenya Medical Research Institute, Centre for Global Health Research, Kisumu, Kenya, [6]National Institute for Medical Research (NIMR), Tanga Medical Research Centre, Tanga, Tanzania, United Republic of, [7]Kilimanjaro Christian Medical Centre, Moshi, Tanzania, United Republic of, [8]School of Medicine, Makerere University College of Health Sciences, Kampala, Uganda, [9]Malaria Alert Center, College of Medicine, University of Malawi, Blantyre, Malawi, [10]Department of Health Systems and Policy, School of Global and Public Health, Kamuzu University of Health Sciences, Blantyre, Malawi, [11]Center for Vaccine Development and Global Health, University of Maryland School of Medicine, Baltimore, MD, United States, [12]Department of Pharmaceutics, School of Pharmacy, Muhimbili University of Health and Allied Sciences, Dar es Salaam, Tanzania, United Republic of, [13]Department of Clinical Pharmacy and Pharmacology, School of Pharmacy, Muhimbili University of Health and Allied Sciences, Dar es Salaam, Tanzania, United Republic of, [14]Department of Global Public Health, Karolinska Institute, Karolinska University Hospital, Stockholm, Sweden, [15]Department of Clinical Pharmacy and Pharmacy Administration, Faculty of Pharmacy, University of Maiduguri, Maiduguri, Nigeria, [16]Department of Obstetrics and Gynaecology, University of Maiduguri Teaching Hospital, Maiduguri, Nigeria, [17]Department of Clinical Sciences, Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [18]Department of Medicine, Stanford University, Stanford, CA, United States, [19]Department of Medicine, University of California, San Francisco, San Francisco, CA, United States

High-grade *Plasmodium falciparum* resistance to sulfadoxine-pyrimethamine (SP) in East and Southern Africa prompted a series of trials comparing intermittent preventive treatment in pregnancy (IPTp) with SP to dihydroartemisinin-piperaquine (DP), a highly efficacious artemisinin-based combination therapy. We conducted an individual participant data random-effects meta-analysis of 9 trials involving 7,533 HIV-uninfected women with singleton pregnancies that led WHO to continue recommending IPTp-SP. We previously reported that IPTp-SP was associated with increased fetal growth and newborn birthweight despite a lower risk of malaria outcomes with IPTp-DP. Here, we provide new findings regarding the impact of these regimens on maternal, birth, and infant outcomes. Compared to IPTp-SP, IPTp-DP was associated with a 69% [95% CI: 45%–82%] lower incidence of clinical malaria during pregnancy, a 56% [26%–74%] lower risk of placental parasitemia detected by histopathology, microscopy, RDT, or PCR, and a 17% [0%–31%] lower incidence of moderate maternal anemia (Hb<9 g/dL). Effects were similar in primi- and multi-gravidae (p-interaction≥0.53). Compared to IPTp-DP, IPTp-SP was associated with higher mean weekly maternal weight gain (30 g [14-46]). At birth, the risk of small-for-gestational age was 14% [3%–24%] lower in the IPTp-SP arm. Among multigravidae, underweight and stunting at birth were 39% [14%-56%] and 24% [8%–37%] lower with IPTp-SP. These effects were not observed in primigravidae: 31% [-10%–92%] and 16% [-8%–46%] higher in the IPTp-SP arm, respectively. By age ~2 months, the risk of stunting remained lower in the IPTp-SP arm in multigravidae 18% [3%–30%], but not in primigravidae (-16% [-7%–46%]) (p-interaction<0.001). The risk of being underweight by ~2 months was 27% [6%–44%] lower in the IPTp-SP arm, with no differences between gravidity subgroups (p-interaction=0.79). IPTp-DP was associated with reductions in malaria, regardless of gravidity. However, IPTp-SP was associated with improved birth and infant outcomes, particularly in multigravidae. Trials evaluating a combined regimen of DP+SP for IPTp are warranted.

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SAFETY AND FEASIBILITY OF INTEGRATING MASS DRUG ADMINISTRATION FOR HELMINTH CONTROL WITH SEASONAL MALARIA CHEMOPREVENTION IN SENEGALESE CHILDREN: A RANDOMIZED CONTROLLED, OBSERVER-BLIND TRIAL

Muhammed Olanrewaju Afolabi[1], Doudou Sow[2], Schadrac C. Agbla[3], El Hadji B. Fall[4], Fatimata Bintou Sall[4], Amadou Seck[4], Isaac Akhénaton Manga[5], Ibrahima Mbaye[4], Mor Absa Loum[6], Baba Camara[7], Diatou Niang[7], Babacar Gueye[8], Doudou Sene[8], Ndéye M'backé Kane[8], Bocar Diop[8], Awa Diouf[4], Ndeye Aida Gaye[4], Marie Pierre Diouf[4], Aminata Colle Lo[5], Brian O. Greenwood[1], Jean Louis Ndiaye[4]

[1]London School of Hygiene & Tropical Medicine, London, United Kingdom, [2]Université Gaston Berger de Saint-Louis, Saint-Louis, Senegal, [3]University of Liverpool, Liverpool, United Kingdom, [4]Université de Thies, Thies, Senegal, [5]Université Cheikh Anta Diop, Dakar, Senegal, [6]Université de Thies,, Thies, Senegal, [7]Saraya Health Centre, Saraya, Senegal, [8]Ministère de la Santé et de l'Action Sociale, Dakar, Senegal

The overlap in the epidemiology of malaria and helminths has been identified as a potential area to exploit for the development of an integrated control strategy to achieve the WHO targets of eliminating malaria and helminths by 2030. We conducted a randomized controlled, observer-blind trial, to assess the feasibility and safety of combining mass drug administration (MDA) for schistosomiasis and soil-transmitted helminths (STH) with seasonal malaria chemoprevention (SMC) among children living in Senegal, West Africa. Children aged 1-14 years were randomized 1:1:1, to receive Vitamin A and Zinc supplements on Day 0 (control group); or praziquantel on Day 0 (treatment group 1); or albendazole and praziquantel on Day 0 (treatment group 2), followed by SMC drugs (sulphadoxine-pyrimethamine/amodiaquine) on Days 1-3 in the control and treatment groups. Safety assessment was performed by collecting adverse events from all children for six subsequent days following drug administration. Pre- and post-intervention, blood, urine, and stool samples were collected from the study participants for determination of haemoglobin concentration, microscopy, and PCR assays. From 9-22 June 2022, 627 children were enrolled and randomized as described above. Mild-to-moderate, transient vomiting was observed in 12.6% (26/206) in treatment group 2, 10.6% (22/207) in treatment group 1, and 4.2% (9/214) of children in the control group (p=0.005). No statistical difference was observed in the prevalence of malaria-helminth co-infection before and after intervention (p=0.26). Malaria parasitaemia was much higher in the control group than in the intervention arms (p=0.03). Children who received praziquantel and SMC drugs had a lower risk of developing severe anaemia than those who received SMC drugs alone. Integration of MDA for helminths with SMC drugs was safe and feasible among Senegalese children. These findings boost the public health recommendations for a paradigm shift from parallel, top-down disease control programs to integrated, locally relevant, evidence-based, and sustainable child health policies and their delivery.

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DEVELOPMENT AND PRE-TEST OF A RISK BENEFIT ASSESSMENT TOOL TO SUPPORT PROGRAMMATIC DECISION-MAKING REGARDING *PLASMODIUM VIVAX* RADICAL CURE TREATMENT OPTIONS IN LATIN AMERICA AND THE CARIBBEAN

Xue Wu[1], Sonia Herrera[1], Corey Rathe[1], Leopoldo Villegas[1], Angela Devine[2], Kamala Thriemer[3], Ivan Cardenas[4], Mario Olivera[5], Maria Paz Ade[6], Roberto Montoya[6], michelle Hsiang[1]

[1]U.S. President's Malaria Initiative Impact Malaria, Washington, DC, United States, [2]Menzies School of Health Research and Charles Darwin University, Darwin, Australia, [3]The University of Melbourne, Melbourne, Australia,

[4]Ministry of Health, Bogota, Colombia, [5]National Institute of Health, Bogota, Colombia, [6]Pan American Health Organization, Washington, DC, United States

In Latin America and the Caribbean, where *Plasmodium vivax* is the dominant malaria species, there are efficacy, adherence, and safety challenges associated with the radical cure options currently recommended (or implemented) in the region. New and/or alternative regimens include double dose PQ (7 mg/kg total dose over 14 days, ddPQ14), PQ administered over 8 weeks (6 mg/kg total administered weekly, PQ8wks) and tafenoquine (300mg single-dose, TQ, not yet recommended by the World Health Organization, but is being incorporated in Brazil). Different delivery strategies can also improve safety and effectiveness, including supervision of treatment, as well glucose-6-phosphate dehydrogenase (G6PD) rapid point-of-care testing to prevent G6PD deficiency-related hemolysis that can occur with any radical cure regimens. In consideration of these options, we developed a risk benefit assessment (RBA) tool to support decision-making regarding the selection of a safe and effective treatment scheme to use at national or subnational levels. The RBA tool requires inputs of the malaria epidemiological data (i.e., the number vivax cases, # relapses and/or recurrences episodes, # hemolytic events, etc.) from the national health information system. A decision tree approach is used to compare estimated number of recurrences for different radical cure treatment schemes. An interactive ShinyApp tool, in English or Spanish, https://ucsf-mei.shinyapps.io/RiskBenefitTool_en/ or https://ucsf-mei.shinyapps.io/RiskBenefitTool_es/, respectively, enables the user to provide input values regarding the local epidemiology of malaria, G6PD deficiency, and hemolytic risk. To inform how radical cure approaches can be tailored for local contexts, we share modeled results from low and high hemolytic risk settings in Colombia. A main limitation of the tool includes scarce data on hemolysis and recurrence risks associated with different treatment regimes. Nonetheless, the tool provides a framework upon which these variables and/or new data may be incorporated in the future.

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MALARIA HEALTHCARE SYSTEM OF PAKISTAN AMIDST CLIMATE CRISES: A SWOT ANALYSIS

Saba Siddiqui[1], Huma Syed Hussain[1], Abid Hasan[2], Momin Kazi[1], Najia Ghanchi[1], Javeria Samad[1], Muhammad Mukhtar[3], Altaf Ahmed Lal[4], Mohammad Asim Beg[1], Farah Naz Qamar[1]

[1]Aga Khan University Hospital, KARACHI, Pakistan, [2]Zenysis Technologies, KARACHI, Pakistan, [3]Directorate of Malaria Control, Islamabad, Pakistan, KARACHI, Pakistan, [4]Global Health and Pharmaceuticals, Atlanta, GA, United States

Pakistan continues to bear a heavy burden of malaria. By 2021, case incidence was reduced by 40% from 2015. The 2022 floods resulted in a fivefold increase in malaria cases, with 2.1 million new infections and damage to over 1000 health facilities. Sindh had 53.5% of flood-related cases, with 117,282 from Thatta district. About 28.9% of Pakistanis reside in malaria-prone areas, with P.vivax accounting for 80% of all malaria cases. This SWOT analysis of Pakistan's healthcare system focuses on critical issues that must be addressed to combat malaria.Our analysis identifies challenges in the healthcare system, such as data fragmentation, poor resource allocation, noncompliance with treatment guidelines, and drug shortages, mainly primaquine. Investing in the current infrastructure of primary care clinics, microscopy facilities, and health information systems is necessary. True disease burden measurement is critical for executing control strategies but is limited by insufficient training and lab facilities in endemic areas. By integrating health information systems with real-time monitoring, mHealth can enhance disease surveillance, optimize resource allocation, and alleviate drug shortages. Furthermore, electronic prescriptions can help assure treatment adherence. However, rising temperatures, political unrest, and unrestricted access to antimalarials pose a considerable threat. The approval of the malaria vaccine, together with global commitment to malaria elimination provides a chance to develop an advanced malaria care plan that uses tested and field-usable methods for ending malaria in Pakistan. To address malaria and climate-related health challenges, local and global partners must invest in monitoring and diagnostic infrastructure, training on treatment standards, and an electronic disease surveillance dashboard. This improves public health and resource allocation. Our analysis recommends stakeholders assist Thatta to become a model for malaria elimination strategies. In conclusion, climate issues in Pakistan necessitate urgent upgrading of all malaria control strategies to eliminate malaria by 2035.

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A SCOPING REVIEW OF PATIENT ADHERENCE TO ANTIMALARIAL DRUGS

Manash Shrestha[1], Phone S. Hein[2], Bijaya Shrestha[3], Kristin Banek[4], Caroline A. Lynch[1]

[1]Medicines for Malaria Venture (MMV), Geneva, Switzerland, [2]Asia Pacific Malaria Elimination Network (APMEN), Singapore, Singapore, [3]Center for Research on Education, Health and Social Science (CREHSS), Kathmandu, Nepal, [4]Institute for Global Health and Infectious Diseases, University of North Carolina, Chapel Hill, NC, United States

Patient adherence has been a constant global challenge for effective antimalarial treatment and prophylaxis. This scoping review was carried out to map out patient adherence to antimalarial drugs and explore recent evidence (2013-2023) from countries where malaria is still a public health problem. A comprehensive search was performed using PubMed, EMBASE, and CINAHL databases. Reference lists from the included studies were also hand-searched. Covidence software was used to conduct the different steps of this review. Three independent reviewers screened the title and abstracts, reviewed the full-texts, and extracted the data. In total, 132 studies were included in the final review, which comprised studies measuring patient adherence to falciparum antimalarials (n=42), vivax radical cure (n=15), both falciparum antimalarials and vivax radical cure (n=7), intermittent preventive treatment of malaria for pregnant women (n=23), mass drug administration (n=14), antimalarial travel chemoprophylaxis (n=21), and other forms of antimalarial chemoprophylaxis (n=10). Most studies were from the African region (82; 62.1%), followed by Asia-Pacific (24; 18.2%), North America (11; 8.3%) and Latin America (8; 6.1%). The adherence reported ranged from 4-100%, with a majority of studies reporting 50-90% adherence (67; 55.4%). Around half of the studies were observational (n=67), and patient self-reporting was the most-used technique to measure adherence, either through an interview (n=21), as a questionnaire (n=38), or combined with manual pill count (n=26) and other techniques (n=24). Only 15 studies (11.3%) reported any intervention to improve patient adherence, with SMS reminders (n=5), home visits or partial supervision (n=5), and drug packaging (n=2) being the major interventions, with relatively mixed success. These findings suggest that although there have been improvements in new products addressing patient adherence in the last decade, this remains a significant barrier. Effective interventions will require deeper consideration of underlying factors and practical challenges to bring about positive behavior change.

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NORMALIZED COMPONENTS OF HEALTH SYSTEMS STRENGTHENING IN DELIVERING MALARIA TREATMENT SERVICES: A 3-YEAR IMPLEMENTATION STUDY

Annisa Rahmalia[1], Liony Fransisca[2], Enny Kenangalem[2], Reynold R. Ubra[3], Faustina H. Burdam[2], Koen Peeters Grietens[4], Benedikt Ley[1], Ric N. Price[1], Jeanne R. Poespoprodjo[2]

[1]Menzies School of Health Research, Darwin, Australia, [2]Yayasan Pengembangan Kesehatan dan Masyarakat Papua (YPKMP), Timika, Indonesia, [3]Mimika Regency Health Office, Timika, Indonesia, [4]Institute of Tropical Medicine, Antwerp, Belgium

In Indonesia, the Papua provinces carry the highest malaria burden. Health systems strengthening activities were rolled out in an implementation study at five public health clinics in southern Papua to improve malaria case management:(1) providing patients with a unique identifier to identify recurrences, (2) monitoring artemisinin combination therapy (ACT) and primaquine doses accuracy, (3) observing the first doses of both antimalarial drugs, (4) patient education to motivate adherence,

and (5) home visits by community health workers (CHWs) to encourage treatment adherence. As part of the study, periodic continuous quality improvement (CQI) meetings were held with quantifiable targets set by the health workers and clinic managers themselves. Qualitative methods were used to study the processes of the implementation. The Normalization Process Theory (NPT) was used to assess the level of integration of the intervention into the system. Between October 2019 and August 2023, 93 participant observations, 11 focus group discussions (FGDs), and 68 interviews were conducted. The intervention includes setting up a desk called '*malaria corner*' at the clinics to deliver the activities. In the 3 years of implementation, health workers quickly integrated dose checking but observation of the first dose was hindered by scarcity of drinking water and snacks and a prolonged shortage of antimalarials in 2022. Adherence education was given to patients and their caregivers with varying degrees of detail. Existing CHWs delivery of adherence monitoring at home was improved by additionally providing them with incentives. While not all activities are normalized, several components have been modified and embedded into routine practice, although this was limited by personal and structural barriers and other programmatic activities. Our study highlights the complexities of strengthening health system, which is a crucial step in decreasing malaria transmission through better case management.

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TRACKING AND SCALING UP OF SERVICE PROVISION TO FOREST GOERS AND MOBILE AND MIGRANT POPULATIONS FOR CONTROLLING OF LOCAL MALARIA TRANSMISSION IN FORESTED AREAS OF MYANMAR

Kyaw Myint Tun[1], Khin Mon Mon[1], Hnin Su Su Khin[1], Theodora Meerkerk[1], Nu Nu Khin[2], Gunawardena Dissanayake[3], Jimee Hwang[4], Saw Naung Naung[1], Khant Maung Maung[1], Ersin Topcuoglu[5], Danielle Awabdeh[5]

[1]PMI Eliminate Malaria, University Research Co., LLC, Yangon, Myanmar, [2]U.S. President's Malaria Initiative, USAID, Yangon, Myanmar, [3]U.S. President's Malaria Initiative, USAID, Bangkok, Thailand, [4]U.S. President's Malaria Initiative, Malaria Branch, U.S. Centers for Disease Control and Prevention, Atlanta, GA, United States, [5]PMI Eliminate Malaria, University Research Co., LLC, Chevy Chase, MD, United States

The forest goers and the mobile and migrant populations (MMPs) in Myanmar are a risk group for malaria. The U.S. President's Malaria Initiative (PMI) in Myanmar funded University Research Co., LLC and its consortium of partners in collaboration with the National Malaria Control Program to improve access to these essential services among marginalized and at-risk groups, including forest goers and MMPs. We assessed the odds of positive malaria rapid diagnostic test (RDT) among forest goers and MMPs compared to static resident populations, considering the geographic area, programmatic coverage, age, sex, and provider type. This analysis used de-identified, programmatic malaria case data (from both passive and active detection) from January 2020 to December 2023 from 31 townships of Kayin and Rakhine States and the Tanintharyi Region for descriptive analyses, chi-square tests of association, and logistic regression to understand whether forest goers and MMPs were more vulnerable than the static resident populations to being infected with malaria. Overall, there were 864,159 tests and 40,029 positives, revealing a test positivity rate of 4.6%. The proportion of forest goers and MMPs among those tested in 2023 was 21.4%, which increased by 30% than those tested in 2020. Compared to static residents, forest goers, and MMPs were at a 9.7 times greater odds ratio (95% CI: 9.5-9.9) of positive RDT after controlling for other covariates, including sex, age group, provider type, and geographic areas. Stratified analyses showed that forest goers and MMPs had 10.0 times higher odds ratio (95% CI: 9.8-10.2) of positive RDT than those of static residents after adjusting sex and 11.0 times higher odds ratio (95% CI: 10.8-11.3) after adjusting age group. Among the migrants, males had a 2.0 times higher odds ratio than females (95% CI: 1.9-2.1). Findings support the importance of designing specific strategies to reach forest goers and MMPs with malaria services. Additionally, forest goers and MMP-specific services should be scaled up, and the reasons why male forest goers and MMPs were at higher risk than female forest goers and MMPs should be explored.

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USING A MULTI-PRONGED APPROACH TO TARGETING *PLASMODIUM VIVAX* TO SHARPLY REDUCE INCIDENCE

Sokomar Nguon[1], Samphornarann Top[1], Jeanne Rideout[1], Polo Chin[1], Phally Chhun[1], Say Mang[1], Rida Slot[2], Tyson Volkman[2], Ly Po[3], Siv Sovannaroth[3], Dysoley Lek[3], Tha Meas[3], Virak Khieu[3], Huy Rekol[3], Dy Khoy[4], Ke Kimmen[5]

[1]Cambodia Malaria Elimination Project 2, Phnom Penh, Cambodia, [2]PMI/USAID, Phnom Penh, Cambodia, [3]Center for Parasitology, Entomology and Malaria Control, Phnom Penh, Cambodia, [4]Pursat Provincial Health Department, Phnom Penh, Cambodia, [5]Provincial Health Department, Pursat, Cambodia

By the end of 2023, Cambodia had all but eliminated *Plasmodium falciparum (Pf);* from late October 2023 through March 2024, only 3 cases were recorded in the country, and the bulk of malaria cases are now due to *P. vivax (Pv)*. CNM and USAID/PMI through the CMEP2 Project applied lessons learned from *Pf* elimination efforts to develop a multi-pronged strategy to eliminate *Pv* in 14 operational districts in 6 Provinces. The strategy layered multiple activities to produce maximum effect: (i) To combat relapses, *Pv* radical treatment was implemented for *Pv* patients with normal Glucose 6 Phosphate Dehydrogenase (G6PD) test results. A 14-day primaquine (PQ) regimen initiated in early 2021 was shifted to a 7-day PQ regimen in mid-2023 to improve completion rates. In May 2023, CNM and CMEP2 began piloting an 8-week PQ regimen in 8 health facilities (HFs) in 2 ODs of Pursat Province for *Pv* patients with deficient and intermediate G6PD test results. (ii) Integrated Drug Efficacy Surveillance (iDES)has been implemented in 10 HFs in 3 ODs of Pursat and Battambang Provinces since the beginning of 2023. Every individual diagnosed with malaria is enrolled for comprehensive follow-up for either 42 days (*Pf, P. malariae [Pm], & P. knowlesi [Pk]*) or for 90 days (*Pv & P. ovale [Po]*). In 2023,100% of patients enrolled in iDES following *Pf/Pm/Pk* infection and 79% of those enrolled following *Pv* infection completed the full course of treatment. No one tested positive for malaria during the follow-up period. (iii) Preventive interventions targeting forest goers were established due to the elevated infection risk in this group secondary to exposure to higher transmission areas in forested areas. Through 24/7 stationary teams at identified potential entry/exit points, all forest goers were tested for malaria, and either treated if positive or given artesunate pyronaridine for 3 consecutive days as intermittent preventive treatment (IPT) if negative. As Cambodia works to achieve elimination of all malaria species by 2025, introducing and sustaining multi-pronged intervention efforts targeting specific populations will be important, particularly for the elimination of Pv.

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PRIVATE COMMUNITY PROVIDERS' EXPERIENCES ON *PLASMODIUM VIVAX* RADICAL CURE TOOLS: EVIDENCE FROM MYANMAR

May Me Thet[1], Khine Khine Saw[1], Moe Myint Oo[1], Zwe Thihaa Kyaw[2], Wah Wah Thaw[2], Khin Zarli Aye[2]

[1]Population Services International Myanmar, Yangon, Myanmar, [2]PATH Myanmar, Yangon, Myanmar

The Partnership for Vivax Elimination (PAVE) supports Myanmar's national malaria control program to achieve malaria elimination goals, focusing on effective Plasmodium vivax case management. PAVE developed a new set of tools including Standard Operating Procedures, Counseling Form, and Directly Observed Treatment (DOT) form to enhance P. vivax treatment. Training on these tools was provided to 420 private community malaria providers across Mandalay, Kachin, Mon and Shan regions in 2022. A qualitative assessment, involving 30 in-depth interviews, assessed providers' experiences with the new tools. Data were analyzed using thematic analysis. Findings indicated that most providers could effectively utilize the forms, although some initially faced challenges, often resolved with supervisor assistance. Providers perceived that the forms ensured proper Primaquine dosing and follow-up visits, aligning with National Treatment Guidelines. Challenges to having four follow-up visits included

transportation difficulties and distance, resulting in some providers resorting to phone calls instead of physical visits. Adherence to urine collection in the initial treatment phase was generally high, but declined over time. Patients and providers lacked clarity regarding sulfur-containing medications. However, providers recognized numerous benefits of using the Counseling Form, including improved medication adherence and understanding of treatment duration and side effects. Feedback on the DOT form was positive, with providers highlighting its role in preventing missed doses and assisting patients' understanding of medication regimens. Suggestions for improvement included developing a manual to ensure consistency in providers' form completion. The study highlights private providers' favorability of new tools for managing P. vivax with Primaquine, indicating potential benefits. It underscored the importance of continued support and refinement, such as developing comprehensive manuals for consistent form utilization. This research signifies progress towards effective malaria elimination strategies in Myanmar.

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ADVANCING MALARIA ELIMINATION IN BRAZIL: PRIORITIES AND STRATEGIES FOR RESEARCH AND ACTION

Cassio Peterka[1], Marcela Dourado[2], Djane Baia[3], Pablo Fontoura[2]
[1]Amapá State Government, Macapá, Brazil, [2]Ministry of Health, Brasilia, Brazil, [3]University of the Amazonas State, Manaus, Brazil

Malaria constitutes a notable public health challenge in Brazil, particularly in the Amazon region, which accounts for 99% of reported autochthonous cases. In 2022, Brazil initiated the National Malaria Elimination Plan. Subsequently, in June 2023, the Brazilian MoH convened a meeting involving members of the scientific academy and state representatives to deliberate on and establish research priorities concerning malaria. This initiative underscores our unwavering dedication to eradicating this disease. The creation of the Research Priorities Matrix aimed at identifying primary knowledge gaps and allocating resources toward research endeavors supportive of malaria elimination efforts. Prioritization of technological advancements in case surveillance, encompassing data analysis and predictive modeling, holds paramount importance in expediting the detection and timely treatment of Plasmodium infections across diverse transmission settings. By focusing on these pivotal domains, we can ensure targeted and efficacious endeavors toward achieving our objective of malaria elimination. Consequently, there will be enhanced precision in detecting parasite reservoirs, including asymptomatic and subpatent infections, particularly in regions striving for malaria elimination. Furthermore, advancements in diagnostic techniques and novel therapeutic approaches will aid in mitigating relapses attributed to *Plasmodium vivax*, the species accountable for roughly 85% of malaria cases in the country. The development of novel tools for entomological surveillance necessitates efficacious management of Anopheles vectors, including the evaluation of resistance to presently utilized insecticides. The endeavor to eliminate malaria in Brazil represents a viable and sustained strategy contingent upon substantial political contributions and reinforced commitments among federal entities. Therefore, the delineation of research priorities assumes critical significance in guiding concerted actions toward malaria elimination in Brazil, ensuring effective allocation of financing and resources to support these endeavors.

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HEALTH EDUCATION FOR MALARIA ELIMINATION IN BRAZIL - MALARIA LEADERSHIP COURSE

Pablo Secato Fontoura[1], Renata Castro Barros[1], Grace Godim Mafra[2], Marcela Lima Dourado[1], Cassio Roberto Leonel Peterka[3], Alexander Vargas[1], Cláudia Souza Ferreira Martins[2]
[1]National Malaria Control Program, Ministry of Health of Brazil, Brasília, Brazil, [2]Oswaldo Cruz Foundation, Rio de Janeiro, Brazil, [3]Superintendência de Vigilância em Saúde, Macapá, Brazil

In Brazil, despite the significant reduction in cases over the last decade, malaria is an example of partially successful elimination, mainly due to the spatio-temporal fluctuation cases malaria associated with the weakening of malaria health services in states and municipalities. In this context, it was proposed a course to train leaders to fight against malaria - *Leadership Course to Malaria Control and Elimination*. The primary objective of this course is to strengthen local health services by multidisciplinary training health professionals who work directly to malaria elimination in the Brazilian Legal Amazon. The training courses will be held in 9 states of the Brazilian Amazon organized by the National Malaria Control Program in association with representatives of the malaria research institutions. The methodology consists of problematizing heterogeneous malaria transmission scenarios in terms of management, policy, surveillance, diagnosis and treatment and health education to malaria elimination. To date, two malaria leadership courses have been held. These courses were attended by Municipal Supporters for Malaria Prevention, Control and Elimination from 34 Amazonian municipalities with a high malaria burden in Brazil, and 63 health professionals representing the states of the Brazilian Amazon that are close to malaria elimination - Maranhão, Mato Grosso and Tocantins. The average age of the participants was 39 years old (range: 29 to 69 years), with 53.4% of the participants being female. Around 81.8% of the participants had a university degree, with 64 (72.8%) having completed at least one postgraduate course. There was a homogeneous participation of health professionals working in malaria management, diagnosis and treatment and entomological surveillance and vector control. As a final evaluation of the participants, all of them reported that they would use the knowledge acquired during the courses in their work routine in the fight against malaria. It is expected that this course provides technical and scientific knowledge to develop sustainable and feasible local actions to support malaria elimination in Brazil.

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COVERAGE AND IMPACT OF A PROGRAMMATIC MASS DRUG ADMINISTRATION CAMPAIGN FOR MALARIA IN SOUTHERN MOZAMBIQUE USING ROUTINE SURVEILLANCE DATA

Maria Tusell[1], Arsénio Nhacolo[2], Laura Fuente-Soro[1], Jacopo Vecchio[1], Amancio Nhangave[3], Khalid Bapu[2], Felimone Amone[2], Orcídio Chivite[4], Nteruma Catorze[5], Valencia Novele[5], Wilson Simone[2], Ismail Chiposse[3], Alfa Moiane[3], Alberto Gomo[4], Odety Chissico[4], Donaldo Artur[4], Mercia Dimene[6], Samira Sibindy[6], Baltazar Candrinho[6], Pedro Aide[2], Caterina Guinovart[1]
[1]Barcelona Institute for Global Health (ISGlobal), Barcelona, Spain, [2]Manhiça Health Research Centre, Manhiça, Mozambique, [3]Gaza Provincial Directorate of Health, Xai-Xai, Mozambique, [4]Manjacaze District Directorate of Health, Manjacaze, Mozambique, [5]Zavala District Directorate of Health, Zavala, Mozambique, [6]National Malaria Control Program, Ministry of Health, Maputo, Mozambique

Between December 2022-February 2023, a programmatic Mass Drug Administration (pMDA) was implemented in Mozambique by the National Malaria Control Program in the administrative post of Chidenguele (Manjacaze district, Gaza province), where the estimated population according to administrative data is 59,271. All eligible individuals aged ≥6 months were targeted to receive two rounds of the antimalarial dihydroartemisinin-piperaquine. This study evaluates the coverage of the pMDA and its impact on malaria incidence. Coverage was estimated using programmatic data collected during the pMDA. Impact was evaluated

through a controlled interrupted time series analysis using selected health facility catchment areas from the neighbouring district of Zavala (Inhambane province) as the comparison area. We assessed whether there was a change in malaria incidence after the introduction of the intervention in the pMDA versus the comparison group and whether there was a change in the malaria trend over time after the intervention compared to the pre-intervention period in the pMDA versus the comparison group. Negative binomial regression was performed, using monthly malaria case counts from the routine surveillance system as the dependent variable and adjusting for confounders. Population coverage (individuals reached [absent+present]/total population) was 63.4% in round 1 (R1), and increased to 92.0% in round 2 (R2) after optimising the delivery strategy based on lessons learnt in R1. Programmatic coverage (individuals treated/target population) increased from 41.2% in R1 to 69.7% in R2. Absences and exclusions in R2 accounted for 8.7% and 6.9% of the target population, respectively, whereas 6.7% refused to participate and 8.0% were not reached. Preliminary results from the impact evaluation will be available to be presented at the Meeting. Reaching 80% programmatic coverage, as recommended by WHO, is difficult even with high rates of population coverage, mainly due to absences and exclusion criteria. Routine data is an important source of information that can be used to evaluate the impact of interventions implemented programmatically.

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ENHANCING THE DESIGN OF RANDOMIZED TRIALS IN MALARIA ELIMINATION SETTINGS: A SIMULATION STUDY OF THE RING TRIAL DESIGN

Anna T. Nguyen[1], Arusha Patil[1], Davis Mumbengegwi[2], Michelle S. Hsiang[3], Jade Benjamin-Chung[1]

[1]Stanford University, Stanford, CA, United States, [2]University of Namibia, Windhoek, Namibia, [3]University of California, San Francisco, San Francisco, CA, United States

In malaria elimination settings, chemoprevention and vector control interventions are often reactive and focal: they target people who live in "rings" around index cases. Usually, cluster-randomized trials (CRTs) are used to evaluate ring interventions, but they may have low statistical power due to strong spatiotemporal clustering and low incidence in elimination settings. An alternative design that may have higher statistical power is the ring trial design, which was used to evaluate the Ebola vaccine but has not yet been used for malaria interventions. This design randomizes rings as index cases are detected. We compared statistical power of cluster-randomized and ring trials in a simulation study. We fit cluster-level malaria hazard functions using data from a trial conducted in a setting with low malaria incidence (50-100 cases per 1000 person-years) and strong spatiotemporal clustering in Namibia (NCT02610400). We simulated a ring intervention delivered within 500 meters of index cases in the intervention arm. In CRTs we randomized 56 administrative areas as clusters at baseline. In ring trials, we randomized rings as index cases occurred. For each design, we estimated intention-to-treat effects with robust standard errors and statistical power in 1,000 simulated trials. We examined different levels of intervention effectiveness, spillover effects, baseline incidence, spatial clustering, and treatment coverage. For all baseline incidence levels, ring trials had statistical power greater than or equal to CRTs. In transmission settings with incidence of 50 per 1,000 person-years, CRTs were powered at 80% to detect a minimum reduction in incidence of 60%, while ring trials were powered to detect a minimum reduction of 44%. For a lower effect size where incidence was reduced by 30%, power in both trial designs was less than 80% but power was 25.5% higher in the ring trial (54.5%) vs CRT (29.0%). CRTs had lower false positive rates than ring trials with larger differences at high baseline incidence. Our findings suggest that ring trials are a promising alternative to CRTs for evaluating ring interventions in malaria elimination settings.

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STRATEGIES TO ACHIEVED *PLASMODIUM FALCIPARUM* ELIMINATION IN CAMBODIA: PRACTICAL APPROACHES AND FINDINGS

Sovannaroth Siv[1], Rafael Jairah Jr. Matoy[2], Alexa M. Kugler[2], Elijah Filip[2], Bunmeng Chhun[2], Rekol Huy[1]

[1]National Centre for Parasitology, Entomology and Malaria Control, Phnom Penh, Cambodia, [2]Clinton Health Access Initiative, Phnom Penh, Cambodia

In 2018 there were 18,458 *Plasmodium falciparum* cases in Cambodia. With World Health Organization support, Cambodia began implementing targeted strategies to interrupt remaining transmission and reach *P. falciparum* elimination. Strategies were implemented in a phased approach: Intensification Plan 1 (IP1, Oct 2018 to Oct 2019), Intensification Plan 2 (IP2, Nov 2019 to Dec 2020), and Last Mile (LM, Dec 2020 to Dec 2023). The Village Malaria Worker (VMW) program was reactivated in 2018 after four years of disruption. As a result, testing increased by 15% between 2017 and 2018 (213,585 to 245,563). VMW testing constituted 55% of all tests in 2018, and the number of cases decreased by 31%, from 27,077 to 18,458. IP1 was launched in 2018 and included the 30 health center catchment areas (HFCAs) with the greatest malaria burden. During IP1, 4,725 P. falciparum cases were detected. In 2019, IP2 expanded to 36 HFCAs. During IP2, 1,092 P. falciparum cases were detected. A Controlled Interrupted Time Series (CITS) analysis demonstrated an accelerated decline in cases in IP1 and IP2 areas compared to non-IP areas. In 2021, LM was implemented to ensure each locally acquired P. falciparum case triggered a comprehensive investigation and tailored response. By 2023, 185 villages implemented full or partial LM. An analysis of the impact of LM showed the largest reduction in total cases in LM areas compared to partial and non-LM areas. In 2023, there were 34 cases nationwide and only 3 cases reported in the 4th quarter, a 99% case reduction in 5 years. From 2018-2023, there was a significant increase in malaria testing alongside a dramatic reduction in *P. falciparum* cases. The strategies of each elimination stage helped the country reduce the number of *P. falciparum* malaria cases to a negligible number, paving the way to malaria elimination.

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IMPROVING ADHERENCE AND RADICAL CURE OVERAGE FOR *PLASMODIUM VIVAX* THROUGH THE IMPLEMENTATION OF G6PD TESTING AT POINTS OF CARE IN CAMBODIA

Desoley Lek[1], Samphor Narann Top[2], Jayme Hughes[3], Sokomar Nguon[2], Sovann Peng[4], Bunmeng Chhun[3], Hok Kry[1], Bun Sour[1], Koung Lo[1], Vannak Keo[5], Sles You[1], Voeurng Bunreth[1], Rafael Jairah Jr. Matoy[3], Elijah Filip[3], Rekol Huy[1]

[1]National Centre for Parasitology, Entomology and Malaria Control, Phnom Penh, Cambodia, [2]University Research Co, Cambodia, Phnom Penh, Cambodia, [3]Clinton Health Access Initiative, Phnom Penh, Cambodia, [4]Catholic Relief Services, Cambodia, Phnom Penh, Cambodia, [5]FHI360 Cambodia, Phnom Penh, Cambodia

Cambodia aims to eliminate malaria by 2025. Following a 99% drop in *Plasmodium falciparum* (Pf) cases since 2018, *P. vivax* (Pv) infections now represent 95% of the 1,384 cases in 2023. Unlike Pf, Pv has a hypnozoite stage that requires radical cure with primaquine (PQ) to prevent relapse and achieve elimination. Due to the possible life-threatening side effects of PQ in G6PD-deficient patients, the WHO recommends G6PD testing to guide the administration of PQ. In 2020, the National Center for Parasitology Entomology and Malaria Control (CNM) enrolled males with mono or mixed Pv weighing ≥20 kgs in a radical cure pilot program in 88 health facilities (HF) across 4 provinces over 13 months. The pilot aimed to assess the feasibility of implementing qualitative point-of-care G6PD testing and a 14-day PQ scheme. Among 2,861 enrolled patients, 1,281 (45%) were G6PD tested, 959 (33.5%) had normal G6PD activity and received PQ, though just 78% completed treatment. HF staff also reported difficulty completing referrals for G6PD testing due to the high costs and time associated with travel to the HF. In 2021, CNM expanded the radical cure program to 324

HFs in 21 provinces and switched to quantitative G6PD tests so non-pregnant/breastfeeding females were now eligible. To address the gaps from the pilot, CNM introduced referral incentives for CHWs to accompany patients to HFs in 2022 and transitioned from a 14- to 7-day PQ scheme in 2023. As a result, 60% (678/1,129) of all eligible patients initiated radical cure in 2023. The proportion of patients successfully referred to HF increased from 42% in 2021 to 88% in 2023 while adherence increased from 87% to 96%. However, some coverage gaps remain, including 187 patients who were not G6PD tested and 264 (28%) G6PD-deficient patients who were ineligible for the 7-day PQ scheme. In response, CNM piloted 8-week PQ radical cure (0.75 mg/kg target dose) for deficient or intermediate patients and plans to implement this nationwide in 2024. Cambodia's experience demonstrates the feasibility of implementing successful radical cure strategies and serves as an example for other countries striving for malaria elimination.

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THE ACCEPTABILITY AND FEASIBILITY OF ROUTINE CLINICAL FOLLOW-UP FOR SHORT COURSE RADICAL CURE TREATMENT FOR *PLASMODIUM VIVAX* MALARIA: A COMPARATIVE ANALYSIS OF CAMBODIA AND ETHIOPIA

Sarah Cassidy-Seyoum[1], **Muthoni Mwaura**[1], Keoratha Chheng[2], Muhaba Kejela[3], Kansite Korra[3], Tsegabehan Wodaj[4], Agnes Meershoek[5], Tamiru S. Degaga[1], Lorenz von Seidlein[2], Rupam Tripura[2], Bipin Adhikari[2], Benedikt Ley[1], Dysoley Lek[6], Ric N. Price[1], Nora Engel[5], Kamala Ley-Thriemer[1]

[1]Menzies School of Health Research, Casuarina, Australia, [2]Mahidol Oxford Tropical Medicine Research Unit, Bankok, Thailand, [3]Department of Sociology and Social Anthropology, Arba Minch University, Arba Minch, Ethiopia, [4]Department of English Language and Literature, Arba Minch University, Arba Minch, Ethiopia, [5]Care and Public Health Research Institute, Maastricht University, Maastricht, Netherlands, [6]National Center for Parasitology, Entomology and Malaria Control, Phnom Penh, Cambodia

Plasmodium vivax malaria requires a combination of drugs targeting both the blood and liver stages (hypnozoites) of the parasite (radical cure). The only available antimalarial drugs to clear hypnozoites are primaquine and tafenoquine, which can cause hemolysis in glucose-6-phosphate dehydrogenase-deficient patients. Although higher, shorter doses of primaquine have been proven more effective, they also increase the risk of hemolysis. Therefore, to support safety and improve adherence to a complete course of treatment, a clinical review three days after commencing primaquine has been proposed. It is unclear what type of clinical or laboratory-based assessments are acceptable and feasible for health providers and patients in different contexts and how such follow-up visits can be integrated into routine malaria care. Between March and September 2023 we conducted focus group discussions (FGDs) with *P. vivax* malaria patients and healthcare providers. The FGDs incorporated an interactive visual exercise for participants to explore and co-design a suitable day 3 review for their setting. Ten FGDs were completed in three health facility catchment areas in Pursat, Cambodia and six FGDs in four health facility catchment areas in Arba Minch, Ethiopia (total of 57 participants). Preliminary results suggest that distance, infrastructure, continuity of care, workload, and quality of care impact upon perceptions of where and how the follow up should be conducted. Collaboration across health care levels and the involvement of community health workers in monitoring as well as community engagement and sensitization regardless of chosen location were regarded as critically important. Selected procedures for the review were shaped by participants' perceptions of shorter, higher primaquine doses and how respondents understood the day 3 review visit, capacity, and costs to patients and health system. The findings and methodological approach outlined will inform the design and implementation of suitable follow-up strategies to increase safe delivery of novel radical cure options in different epidemiological and health system contexts.

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IMPLEMENTATION OUTCOMES OF 1-3-7 FOCUS INVESTIGATION FOR MALARIA IN A LOW TRANSMISSION SETTING IN SOUTHERN PROVINCE, ZAMBIA: A MIXED METHODS STUDY

Anne C. Martin[1], Japhet Matoba[2], Caison Sing'anga[2], Mukuma Lubinda[2], Sydney Mweeta[2], Xinyue Chen[3], Ignatius Banda[4], Busiku Hamainza[4], Harry Hamapumbu[2], Edgar Simulundu[2], William J. Moss[1]

[1]Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [2]Macha Research Trust, Choma, Zambia, [3]Department of International Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [4]National Malaria Elimination Centre, Zambia Ministry of Health, Lusaka, Zambia

Since 2016, eleven countries have been certified as malaria free, but none of these are in continental Africa, where elimination challenges are unique. The 1-3-7 surveillance approach requires case reporting, case investigation/classification, and focal classification/responsive follow-up to be completed one, three, and seven days, respectively, after index case diagnosis. This approach is not dissimilar from reactive case detection as done in Zambia today, but it adds real-time short-messaging-service (SMS) reporting for each malaria case at each step (rather than weekly reporting) and with it, layers of monitoring, accountability, and data transparency. China, Thailand, Myanmar, and other countries deploy 1-3-7, citing high fidelity to deadlines and broad acceptability, yet 1-3-7 has yet to be widely deployed in Africa. This mixed-methods study evaluated implementation and service outcomes of 1-3-7 in a rural area of Choma District Zambia. Select outcomes were fidelity, feasibility, acceptability, efficiency, and equity. These were assessed through quantitative analysis of program metadata and qualitative analysis of semi-structured interviews with program personnel. Barriers and enablers to success and potential scale-up were also assessed. Fidelity was moderate with 61% of cases being reported. Network coverage was a common challenge to feasibility that likely affected reporting rates and fidelity. A large portion (64%) of cases diagnosed at the health facility were not eligible for follow-up per 1-3-7 criteria. Distance from the health center was a key barrier to feasibility and equitable reach of services. Reporting times were faster in areas where transmission was thought to be higher and slower in areas with poor network coverage. The program was widely accepted, and these results provided nuanced guidance to make modifications for 1-3-7 ahead of future scale-up.

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TRAVELERS WITH SUBMICROSCOPIC *PLASMODIUM SPP*. INFECTIONS CONTRIBUTE TO MAINTAIN RESIDUAL MALARIA IN TWO RURAL COMMUNITIES FROM THE PERUVIAN AMAZON REGION

Roberson Ramirez[1], Pamela Rodríguez[2], Ananias A. Escalante[3], Joseph M. Vinetz[4], Katherine Torres[5], Dionicia Gamboa[6]

[1]Amazonian International Center of Excellence for Malaria Research, Laboratorios de Investigación y Desarrollo, Facultad de Ciencias e Ingeniería, Universidad Peruana Cayetano Heredia, Lima, Peru, [2]Laboratorio de Malaria: Parásitos y Vectores, Laboratorios de Investigación y Desarrollo, Facultad de Ciencias e Ingeniería, Universidad Peruana Cayetano Heredia., Lima, Peru, [3]Institute for Genomics and Evolutionary Medicine (IGEM), Temple University., Philadelphia, PA, United States, [4]Amazonian International Center of Excellence for Malaria Research, Laboratorios de Investigación y Desarrollo, Facultad de Ciencias e Ingeniería, Lima, Peru. Section of Infectious Diseases, Department of Internal Medicine, Yale School of Medicine., New Haven., CT, United States, [5]Amazonian International Center of Excellence for Malaria Research, Laboratorios de Investigación y Desarrollo, Facultad de Ciencias e Ingeniería, Universidad Peruana Cayetano Heredia, Instituto de Medicina Tropical "Alexander von Humboldt"., Lima, Peru, [6]Amazonian International Center of Excellence for Malaria Research. Laboratorios de Investigación y Desarrollo, Facultad de Ciencias e Ingeniería, Universidad Peruana Cayetano Heredia., Lima, Peru

The movement of parasites through human mobilization is an important challenge to malaria elimination programs worldwide. This study aims

to identify malaria parasites in followed-up travelers going in and out of two rural communities in the Peruvian Amazon region to understand their contribution to maintaining residual malaria. Weekly active and passive case detections, as well as screening populations, were performed from February to August 2021 and 2022 in Libertad and Urcomiraño communities in Mazan district. Whole blood samples were collected for microscopy and qPCR diagnostics. A questionnaire specifically designed for travelers was applied to gather information about travel duration and reasons, visited communities, and other relevant sociodemographic information. From 678 people included in the study, 21.2% (144/678) traveled from Libertad and Urcomiraño to other communities in the last month. 119 malaria cases were detected during the study period, 89% (106/119) were submicroscopic infections, and 21.0% (25/119) were detected in travelers. Human mobilization was heterogeneous, and strong connectivity with other communities and creeks was found in the social network analysis; Mazan (out-degree=80) was the most frequent destination. Family affairs (40%) were the principal reason for traveling. Multilevel logistic regression analysis using balancing methods showed that male individuals over the age of 18 with travel history in the last month were the risk factors associated with malaria (p-value<0.001). These results show that human mobilization has an important role in the maintenance of residual malaria, and they will become the reservoirs of transmission and a challenge for the current elimination plan in the Peruvian Amazon.

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INCREASING CERTAINTY AROUND IMPACT OF SEASONAL MALARIA CHEMOPREVENTION: A MODELING FRAMEWORK USING ROUTINE DATA SOURCES IN BURKINA FASO

Monica Anna de Cola[1], Benoit Sawadogo[2], Cheick Campaore[2], Chuks Nnaji[3], Sidzabda Kompaore[4], Arantxa Roca-Feltrer[5], Sol Richardson[6], Christian Rassi[3], Patrick Walker[1], Lucy C. Okell[1]

[1]Imperial College London, London, United Kingdom, [2]Malaria Consortium, Burkina Faso, Ouagadougou, Burkina Faso, [3]Malaria Consortium, London, United Kingdom, [4]Ministry of Health, Burkina Faso, Ouagadougou, Burkina Faso, [5]PATH, Mozambique, Maputo, Mozambique, [6]Tsinghua University, Beijing, China

Evidence from randomized controlled trials has demonstrated that Seasonal Malaria Chemoprevention (SMC) can prevent around 75% of malaria cases. However, there has been an increase in cases reported at health facilities in Burkina Faso since SMC implementation. This could indicate changes in treatment seeking or reporting, or that SMC is not achieving the desired effect. We developed a framework utilizing a mathematical model to assess whether impact is consistent over time, across different metrics, and among various population subgroups. We calibrated the Imperial College transmission model to slide prevalence in 65 districts in Burkina Faso (household surveys 2010-2018) using maximum likelihood. Included in the model were rainfall, net use, and treatment. We allowed for increases in treatment seeking by using the total consultation rate for all illness in the dry season when there are negligible malaria cases. We found that children in districts with SMC administration had significantly lower odds of prevalent malaria infection during the SMC protective period (OR 0.38, 95% CI 0.29-0.52, p<0.001) and remained lower for up to two months post-SMC. Modeled prevalence estimates by district aligned with prevalence data, indicating the anticipated impact of SMC at 70% coverage (Spearman correlation coefficient: r=0.66, p>0.001; r=0.76, p>0.001; r=0.60, p>0.001; in 2010, 2014, 2017, respectively). Health facility case data increased in total over time but the proportion of cases in children under-five years old who receive SMC decreased when compared to those aged 5-14, who do not receive SMC. The decrease in the proportion of cases under-five coincided with the introduction of SMC at different years in different districts (2014-2019). These findings correlate with modeled predictions (r = 0.60, p>0.001). We found evidence of seasonal variation in the proportion of cases under-five pre-SMC, that might obscure impact of SMC. We were able to replicate these patterns by including non-malarial fevers that would be counted as cases at the health facility due to incidental asymptomatic parasitaemia. Estimated cases averted will be presented.

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MOLECULAR INVESTIGATION OF RECURRENT *PLASMODIUM MALARIAE* INFECTION IN THE DEMOCRATIC REPUBLIC OF THE CONGO

Wenqiao He[1], Rachel Sendor[2], Melchior Kashamuka[3], Kristin Banek[3], Joseph Losoma Atibu[3], Zachary R. Popkin-Hall[1], Jonathan J. Juliano[1], Antoinette Tshefu[3], Jonathan B. Parr[1]

[1]Division of Infectious Diseases and Institute for Global Health and Infectious Diseases, University of North Carolina at Chapel Hill, Chapel Hill, NC, United States, [2]Department of Epidemiology, Gillings School of Global Public Health, Chapel Hill, NC, United States, [3]Kinshasa School of Public Health, Kinshasa, Congo, Democratic Republic of the

Plasmodium malariae is a neglected malaria parasite associated with clinically significant symptoms. Recent surveys confirm wide distribution of *P. malariae* across African countries where it often co-circulates with *P. falciparum.* Persistent *P. malariae* infection has been reported decades after initial confirmed infection. In this study, we are developing a multilocus sequencing approach to differentiate *P. malariae* re-infection from persistent infection in a longitudinal study conducted from 2015 to 2017 in Kinshasa, Democratic Republic of the Congo (DRC). Among 9,089 samples from 1,565 participants, 58 participants had recurrent *P. malariae* infections, comprising 131 *P. malariae* infections. Six *P. malariae* infections identified among six individuals from the same health area were selected. We Sanger sequenced partial *P. malariae csp* and *ama1* genes in four samples, then amplified and sequenced six *P. malariae* microsatellite loci in three samples using Oxford Nanopore MinION platform. Sequencing data was analyzed using an in-house pipeline for base-calling, filtering, demultiplexing, trimming, alignment, consensus sequence extraction, and determination of microsatellite repeat number. *Csp* and *ama1* sequencing showed 54 and 3 single-nucleotide differences, respectively, between the four samples tested; *csp* repeat analysis is ongoing. Microsatellite analysis confirmed distinct repeat numbers in three of six loci between all three samples tested. Optimization of a pooled *csp* and microsatellite Nanopore sequencing approach and analysis pipeline is underway. We will evaluate the assay performance by comparing results to Illumina whole-genome sequencing data generated from 13 infections derived from 5 participants. After methods optimization, we will apply it to all *P. malariae* samples. Our preliminary findings suggest that *csp* and microsatellites can be used to differentiate re-infection from relapse within one health area of Kinshasa, DRC. Combining sequencing results with available clinical and demographic data, we aim to improve our understanding of recurrent *P. malariae* infections in the DRC.

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MATHEMATICAL MODELING TO OPTIMIZE THE COHORT MONITORING TO ESTIMATE INCIDENCE RATE AND TIME TO FIRST INFECTION IN MALARIA-ENDEMIC SETTINGS

Yura K. Ko[1], Wataru Kagaya[2], Jesse Gitaka[3], Chim W. Chan[4], Bernard N. Kanoi[3], Akira Kaneko[1]

[1]Karolinska Institutet, Solna, Sweden, [2]Nagasaki University, Nagasaki, Japan, [3]Mount Kenya University, Thika, Kenya, [4]Osaka Metropolitan University, Osaka, Japan

It is important to evaluate the effectiveness of interventions against malaria. Key epidemiological endpoints are incidence rate and time to first malaria infection, typically estimated through longitudinal cohort study. However, the methods used to evaluate the endpoints are not standardized, such as the frequency of monitoring and the type of tests. We used the Imperial malaria transmission model to simulate different scenarios in malaria endemic settings (Entomological Inoculation Rate [EIR] = 10). Assuming a 30% reduction in an individual's EIR by intervention in a closed population of 10,000 with 10% intervention coverage, we estimated protective effectiveness (PE) defined as relative risk reduction by comparing cumulative incidence rates and time to first infection between intervention and control groups over 18 months post-intervention. We explored four scenarios with different visit intervals (7-, 14-, 28-, and 60-day intervals) for active

case detection alongside passive case detection, where participants seek healthcare upon developing symptoms. Through 100 simulations for each scenario, we observed that the cumulative incidence of infection decreased with longer visit intervals for both rapid diagnostic tests (RDT) and polymerase chain reaction (PCR). However, the estimated PEs did not vary across different visit interval scenarios. The median estimated PEs were 0.14, 0.13, 0.16, and 0.16 by RDT and 0.12, 0.12, 0.14, and 0.14 by PCR among 7-, 14-, 28-, and 60-day intervals, respectively, in terms of cumulative incidence. For the outcome of time to first infection, the median estimated PEs were 0.11, 0.14, 0.14, and 0.14 for RDT and 0.11, 0.15, 0.15, and 0.15 for PCR, respectively, for the intervals. Our results suggest that intensive monitoring, such as weekly or biweekly visits, may not always be necessary for evaluating malaria interventions. The results likely depend on the sample size and transmission intensity of the target population. Investigators should optimize cohort monitoring approaches during the study design phase, taking logistical aspects into account in field settings.

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ASSESSING ENTOMOLOGICAL MEASURES OF INDIVIDUAL *PLASMODIUM FALCIPARUM* INFECTION RISK

Max McClure[1], Emmanuel Arinaitwe[2], Moses R. Kamya[2], Philip J. Rosenthal[1], Joaniter Nankabirwa[2], Maxwell Kilama[2], Grant Dorsey[1], Bryan Greenhouse[1], Isabel Rodriguez-Barraquer[1]

[1]UCSF, San Francisco, CA, United States, [2]Infectious Diseases Research Collaboration (IDRC), Kampala, Uganda

While the aggregated entomological inoculation rate (EIR) is often associated with parasite prevalence at the population level, it is unclear whether mosquito data collected at finer spatial and temporal scales reflect heterogeneity in infection risk. With data from a longitudinal cohort that includes passive and active surveillance of participants and household-level mosquito captures and sporozoite rates (SR), we tested associations between multiple spatiotemporal models of EIR and individual risk of P. falciparum infection. We used data collected from 454 children aged 0.5-5 years in 244 households between 2011-2017 in three Ugandan districts: low-EIR Jinja, intermediate-EIR Kanungu and high-EIR Tororo. Vector densities and SRs were assessed monthly in each household using CDC light traps. Modeling each site separately, we predicted the distribution of EIRs at each household using combinations of spatial, temporal and spatiotemporal smooths of each entomological metric. We then assessed the association between predicted household-level EIR and individual malaria incidence using Poisson generalized additive mixed effects models. Data from Jinja were fit better (AIC) by a model that used fine-scale estimates of vector density and averaged SR temporally over the study period, while data from Kanungu and Tororo were fit better by a model that used fine-scale estimates of SR and averaged vector density temporally. The EIR-incidence relationship varied between sites, with positive associations in the Kanungu (IRR 1.85, 95% CI 1.45-2.35) and Jinja models (IRR 1.68, 95% CI 1.28-2.20) and no association in the Tororo model (IRR 1.25, 95% CI 0.95-1.65). These results show the relationship between EIR and malaria incidence may depend on local transmission dynamics and be strongest at intermediate-EIR sites. They also underscore the challenges of using individual captures to estimate individual malaria exposure.

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SPACE-TIME SENSITIVE MODELING OF SUBCLINICAL MALARIA PREVALENCE AT THE VILLAGE LEVEL IN LOW ENDEMIC AREAS OF MYANMAR USING RANDOM FOREST MODEL

Poe Poe Aung[1], Zaw Win Thein[2], Milena Pavlova[3], Wim Groot[3], Regien Biesma[4], Thura Htay[2], Zin Min Tun Tun[2], Aye Kyawt Paing[2], Kay Thwe Han[5], Nay Yi Yi Lin[6], Zaw Lin[6], Varada Shevade[7], Tatiana Loboda[7]

[1]Malaria Consortium, Bangkok, Thailand, [2]Duke Global Health Institute, Myanmar Program, Duke University, Yangon, Myanmar, [3]1Department of Health Services Research, CAPHRI, Maastricht University Medical Center, Faculty of Health, Medicine and Life Sciences, Maastricht University, Maastricht, Netherlands, [4]Global Health Unit, Department of Health Sciences, University Medical Center Groningen, Groningen, Netherlands, [5]Department of Medical Research, Ministry of Health, Yangon, Myanmar, [6]National Malaria Control Program, Ministry of Health, Nay Pyi Taw, Myanmar, [7]Department of Geographical Sciences, University of Maryland, Baltimore College Park, Baltimore, MD, United States

Malaria elimination progress in Myanmar has slowed down in recent years. Predicting malaria risks helps identifying targeted strategies in resource limited setting. This study estimated the temporal variation in subclinical malaria prevalence at village level in low endemic areas and quantified the impact of different socio-demographic, climatic and environmental drivers. A cross-sectional study was conducted using lot-quality assurance sampling in 67 villages across two townships in low endemic area of Myanmar. Data was collected in the wet and dry season of 2018-2019. Finger prick blood was collected from 11,128 asymptomatic individuals to detect subclinical malaria using ultra-sensitive polymerase chain reaction. We analyzed the effect of occupational risk, human utilization of landscape, climatic and environmental factors over-time to predict the subclinical malaria prevalence using machine learning approach (random forest model). The prevalence of subclinical malaria at individual level was 0.9% (*Plasmodium falciparum)*, 3.9% (*P. vivax)* and 4.9% (any malaria). At village level, <2% of people were subclinical malaria positive (ranged 0.7%-52%). Random forest model using potential and least correlated factors explained 41.5% (*P. falciparum),* 58.4% (*P. vivax)* and 58.3% (any malaria) of variance. Despite relatively low overall performance, the error rate of the models is very low. Fraction of croplands within a 2km buffer around villages was found to be the most important predictor for subclinical malaria, followed by frequency of interaction with water bodies, logging activities, fraction of managed and natural forest within the 2km buffer, greenness of vegetation and farming as the self-reported occupation. Findings suggest that in low endemic areas, the influence of village level socio-demographic, climatic and environmental factors is not sufficient to reliably predict the rate of subclinical malaria. Though the models' predictive power is limited, the very low error rate indicates high validity predictions. Incorporating temporal potential predictors in higher malaria endemicity will likely improve model performance.

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PREVALENCE OF SUBCLINICAL MALARIA AND ITS POTENTIAL RISK FACTORS IN LOW TRANSMISSION SETTING IN MYANMAR: A COMMUNITY-BASED SURVEY

Zaw Win Thein[1], Wandee Sirichokchatchawan[1], Thura Htay[2], Zin Min Tun[2], Aye Kyawt Paing[2], Kay Thwe Han[3], Nay Yi Yi Lin[4], Zaw Lin[4], Poe Poe Aung[5]

[1]Chulalongkorn University, Bangkok, Thailand, [2]Duke Global Health Institute, Myanmar Program, Duke University, Yangon, Myanmar, [3]Department of Medical Research, Ministry of Health, Yangon, Myanmar, [4]National Malaria Control Program, Ministry of Health, Nay Pyi Taw, Myanmar, [5]Malaria Consortium, Bangkok, Thailand

Despite ongoing efforts, malaria remains a global significant public health issue. Myanmar continues to experience a persistent prevalence of malaria infections in subnational elimination areas. Subclinical malaria contributes the persistent infection through asymptomatic carriers. This study identified the prevalence and potential risk factors for subclinical malaria in low transmission areas. A cross-sectional study was conducted using lot quality assurance sampling in 64 villages in two townships of Mandalay Region, Myanmar. Samples and data were collected from 11,128 asymptomatic individuals between Feb-Dec 2018. The questionnaire identified socio-demographic characteristics, travel history, mosquito exposure, population mobility and malaria history. Malaria infection was detected by rapid diagnostic test (RDT) and ultrasensitive polymerase chain reaction (usPCR) as a gold standard. Multiple logistic regression was performed to examine the potential risk factors for the prevalence of subclinical malaria infections. A total of 11,114 were involved in the final analysis (7,676 from Singu and 3,438 from Thabeikkyin). The mean age of participants was 31.3 years (SD 18.81) and the majority were dependent (25.2%). The prevalence of subclinical malaria by usPCR was 4.9% any malaria, 0.9% *P.falciparum*

and 3.9% *P.vivax.* Prevalence was very low for RDT (0.13% any malaria, 0.08% *P.falciparum* and 0.05% *P.vivax).* Multiple logistics regression shows that younger age group (< 29 years) (aOR=1.30, 95%CI=1.08-1.58), male (aOR=1.62, 95%CI=1.34-1.96), outdoor workers (aOR=1.40, 95%CI=1.14-1.72), having activities related to higher mosquito exposure (aOR=3.70, 95%CI=2.61-5.23), and having taken anti-malaria drug in the past 2 months (aOR=2.34, 95%CI=1.50-3.64) were significantly associated with subclinical malaria. The evidence showed the undeniable significance of subclinical malaria in low transmission areas. Policymakers should continue surveillance of subclinical malaria and tailor interventions to effectively control malaria transmission in high-risk populations to achieve malaria elimination.

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BLOOD TRANSFUSIONS FOR CHRONIC MALARIA ANEMIA IN PRISONERS OF WAR ON THE THAI-BURMA RAILWAY 1943-1945

G. Dennis Shanks[1], Madeleine Payne[2], Colin J. Sutherland[2]

[1]ADF Malaria and Infectious Disease Institute, Enoggera, Australia, [2]London School of Hygiene and Tropical Medicine, London, United Kingdom

Blood transfusion is an important intervention for severe anemia caused by chronic relapsing vivax malaria. Sixty thousand Allied Prisoners of War (POW) worked on the Imperial Japanese Army's railroad from Thailand to Burma during 1942-5. From mid-1943 a blood transfusion service was run by the prisoners themselves to rescue severely ill fellow prisoners who were otherwise unlikely to survive the war. Approximately 1/3 of all POW did not survive the war largely due to a combination of starvation, ill-treatment and infectious diseases including cholera and malaria. Extant transfusion records (1251 recipients, 1189 donors) in ledger books held by the UK National Archives at Kew were accessed and analyzed. Survival to the end of the war in September 1945 was determined from Commonwealth War Graves Commission records. The records examined indicate that freshly donated whole blood was manually defibrinated and transfused following cross matches based on POW medic sera. Overall survival to the end of the war was 74% of recipients and 88% of donors. Post-war survival rates for the main diseases associated with anemia in transfusion recipients were 53% for malnutrition, 59% for dysentery, 68% for skin ulcers, and 90% for malaria. Most donors died in transport ships sunk on their way to Japan. By 1945 the vast majority of blood transfusions were given for severe anemia caused by chronic (nearly monthly) relapsing vivax malaria. Although the POW situation was admittedly extreme, eliminating residual liver parasites to prevent chronic vivax relapses and the resulting severe anemia does directly contribute to adult survival from *Plasmodium vivax*.

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PERFORMANCE METRICS DURING A MALARIA OUTBREAK RESPONSE; LESSONS FROM EVALUATION IN AN ARID, EPIDEMIC-PRONE COUNTY IN KENYA

Jedidah W. Kiprop[1], Wachira Muguku[1], Abdi Sorakotol[2], Emmanuel Sigei[1], Martha A. Wario[2], Lydia Khalayi[1], Fredrick O. Odhiambo[1], Maurice Owiny[1], Ahmed Abade[1]

[1]Field Epidemiology and Laboratory Training Program, Ministry of Health, Nairobi, Kenya, [2]Marsabit County Health Department, Marsabit, Kenya

In January 2024, Marsabit County experienced a malaria outbreak with 750 confirmed cases, 12% (90) of whom had severe malaria. This was following heavy rains that displaced 1000 households and increased mosquito population. Epidemic preparedness and response (EPR) strategies were in place, but how the strategies performed was not known. We sought to measure the response timeliness and identify bottlenecks and enablers of system performance.We used the WHO guidelines to conduct an Early Action Review (EAR). We reviewed county weekly malaria surveillance data, EPR dashboard trends since December, and outbreak reports at the Marsabit Public Health Emergency Operations Centre. We calculated the time to detect, notify, and respond to the outbreaks against a benchmark of the 7-1-7 global target of detection within seven days from emergence, notification within one day of detection, and completion of early response actions within seven days of notification. Key informant interviews (KII) were held with the surveillance and response team members. Data analysis was done thematically. The outbreak was detected in 16 days, notified in 7 days, and early response activities were completed in 41 days. Staff capacity included case management, entomologic surveillance, genomic surveillance, and laboratory quality assurance. Stakeholders supported vector control and laboratory diagnosis. Primary health facilities did not monitor EPR dashboards, lacked rapid response teams, pan-specie malaria rapid diagnostic tests, and under-reported cases. Malaria surveillance is additionally challenged by nomadic pastoralism, cross-border disease spread, and changing vector and parasite distribution. KII elicited bottlenecks and enablers for the observed performance and proposed recommendations to strengthen future responses. Through an EAR, we identified areas for outbreak response improvement including facility-level EPR data monitoring, feedback, and support for clinical and diagnostic supplies decision-making. There is a need to strengthen malaria surveillance, diagnosis, and treatment in epidemic-prone border counties.

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ASSOCIATION OF A LONG-TERM CARRIAGE OF SUBPATENT *PLASMODIUM FALCIPARUM* PARASITES WITH CLINICAL MALARIA ATTACKS IN A LOW TRANSMISSION AREA IN SENEGAL

Babacar Souleymane Sambe[1], Ibrahima Sarr[1], Aissatou Diagne[1], Arona Sabène Diatta[1], Serigne Ousmane Mbacké Diaw[1], Joseph Faye[1], Nafissatou Diagne[2], Adja Fatou Mbodj[1], Rokhaya Sane[1], Babacar Diouf[1], Alassane Thiam[1], Arfang Dianmanka[3], Papa Mbacke Sembene[3], Fatoumata Diene Sarr[1], Ibrahima Dia[1], Inès Vigan-WOmas[1], Cheikh Sokhna[2], Aissatou Toure-Balde[1], Makhtar Niang[1]

[1]Institut Pasteur de Dakar, Dakar, Senegal, [2]Institut de Recherche pour le Developpement, Dakar, Senegal, [3]Universite Cheikh Anta Diop, Dakar, Senegal

In low malaria transmission areas, asymptomatic *Plasmodium*-infected individuals contribute to the low-level of residual malaria. In Dielmo (Senegal), long-term monitoring of malaria exposed populations upon the implementation of preventive and control measures has been key to document the reduction of malaria incidence to the point that elimination is being envisaged. Attention is now directed towards the asymptomatic *Plasmodium* carriers that are believed to sustain clinical malaria cases. This study comprehensively elucidates the contribution of asymptomatic *P. falciparum* carriage in clinical malaria in such low-transmission areas. The research followed from June 2014 to December 2022 two cohorts of qPCR-positive (Group 1) and negative (Group 2) individuals (44 in each group) matched by age and sex, and their movements and malaria episodes were tracked. Blood samples were taken biannually for parasite detection using Cytochrome b-based qPCR. The spatial and temporal analysis utilized QGIS and R. Associations between the asymptomatic carriage and the occurrence of clinical malaria were examined using logistic regression model. Temporal follow-up of individual' infections revealed *P. falciparum* carriage was more frequent in Group 1 individuals. The qPCR positivity rate was significantly higher in Group 1 (19.69% vs 10.77%, p-value < 0.001), and an increased infection risk nearby asymptomatic carriers were found within households (odds ratio: 1.44, p = 0.53) and in neighbouring households (odds ratio: 2.64, p = 0.04). The high rates of neighbourhood infections correlated with increased individual infection risk and households with low asymptomatic carriers had fewer malaria cases (average 0.40 per household) compared to those with higher rates (1.33 to 1.5 per household). The study findings provide detailed evidence linking long-term subpatent *Plasmodium* carriage to residual malaria transmission and valuable insights to guide targeted interventions against asymptomatic carriers for elimination purposes.

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EFFECTS OF INTERMITTENT PREVENTIVE TREATMENT FOR MALARIA IN PREGNANCY ON INFANT GROWTH THROUGH AGE 1 YEAR

Yanwei Tong[1], Kalani Ratnasiri[1], Suhi Hanif[1], Anna T. Nguyen[1], Michelle E. Roh[2], Grant Dorsey[2], Abel Kakuru[3], Prasanna Jagannathan[1], **Jade Benjamin-Chung**[1]

[1]Stanford University, Stanford, CA, United States, [2]UC San Francisco, San Francisco, CA, United States, [3]Infectious Diseases Research Collaboration, Kampala, Uganda

Intermittent preventive treatment for malaria in pregnancy (IPTp) can improve birth outcomes, but little is known about whether it confers benefits to postnatal growth. We investigated the effect of IPTp on infant growth in Uganda and its pathways of effects using causal mediation analyses. We analyzed data from 663 infants born to mothers enrolled in a randomized trial of monthly IPTp with dihydroartemisinin-piperaquine (DP) vs sulfadoxine-pyrimethamine (SP) (NCT 02793622). Weight and length were measured from 0-12 months of age. Using generalized linear models, we estimated crude effects for DP vs. SP on length-for-age (LAZ) and weight-for-length Z-scores (WLZ) stratified by gravidity. We estimated average causal mediated effects (mean difference in Z) for placental malaria, gestational weight change, maternal anemia, preterm birth, birth length, and birthweight. We also assessed maternal inflammation as a potential mediator using the Olink Target 96 inflammation panel and maternal plasma samples collected at delivery in a random subsample (N=264). We adjusted mediation models for infant sex, gravidity, gestational age at enrollment, maternal age, maternal parasitemia at enrollment, education, and wealth. We found that SP increased LAZ by 0.18-0.28 Z from birth through age 4 months compared to DP, while DP increased WLZ by 0.11-0.28 Z from 2-8 months compared to SP among infants of multigravida. We did not observe an effect among primigravida. Mediators of SP included increased birth weight and length and maternal stem cell factor at delivery. Mediators of DP included placental malaria and birth length, maternal IL-18 and CD6 at delivery. In summary, SP improved growth by increasing birth size, DP improved growth by reducing placental malaria, and both influenced growth by reducing certain markers of maternal inflammation. In malaria endemic settings, IPTp may prevent infant growth failure among multigravida in the period of exclusive breastfeeding (0-6 months), when few other interventions are available. IPTp with combined SP+DP may improve child growth through multiple pathways among multigravida.

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MALARIA BURDEN IN INFANTS LIVING IN A HIGH MALARIA TRANSMISSION SETTING IN UGANDA

Miriam Aguti[1], Abel Kakuru[1], Jimmy Kizza[1], Blake Shaw[2], Harriet Adrama[1], Peter Olwoch[1], Moses Kamya[1], Grant Dorsey[3], Prasanna Jagannathan[2], Haley Hedline[2], Joaniter Nankabirwa[1]

[1]Infectious Diseases Research Collaboration, Kampala, Uganda, [2]Stanford university, Stanford, CA, United States, [3]University of Carlifonia, San Francisco, San Francisco, CA, United States

An affordable malaria vaccine, R21, will begin to be deployed in Uganda and other African countries with high malaria transmission in 2024, with standard dosing beginning at 6 months of age. However, in infants under 9 months of age, the vaccine provides minimal protection and the only widely used tool for malaria prevention during this period is insecticide treated bed nets. To better understand the burden of malaria during this period, we enrolled a birth cohort of infants born to HIV-uninfected pregnant mothers enrolled in a clinical trial of chemopreventive regimens in pregnancy in Busia, Uganda, a high, perennial transmission setting. Infants are followed for all medical care in a dedicated study clinic, and routine assessments are conducted every 4 weeks. Parasitemia is measured by microscopy and quantitative PCR during routine visits, and symptomatic malaria measured by passive surveillance. At all visits, infants with fever and a positive thick blood smear are diagnosed and treated for malaria. The primary outcome is malaria incidence during the first 12 months of life. Between November 2022 and January 2024, a total of 832 infants aged 4-8 weeks were enrolled into the birth cohort and 267 had reached one year of age. During 457.3 person years (PY) of follow-up, a total of 588 episodes of malaria were recorded (1.29 episodes per PY). Of incident cases, 11 (0.2%) met the definition of complicated malaria (0.02 episodes PPY). Malaria incidence increased from 0.82 episodes PPY prior to 6 months of age to 1.97 episodes ppy from 6-12 months of age. Parasite prevalence measured by qPCR increased from 5% at 1-2 months of age to more than 40% by 6 months of age. Among infants who completed one year of follow-up, the cumulative risk of any parasitemia was 95%. The malaria burden prior to vaccination among infants living in this perennial transmission setting is quite high, increasing with age. Young infants in these settings would benefit from additional control interventions, such as monoclonal antibodies, perennial malaria chemoprevention, and or malaria vaccination initiated earlier in life.

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MATHEMATICAL ASSESSMENT OF THE ROLE OF INTERVENTION PROGRAMS FOR MALARIA CONTROL

Maame Akua Korsah, Stuart T. Johnston, Kathryn E. Tiedje, Karen P. Day, Jennifer A. Flegg, Camelia R. Walker

University of Melbourne, Melbourne, Australia

Malaria remains a global health problem despite the attempts to control and eradicate it. There is an urgent need to understand the current transmission dynamics of malaria and to determine the interventions necessary for control. We seek to develop a fit-for-purpose mathematical model to assess the interventions needed to control malaria in an endemic setting. To achieve this, we formulate a malaria transmission model to analyze the spread of malaria in the presence of control interventions. A sensitivity analysis of the model is performed to determine the relative impact of the model parameters on disease transmission. We explore how variations in the recruitment and management of malaria control interventions affect transmission. Results obtained from the study imply that the discontinuation of these interventions has a significant effect on malaria prevalence. Thus, the maintenance of interventions is imperative for malaria elimination. In a scenario study aimed at assessing the impact of long-lasting insecticidal nets (LLINs), indoor residual spraying (IRS), and localized individual measures, our findings indicate that increased LLIN utilization and extended IRS coverage (with longer-lasting insecticides) cause a more pronounced reduction in the prevalence of symptomatic malaria compared to reduced LLIN utilization and shorter IRS coverage. Additionally, our study demonstrates the impact of localized preventive measures in mitigating the spread of malaria when compared to the absence of interventions. Our results indicate that achieving malaria elimination is associated with a high level of utilization and consistent funding of interventions. By identifying key transmission pathways and emphasizing the importance of intervention maintenance, our findings can guide decision-makers and stakeholders in their efforts to control malaria and improve public health.

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ATTRACTIVE TARGETED SUGAR BAITS FOR MALARIA CONTROL IN WESTERN KENYA (ATSB-KENYA): ENROLLMENT CHARACTERISTICS OF COHORT CHILDREN AND HOUSEHOLDS

Alice Kamau[1], Kizito Obiet[2], Caroline Ogwang[2], Daniel McDermott[1], Maia Lesosky[3], Julia Janssen[4], Wycliffe Odongo[4], Julie R. Gutman[4], Jonathan S. Schultz[5], Wycliffe Nicholas[2], Brian Seda[2], Mercy Chepkirui[2], Frank Aduwo[2], Oliver Towett[2], Martin J. Donnelly[1], Eric Ochomo[2], Simon Kariuki[2], Aaron M. Samuels[4], Feiko ter Kuile[1], Sarah G. Staedke[1]

[1]Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [2]KEMRI Centre for Global Health Research, Kisumu, Kenya, [3]Imperial College London, London, United Kingdom, [4]Centers for Disease Control and Prevention, Atlanta, GA, United States, [5]Centers for Disease Control and Prevention,, Atlanta, GA, United States

In western Kenya, we are conducting a cluster-randomized controlled trial to assess the impact of a new vector control tool, Attractive Targeted

Sugar Baits (ATSBs), on malaria burden in a cohort of children aged 1 to <15 years. Characteristics of the cohort, their households, and factors associated with baseline malaria prevalence are described here. Children were randomly selected by cluster (n=70) from a census database. Three consecutive cohorts were enrolled in March-April 2022, September-October 2022, and March 2023. ATSBs were deployed in March 2022. At enrolment, participants were tested for malaria by rapid diagnostic test (RDT). After enrolment a household survey was conducted. Household structures were classified as 'improved' (finished walls and roofs, closed eaves) or 'traditional' (all other construction). Data were analysed using a generalised linear mixed model to assess factors associated with baseline malaria prevalence. Of the 3,705 children screened, 220 declined and 523 were excluded. Overall, 2,962 children were enrolled with a median age of 8.5 years (IQR: 4.8, 11.8); 48% were female. Bednet use was reported more frequently in children aged 1-4 years (96%) than in those aged 5-15 years (84%, p<0.001). In the household survey, only 199/2,595 (8%) households were categorised as 'improved', as most houses had open eaves. While 99% of households owned at least one net, only 51% were adequately covered (at least one net per two household residents). Among 999 children enrolled in the first cohort (baseline), 498 (50%) tested positive by RDT. In an adjusted multivariable analysis, factors associated with RDT positivity included sub-county (Alego-Usonga vs Rarieda, adjusted odds ratio [aOR] 4.8; 95% CI: 2.7-8.5; p<0.001), house construction (traditional vs improved, aOR 2.8; 95% CI: 1.6-5.0; p<0.001), and age (5-<15 years vs 1-4 years, aOR 1.6; 95% CI: 1.1-2.4; p=0.009). The burden of malaria in children remains high in western Kenya. Strategies to ensure high bednet coverage and use, and additional tools such as ATSBs, are needed to intensify malaria control in western Kenya.

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INDOOR RESIDUAL SPRAYING IN SOURCE DISTRICTS IN SOUTHERN MOZAMBIQUE INFLUENCES CROSS-BORDER MALARIA TRANSMISSION IN KWAZULU-NATAL, SOUTH AFRICA

Rajendra Maharaj, Ishen Seocharan, Vishan Lakan
South African Medical Research Council, Durban, South Africa

Cross-border malaria control initiatives are important for malaria elimination efforts especially when low transmission countries share borders with high transmission countries. The main source of imported malaria into KwaZulu-Natal are Gaza and Inhambane in Mozambique. This province is targeting elimination, but low levels of transmission is being driven by imported malaria. To advance the provincial elimination agenda, the National Treasury decided to support indoor residual spraying (IRS) in southern Mozambique. Studies have shown that Gaza and Inhambane provinces in southern Mozambique are the main contributors to the imported cases detected in South Africa. The study area was conducted by analysing malaria cases in Guija district in Gaza province as well as in the districts of Inharrime, Panda and Zavala in Inhambane province, in Mozambique. All districts recorded above 95% IRS coverage. Malaria cases prior to the application of IRS was compared to cases post-spray. The imported malaria cases into KwaZulu-Natal were compared prior and post implementation of IRS in the four identified districts. Malaria data from 2019 to 2023 was used to complete this study. The number of imported malaria cases in KwaZulu-Natal increased from 534 to 602 in 2019 to 2023. Imported cases accounted for 86% of total cases in 2019 whilst in 2023 imported cases comprised 80% of total cases. Since all other factors were consistent over the time periods, it is plausible that IRS in the source districts influenced the number of imported cases entering KwaZulu-Natal. Guija and Zavala, showed no significant differences between baseline and endline prevalence data. Panda and Inharrime recorded statistically significant differences in the prevalence rates for the same period (p-value <0.001). This demonstrated that IRS in at least two districts were influenced by the IRS campaign. Cases in Panda remained static but prevalence in Zavala increased compared to baseline. Transmission in targeted districts in southern Mozambique were influenced by the IRS campaign and led to a decrease in the proportion of imported cases reported in KwaZulu-Natal between 2019-2023.

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HIGH PREVALENCE OF MALARIA AMONG REACTIVE CASES IN COMMUNITIES NEAR THE HOME OF THE INDEX CASES: IMPLICATIONS FOR MALARIA CONTROL

Zerihun Zewde[1], Demeke Daka[2], Teklu Wegayehu[1], Bernt Lindtjørn[1], **Fekadu Massebo**[1]
[1]Arba Minch University, Arba Minch, Ethiopia, [2]Madda Walabu University, Robe, Ethiopia

Detecting and treating all malaria cases is crucial for effectively controlling malaria. This study aimed to assess the burden of malaria in the community from where patients sought treatment. From July to October 2022, we conducted a study in the Sile village of South Ethiopia to identify consecutive malaria patients (index cases) at a health post. We then conducted a cross-sectional community survey to investigate reactive malaria cases. Within 6 days of the index case's identification, all individuals residing in the same household as the index case and 30-35 nearby households were screened using a Rapid Diagnostic Test, with PCR utilised for confirmation. Parasite and gametocyte load were measured microscopically. Ethical approval was obtained from the Arba Minch University Institutional Review Board (IRB/1292/2022). 181 index malaria cases were identified, and of 2009 individuals screened in the community, 104 reactive tested positive (prevalence rate 5.2% (95% CI = 4.2-6.2). There were more men in index cases (58.6%) than in reactive cases (Fisher's exact test, 43.3%; P=0.014). The prevalence of *Plasmodium falciparum* was higher among the index cases (67.4%) than the reactive cases (39.4%; *P*<0.01). The corresponding rates of *P. vivax* cases were 30.4% among index and 45.6% among reactive (P=0.02). All index cases were symptomatic, and 21.2% of reactive cases had symptoms of malaria (P<0.01). The median *P. falciparum* parasite density (N=207/255; 72%) among index cases was 20320 parasites/µl and higher than 5010 among reactive cases (non-parametric test, *P*<0.01). For *P. vivax*, the median parasite density among index cases was 7900, and higher than 1428 among reactive cases (*P*<0.01). We analysed 212/255 slides for gametocytes; 56% did not detect any gametocytes. Of the remaining, the density was low and similar for the *P. falciparum* and *P. vivax* index and reactive cases. Asymptomatic people in the community were more likely to be females (OR=1.8; P=0.04) and were younger (9.7 vs. 18.2 years; P<0.01). Effective malaria control requires more sensitive diagnostic tools to identify cases in the community.

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FEBRILE PATIENTS IN ZAMBIA: WHERE DO THEY SEEK TREATMENT?

Zhiyuan Mao[1], Erica Orange[2], Kafula Silumbe[3], Ruth Ashton[1], Josh O. Yukich[1], Refilwe Yvonne Karabo[1], Busiku Hamainza[4], Megan Littrell[5], Thomas P. Eisele[1]
[1]Tulane University, New Orleans, LA, United States, [2]PATH, Seattle, WA, United States, [3]PATH, Lusaka, Zambia, [4]Zambia National Malaria Control Center, Lusaka, Zambia, [5]PATH, Washington, DC, United States

Zambia has made substantial progress in improving access to malaria diagnosis and treatment throughout the country, with a focus on deploying community health workers (CHWs) in remote rural areas. However, bypassing the nearest CHWs in favor of more distant, higher-level facilities (HFs) is not uncommon. This study aims to assess the utilization of CHWs among individuals with fevers and identify the determinants of CHW use. We analyzed data of a prospective cohort aged 12 months to 14 years from 2022 to 2023, which was part of an attractive targeted sugar bait station trial beginning November 2021 in Western Province, Zambia. Treatment-seeking information was recorded at each participant visit. CHW and HF information, including geolocation, were obtained from DHIS 2 and Zambia Ministry of Health. Travel time for walking to the nearest providers was calculated using the Malaria Atlas Project's travel friction surface. Among 25,638 follow-up visits made to 4,494 enrolled children, 9,992 reported fever, with 4,664 of these reporting seeking treatment at HFs or CHWs. For 76.20% of visits that report treatment seeking, the travel time to the

nearest CHW was shorter than the time to nearest HF. The overall median travel times were 15.44, 47.73, and 43.06 minutes to the nearest CHW, nearest HF, and actual CHW/HF visited, respectively. Excluding visits with reported providers that could not be identified, only 14.35% went to a CHW (N=4,088). The primary regression model showed individuals with >1 complication were less likely to visit a CHW (OR:0.38 [0.23, 0.63]). Notably, for every additional 10 minutes that the nearest HF was farther away than the nearest CHW, the likelihood of choosing a CHW increased by 23% (OR: 1.23; 95% CI: 1.12, 1.36). Spatial variation was evident, with individuals in Luampa (OR: 3.95; 95% CI: 1.62, 9.64) and Nkeyema (OR: 11.87; 95% CI: 4.83, 32.17) being more likely to use a CHW compared to those in Kaoma district. Our study found that CHW utilization among febrile patients remains low ; however, stock-outs at CHW/HF facilities, which were not assessed in our study, may influence treatment-seeking behavior.

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AN UPDATE OF THE EPIDEMIOLOGICAL PARAMETERS OF MALARIA IN SCHOOL AGE CHILDREN IN KOLLE, A RURAL SETTING, MALI

Bouran Sidibe

Malaria Research and Training Centre, Faculty of Medicine, and Odonto-Stomatology, University of Sciences, Technics and Technology of Bamako (USTTB), Bamako, Mali

Kolle, Mali is a malaria research site since 1998. Several clinical trials have been conducted at this site. Currently, there is very few of data on the parasitological and entomological parameters of malaria in this area. This suggests a critical need of updating these parameters. We aimed to update the epidemiological and entomological parameters of malaria transmission in Kolle, and to better inform health policy makers and health care providers to fighting against malaria efficiently. The main objective of this study was to assess the parasitological parameters of malaria transmission during the low-transmission season at the Kollé basic school. A cross-sectional survey was carried out in Kollé, a village in the rural commune of Bancoumana. The main objective was to evaluate the parasitological parameters of malaria transmission at the Kollé primary school. To achieve these objectives, 205 children from different age groups from first to sixth grade and from kindergarten were included to take part in the cross-sectional survey. The study area consisted of all pupils in the first cycle and pre-school at the Kollé basic school. The choice of the primary school was justified by its accessibility at the time of the survey and the fact that most control interventions are focused on children under 5 and pregnant women. It was therefore deemed necessary to know the parasitological parameters of school-age children for future decision-making. The survey took place in January 2023, during the period of low malaria transmission. The survey provided a better estimate of the prevalence of malaria infection in schools and pre-schools. The prevalence of malaria infection obtained during this cross-sectional survey was 19.0%. Children aged 10 and 12 years were the most affected (24.3%,) followed by children under 5 years (22.2%). The proportion of gametocyte carriage varied with age and was higher in with children under 5 years of age (11.1 %). *Plasmodium falciparum* malaria is still the dominant parasite in endemic areas of Kolle, and older children are becoming more vulnerable to malaria infection.

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PSYCHOSOCIAL FACTORS ASSOCIATED WITH CARE SEEKING FOR MALARIA AMONG CAREGIVERS OF FEBRILE CHILDREN UNDER FIVE IN LIBERIA, 2021

Victor S. Koko[1], Eric Gaye[2], Jamesetta Smith[2], Gontopoe Omedo Nuahn[2], Trokon Washington[1], Odell Wannie Kumeh[1], Saratu Olabode-Ojo[2]

[1]Ministry of Health/National Malaria Control Program, Monrovia, Liberia, [2]Breakthrough Action, Monrovia, Liberia

Increasing care-seeking for children with fever is important for malaria control. Measuring psychosocial factors associated with care-seeking is crucial as they may influence behavior. In 2021, the National Malaria Control Program and Breakthrough ACTION conducted a cross-sectional Malaria Behavior Survey in North-Central, South-Central, and Greater Monrovia regions of Liberia (high, moderate, and low malaria prevalence). Through cluster random sampling, 5,822 respondents were selected, including 609 caregivers of children under five who had a fever in the two weeks prior to the survey, and interviewed using a structured questionnaire. Descriptive and multivariate logistic regression analyses were used to measure the association between psychosocial factors and care-seeking. Results revealed that 55% of caregivers sought prompt and appropriate care, defined as care-seeking within one day of fever onset from a health facility or community health worker. Respondents who knew what appropriate care is were three times more likely to report prompt and appropriate care (adjusted odds ratio [aOR]: 3.1, 95% confidence interval [CI]: 1.2-7.9), and those who knew the importance of prompt care-seeking were eight times (aOR: 8, 95% CI: 3.0-21.3) more likely to report the behavior. Both knowledge indicators were gt90% in all areas and subgroups. Perceiving that seeking care is effective (aOR: 1.7, 95% CI: 1.1-2.6) was also associated with care-seeking; this belief was prevalent among 70% of respondents and lowest among those with less than primary education (64%). While not significantly associated with care-seeking, only 34% of respondents believed that most community members seek prompt and appropriate care for febrile children, and 9% believed that community members approve of prompt and appropriate care-seeking. These social norms may influence care-seeking. Social and behavior change programs can sustain high levels of knowledge about prompt and appropriate care-seeking, improve perceived effectiveness of prompt and appropriate care-seeking, and may also benefit from promoting supportive social norms for care-seeking

6402

ANALYSIS OF MALARIA PREVALENCE AND HEALTH SERVICES IN A GOLD MINING SITE IN WESTERN ETHIOPIA: A MIXED METHODS RESEARCH STUDY

Natasha Glendening[1], Werissaw Haileselassie[2], Ming-Chieh Lee[1], Guiyun Yan[1], Daniel M. Parker[1]

[1]University of California - Irvine, Irvine, CA, United States, [2]Addis Ababa University, Addis Ababa, Ethiopia

Malaria is a major public health issue in Ethiopia. Migration can influence the malaria transmission dynamics, with individuals relocating from malaria-free highland regions to malarious lowlands, potentially facing elevated risks of contracting malaria. Migrants may find it difficult to protect themselves against malaria and have limited access to diagnosis or treatment. Settlers in goldmining sites are one type of migrant, and are often neglected in malaria research, yet may have particularly high malaria risk. We conducted a malaria prevalence survey in a new goldmining settlement in the highly malarious Gambella Region, Ethiopia. We conducted interviews to collect socio-demographic data and assess participants' malaria knowledge, attitudes, and practices, aiming to identify correlations between these factors, malaria infections, and bednet access. Interviews were also conducted to comprehend community living conditions and healthcare accessibility. The overall prevalence of *P. falciparum* malaria in the study area was 39.7% (CI: 34.7%-44.4%). Young children were most likely to have malaria, with individuals aged 15-24 having 67% lower odds (aOR: 0.33; CI: 0.13-0.86) of infection compared to those aged 0-4 years old. Meanwhile, those aged 25+ had 75% decreased odds of infection (aOR: 0.25; CI: 0.10-0.65). Having access to bednets showed a protective effect against odds of malaria (aOR: 0.47; CI: 0.22-0.97) but access to bednets was low (12%, n=59). Participants described multiple barriers to access despite a desire for bednets. Individuals who relocated from low elevation areas with high malaria test positivity rates were more likely to test positive for malaria, as were those residing in densely populated households with multiple malaria cases. Conversely, individuals from higher elevations with low malaria test positivity rates, and those living in households with 5-10 occupants and <2 malaria infections, were more likely to have bednets. Future interventions within this goldmining community focusing on bednet distribution and increasing access to diagnosis and treatment would likely help alleviate the malaria burden.

6403

HOUSEHOLD STORM DAMAGE LIMITS ACCESS TO AND USE OF INSECTICIDE TREATED BEDNETS IN MOZAMBIQUE

Kelly M. Searle[1], Dominique E. Earland[1], Albino B. Francisco[2], Valy Muhiro[3], João L. Ferrão[4]

[1]University of Minnesota, Minneapolis, MN, United States, [2]Escola Secundária de Sussundenga, Sussundenga, Mozambique, [3]Centro de Saude Rural de Sussundenga-Sede, Sussundenga, Mozambique, [4]Consultório Associados de Manica, Chimoio, Mozambique

Insecticide treated bednets (ITNs) are the key malaria prevention tool. Mozambique has the fourth highest prevalence of *P. falciparum* malaria worldwide. Manica Province is located in the center of the country and has historically high malaria prevalence with seasonal peaks in incidence following the rainy season. The aim of our study was to determine if household damage in the aftermath of Cyclone Idai was associated with decreased ITN access and use. A cross-sectional community-based survey was administered from December 2019 to February 2020 in Sussundenga village. Additional data regarding demographics, occupation, and employment were collected. Participants were analyzed based on the binary outcome variable of self-reported ITN use on the previous night. Comparisons were made between use and non-use by the following demographic variables: RDT results, age, sex, number of household residents, knowledge of the cause of malaria, head of household employment status (full-time, or part-time/ seasonal), and amount of household damage endured during Cyclone Idai (none, minor, significant, or destroyed). Generalized estimating equations (GEE) logistic regression models were used to identify factors associated with ITN use. GEE logistic regression models were used to account for within household non-independence of household level variables (household cyclone damage, number of household residents, and head of household employment status). In the multivariable analysis household cyclone damage was treated as the primary exposure of interest with other variables (RDT results, age, sex, number of residents per household, knowledge of the cause of malaria, and head of household employment status) included as potential confounders. In this analysis household cyclone damage was shown to be associated with ITN use on the previous night. Minor household damage was associated with a 0.34 times lower odds of ITN use (95% CI: 0.15-0.78), significant damage was associated with a 0.26 times lower odd of ITN use (95% CI: 0.29-1.39), and destruction was associated with a 0.23 times lower odds of ITN use (95% CI: 0.11-0.50).

6404

PLASMODIUM VIVAX OUTBREAK AMONG INDIGENOUS COMMUNITIES IN PLAYA PATAXTTE, IZABAL, GUATEMALA: THE IMPORTANCE OF LOCALIZATION AND RESPONSE

Cecilia Barrientos[1], **Leopoldo Villegas**[2], Ericka Lopez[3], Erick Ramos[1], Deysi Lucero[1], Evelio Saquil[1], Manuel Barrientos[1], Maritza May[1], Cecilia Tiul[1], Elsa Coc[1], Maria Coy[1], Margarita Li[1], Maria Ico[1], Wilmer Augurcia[1], Victor Oxom[1], Israel Ico[1], Isaias Coc[1], Elias Be[1], Marvin Rodriguez[1], Jorge Sacul[1], Cesar Tiul[1], Benjamin Yaxcal[4], Alvaro Choj[4], Romulo Chub[4], Pedro Tut[4], Marcos Marquin[1], Xiomara Bol[4], Madelin Arriaza[1], Hector Soriano[5], Maura Herrera[5], Erik K[5], Karina A[5], Mayra Arana[6], Arly Obando[6], Augusto Zamora[6], Carlos Blanco[7], Erik Castillo[8], Jaime Juarez[9], Luis Marroquin[10], Zoraida Morales[11]

[1]Distrito Municipal de Salud El Estor, El Estor, Guatemala, [2]InterAmerican Development Bank, Washington DC, DC, United States, [3]Distritito Municipal de Salud El Estor, El Estor, Guatemala, [4]Puesto de Salud Playa Pataxte, Playa Pataxte, Guatemala, [5]DDRISS Izabal, Izabal, Guatemala, [6]DDRISS Peten Sur Occidente, Peten Sur Occidente, Guatemala, [7]Sub-Programa de Malaria, Guatemala City, Guatemala, [8]CHAI, El Estor, Guatemala, [9]InterAmerican Development Bank, Guatemala city, Guatemala, [10]InterAmerican Development Bank, Guatemala City, Guatemala, [11]Sub-Programa Malaria, Guatemala city, Guatemala

Guatemala has achieved remarkable progress in reducing malaria; all infections are due to *Plasmodium vivax*. Mid-year internal population movements in the country due to agricultural activities are linked to continued malaria transmission; this poses a threat to achieving the elimination goal. In November 2023, the national surveillance data showed that the El Estor (EE) district in Izabal, Guatemala, reported a high number of malaria cases. We used retrospective data from the surveillance system to describe the response of Mayan communities controlling a malaria outbreak in Playa Pataxte, EE district. A malaria mass screening and treatment of positive cases was coordinated between indigenous health workers and their communities. Malaria cases were confirmed using malaria rapid diagnosis in case of symptomatic cases and blood smears were collected in each person in the community for microscopic diagnosis. Data was collected during household visits using a simplified questionnaire. Malaria prevalence (P) was reported overall and among symptomatic/ asymptomatic people. Between December 4-18, 1157, people were screened for malaria with 35 cases of malaria (all *Pv*) diagnosed and treated in Playa Pataxte, EE. Of these, 25 cases were diagnosed using rapid tests among symptomatic patients. The median age of the cases was 24 years (y) (1 to 64 y). 60% of cases were male and most of them (67%) were in the 14-49 y group (economically active age). Overall, malaria P was 3.02% (P=2.42% in symptomatic vs. P=0.51% in asymptomatic). The outbreak was successfully controlled; all cases received full treatment as per national guidelines. Local communities received logistic and financial support from the national level and all fieldwork was conducted by Mayan people. Playa Pataxte is a remote area located in a forested area inhabited by Mayan people, mostly poor and historically engaged in farming activities. Malaria prevention interventions should be planned before harvesting time to prevent further outbreaks in EE. Community engagement demonstrated the importance of effective localization and response.

6405

PREDICTING MALARIA-SPECIFIC HEALTHCARE ACCESS AND UTILIZATION IN THE DEMOCRATIC REPUBLIC OF THE CONGO: A SYNTHESIS OF GEOSPATIAL, TREATMENT-SEEKING, AND PROVIDER-BASED DETERMINANTS

Alyssa J. Young[1], Ewan Cameron[2], Johanna Karemere Nzgire[3], Julie Hernandez[1], Joshua Yukich[1], Thomas P. Eisele[1], Ruth Ashton[1]

[1]Tulane School of Public Health and Tropical Medicine, New Orleans, LA, United States, [2]Malaria Atlas Project, Telethon Kids Institute, Perth, Australia, [3]Democratic Republic of the Congo Ministry of Health and Sanitation, Kinshasa, Congo, Democratic Republic of the

The Democratic Republic of the Congo (DRC) observes the second highest number of malaria cases and deaths globally. Malaria morbidity and mortality are impacted by access to healthcare, which is influenced by a range of interrelated patient- and provider-based determinants. Increased travel times to healthcare service point locations (HCSPs) have shown to be associated with poorer treatment seeking behavior for malaria. Physical access to HCSPs, however, does not guarantee receipt of necessary, or quality, healthcare services. Health facility service readiness (HFSR), or the ability of an HCSP to diagnose and treat an illness, also impacts an individual's ability to access healthcare. A data-driven understanding of dynamics such as health service availability, treatment-seeking behavior, demographic data, and HFSR is imperative for designing interventions that remove barriers and improve access to malaria care. This research investigates the influence these factors have on healthcare service utilization in the DRC. A gravity model combining HCSP geolocation data, population estimates, friction surface-derived travel times, and reported HCSP service populations is used to re-define HCSP catchment areas in the DRC. A malaria-specific HFSR index is constructed using results from principal component analyses on data from the DRC Service Provision Assessment (SPA). An alternative HFSR index is also created using the same data source, however, adopting a non-weighted, additive approach. These indices are combined with gravity model outputs

and 2013-14 Demographic and Health Survey (DHS) data in a survey-weighted multivariable logistic regression model to investigate prediction capabilities of seeking treatment of fever in the formal sector for children under 5. Receiver operating characteristic (ROC) curves determine which combination of predictors, including HFSR indices, produce the most successful regression model. Results from this research can be used to refine estimates of key malaria indicators and inform targeting of malaria interventions in the DRC to improve access to and seeking treatment for suspected malaria.

6406

LONGITUDINAL ANALYSIS OF THE INFANT GUT MICROBIOTA REVEALS EARLY LIFE PREDICTORS OF MALARIA SUSCEPTIBILITY

Christopher L. Dutton[1], Felicien M. Maisha[2], Madison A. Follis[1], Connie J. Mulligan[1], **Julie M. Moore**[1]

[1]University of Florida, Gainesville, FL, United States, [2]HEAL Africa Hospital, Goma, Congo, Democratic Republic of the

The gut microbiome is both influenced by and can influence susceptibility to malaria infection. The nature of these interactions in infancy, when the gut microbiome rapidly develops and profoundly impacts infant development, is understudied. We addressed this problem in a cohort of 47 infants in Goma, Democratic Republic of Congo. Stool sampled at 6 weeks and 3, 6 and 12 months of age, as well as at "sick" visits and following malaria treatment, was subjected to full length 16S rRNA gene sequencing. Overall, 17 infants had at least one malaria episode. No significant differences in alpha and beta diversity of gut bacteria were found between infants with an active malaria infection and those who never presented with malaria. However, decreases in alpha and beta diversity were observed in post-treatment samples relative to samples at time of diagnosis. Stratification of infants into "malaria" (infection at any time during follow-up) and "no malaria" groups revealed significant differences in abundance for 11 to 20 bacterial species at the four main time points. Infants with high abundance of *Bifidobacterium* were less likely to develop malaria whereas those with high abundance of *Klebsiella* were more susceptible. Of note, *Lactobacillus gasseri* and *Akkermansia muciniphila* tended to be more abundant among malaria-free infants relative to those who developed malaria, reaching significance prior to or at 6 months of age. At one year of age, those infants that carried *Bacteroides xylanisolvens* and *Bifidobacterium catenulatum* were more likely to have remained malaria free. Strikingly, machine learning and a random forest with the Boruta feature selection algorithm applied to 6 week gut microbiota predicted with 73% accuracy which infants would develop malaria in their first year of life, with key discriminating bacteria being Bifidobacteriaceae and Streptococcaceae. These data are consistent with other work that has identified key associations between the human and murine gut microbiota and malaria susceptibility, and provide impetus to functionally characterize gut microbiome signatures that can predict risk for malaria infection in infancy.

6407

PEAK PARASITEMIA AND CLINICAL FEATURES OF EXPERIMENTAL BLOOD STAGE MALARIA INFECTION

Louise Marquart-Wilson[1], Efrosinia Krejany[2], Ria Woo[3], John Woodford[3], Rebecca Webster[3], Stephan Chalon[4], Jörg J. Möhrle[4], Bridget Barber[3], **James S. McCarthy**[2]

[1]University of Queensland, Brisbane, Australia, [2]University of Melbourne, Melbourne, Australia, [3]QIMR Berghofer, Brisbane, Australia, [4]Medicines for Malaria Venture, Geneva, Switzerland

Clinical trials entailing controlled human malaria infections (CHIMI) have become increasingly important to define the activity of investigational antimalarial drugs and vaccines. Where the activity of drugs or vaccines against the blood stages of Plasmodium falciparum is the parameter of interest, there is a need to balance collection of the largest amount of data to define parasite growth while protecting the safety of participants who are at risk of harm if parasite infection progresses beyond an acceptable level. To assist in defining a safe level of target parasitemia that can be reached in such studies before rescue treatment is administered, we undertook a retrospective analysis of peak parasitemia and adverse events among study participants undergoing CHMI via the induced blood stage infection model (IBSM) at a single centre, QIMR Berghofer, Brisbane, Australia. Data from 271 participants enrolled in 21 studies between 2010 and 2023 were available. Parasitemia was quantified using a validated qPCR method, and adverse event data were extracted from study reports. 5 participants were excluded from analysis (1 withdrawal; 4 early rescue treatment). Inoculum size varied from 1,800 parasitised red blood cells (pRBC), (n=129, 2,300 pRBC (n=9) to 2,800 (n=128); treatment day varied from day 7 post-inoculation (n=77), day 8 (n=182) to day 9 (n=7). Among the 266 participants analysed, the median peak parasitemia was 7,598 parasites/mL (Range: 30 - 393,678). 29 subjects had a peak parasitemia >50,000/mL. Adverse events of mild to moderate severity were common across all groups, and consisted of those commonly observed in early malaria (headache [n=197], fever [n=122], myalgia [n=116], leukopenia [n=30] and thrombocytopenia [n=11]). No participants developed clinical or laboratory features of severe malaria, and all adverse events resolved spontaneously. This analysis provides a useful framework for regulatory and scientific evaluation, and safety oversight of this important clinical trial system for drug and vaccine development for malaria.

6408

COMPARISON OF THE PERFORMANCE OF AUTO-REGRESSIVE MOVING AVERAGE (ARIMA) TIME SERIES MODELS AND FB-PROPHET IN THE PREDICTION OF MALARIA INCIDENCE IN UGANDA AT THE NATIONAL AND SUBNATIONAL LEVEL

Benjamin P. Fuller[1], Herbert Kirya Isabirye[2], Richard Ssekitoleko[3], Margaret R. Lawrence[4], Christopher C. Moore[1]

[1]University of Virginia Health, Charlottesville, VA, United States, [2]Uganda Ministry of Health, Kampala, Uganda, [3]World Health Organization, Kampala, Uganda, [4]University of Virginia School of Medicine, Charlottesville, VA, United States

Malaria is caused by a protozoan parasite, genus *Plasmodium*, and is considered endemic throughout East Africa. According to the WHO Malaria report from 2020, there are an estimated 250 million cases each year, with over 94% of global cases in the WHO Africa region. With 5% of global cases, Uganda carries the third largest burden of malarial disease within sub-Saharan Africa. Despite years of focus on combatting malaria by the Ugandan Government and international implementing partners the incidence rate has risen dramatically. In 2023, Uganda instituted an incident command structure at the national level to systematically address the acute rise in case burden. Due to the stochastic nature of the recent rise in cases, appropriate need-based resource allocation has become a major challenge. To better identify areas at risk, we aimed to create an accurate prediction model of malaria incidence in Uganda. Using malaria case logs from DHIS2 dating back to 2020, we created auto-regressive moving average (ARIMA) models of malaria incidence in Uganda on a national and subnational level. We applied the same data to FB-Prophet, a pre-existing algorithm created by Facebook designed to model and predict univariate time series data by combining trends within the data and seasonal variations quickly and accurately. Once the forecasts for January through June of 2024 were complete, we then compared the performance of FB-Prophet with the newly generated ARIMA model. At the national level, the classic ARIMA model outperformed FB-Prophet in 2 out of 3 statistical accuracy tests (Mean absolute error 0.074 vs 0.081, Mean absolute percentage error 22.6 vs 30.2, Root mean square error 0.10 vs 0.085). At the subnational level, ARIMA again outperformed FB-Prophet in 12 (92%) of 13 regions modeled. Overall, both the classic ARIMA time series model and FB-Prophet performed well in forecasting malaria incidence at both the national and subnational level. This prediction capability can be used to assist policy makers to improve need-based resource allocation at the national and regional level throughout Uganda.

6409

MODELLING *PLASMODIUM VIVAX* AND *P. FALCIPARUM* CO-INFECTIONS WITH HETEROGENEITY IN MOSQUITO BITING EXPOSURE

Mathilde Grimée, Aimee Taylor, Michael White
Institut Pasteur, Paris, France

Plasmodium vivax and *P. falciparum*, two of the parasites causing human malaria, co-circulate in regions of Southeast Asia, Eastern Africa, and South and Central America. As anti-malaria efforts in the past decades primarily have targeted *P. falciparum*, the more resilient *P. vivax* has taken over as the predominant parasite. In the affected regions of the world, research is now focusing on identifying effective anti-*vivax* strategies. Modelling can help us identify and explore how both parasites interact on an epidemiological level and how this can impact the effect of these anti-malaria interventions. We present a novel compartmental model that investigates co-infections between these parasites while considering heterogeneity in mosquito biting exposure as a key factor shaping their entangled epidemiology. Heterogeneity in mosquito biting exposure, which has been shown to occur mostly in low-incidence settings, virtually reduces the effective population size, artificially keeping the prevalence over the whole population low, while forcing more coinfections than what we would expect. Through a systematic review we created a robust dataset on the prevalence of *P. vivax*, *P. falciparum*, and coinfections across different settings, which served as the foundation for model development. This data was also used to fit the model through Approximate Bayesian Computation. Finally, we used the model to conduct scenario simulations exploring two potential mechanisms of interaction between *P. falciparum* and *P. vivax* of particular public health relevance. First, we showed the added benefit of treating *P. falciparum* cases with radical cure targeting *P. vivax* liver stages in high-heterogeneity settings. Then, we explored the virtually unanswered question of the mechanisms governing *P. vivax* relapses, by simulating the previously proposed hypothesis that *P. falciparum*-induced fevers can trigger them.

6410

AN INVESTIGATION OF A *PLASMODIUM FALCIPARUM* ODYSSEAN MALARIA CASE IN AN INFORMAL SETTLEMENT, GAUTENG, SOUTH AFRICA, JANUARY 2024

Shanal Nair[1], Charlotte Sriruttan-Nel[1], **Maxwell Mabona**[1], Ntobeko Zondi[1], Noluvuyo Mqulo[2], Sibongile Mngomezulu[2], Basil Brooke[1]
[1]National Institute For Communicable Diseases, Johannesburg, South Africa, [2]Communicable Disease Control, Tshwane District, Pretoria, South Africa

South Africa is nearing malaria elimination, with active transmission confined to low-altitude border regions recognized as endemic. However, occurrences of odyssean malaria persist, particularly in Gauteng Province, a non-malaria endemic area. Odyssean malaria results from malaria parasite-infected mosquitoes inadvertently transported to non-malarious regions through various transportation modes (sea, air, rail, road). On January 22, 2024, a suspected case of odyssean malaria emerged in an informal settlement in Gauteng, reported to the National Institute for Communicable Diseases. This study delineates the case investigation, epidemiology, identifies risk factors, and proposes preventive and control measures. An odyssean malaria case was defined as malaria confirmed by microscopy and/or rapid diagnostic test without travel history, with mechanical transmission excluded. Descriptive analysis of the case investigation was conducted, including clinical and laboratory record review, site visits, patient interviews, and entomological surveys for adult mosquitoes and larvae in potential breeding sites. Odyssean malaria was confirmed in an 11-year-old male presenting with fever, headache, confusion, dizziness, and malaise, with no history of blood transfusions or travel to malaria-endemic areas. *Plasmodium falciparum* trophozoites were detected on microscopy (<0.1% count), accompanied by bicytopenia and anemia. The case's household was in close proximity to busy taxi ranks frequented by commuters from malaria-endemic areas. No *Anopheles* mosquitoes or larvae were found in the household, but *Culex* mosquito larvae were observed in a man-made water well. The case likely contracted malaria from infective mosquitoes inadvertently transported from malaria-endemic regions via taxis, due to the proximity of the taxi rank to the household. Recommendations include covering the well and eliminating stagnant water to reduce mosquito breeding. Healthcare providers in Gauteng should maintain vigilance for malaria, considering it in the differential diagnosis even in patients lacking travel history to malaria-endemic areas.

6411

MALARIA OUTBREAK INVESTIGATION IN MARSABIT COUNTY, KENYA - MARCH 2024

Peter Wachira Muguku[1], Megumi Itoh[2], James Sang[3], Martha Adhi[4], Emmanuel Sigei[1], Lydiah Khalayi[1], Ahmed Abade[1], Fredrick Odhiambo[1]
[1]Kenya Field Epidemiology and Laboratory Training Program, Nairobi, Kenya, [2]U.S. President's Malaria Initiative, US Centers for Disease Control and Prevention, Nairobi, Kenya, [3]National Malaria Control Program, Nairobi, Kenya, [4]County Department of Health, Marsabit, Kenya

Malaria epidemiology in Kenya is heterogenous due to geographic and climactic differences. Marsabit, a semi-arid seasonal malaria zone with wide variation in temperatures (15°C—26°C) and rainfall (200—1000mm per annum), is inhabited by pastoralists. In Dec 2023—Jan 2024, it experienced increased rainfall. Malaria cases increased by 345% (934 from 210 in the same period the previous year). An outbreak investigation was conducted to guide prevention and control efforts. We reviewed surveillance data from Dec 2023—Feb 2024, abstracted data from 12 facilities that surpassed action thresholds (5-year weekly mean + 1.5 Standard Deviation), conducted data quality assessments and descriptive analysis of confirmed cases. We conducted environmental risk assessments, key informant interviews and focus group discussions with community members to assess knowledge, perception and practice on malaria epidemiology, case management, prevention and control. We summarized data using median, frequencies, and proportions. Of 757 malaria cases abstracted, 424 (56.8%) were male, 227 (30.7%) age 10-20 years (median 17, Inter Quartile Range=10, 28 years) and 421 (55.6%) diagnosed by *P. falciparum*-specific rapid diagnostic tests. Reporting accuracy was 90% and test positivity saw a >2-fold increase from 28.8% (Dec) to 65.7% (Jan). Species breakdown by microscopy was as follows: 94.9% *P. falciparum*, 2.7% *P. vivax* and 2.3% *P. ovale*. There were 90 (11.9%) severe malaria cases and 5 deaths (Case Fatality Rate 0.7%). Proximity to a national park, stagnant water, and sleeping outside during herding were observed and identified by community as potential environmental risk factors. An outbreak was confirmed with predominance of *P. falciparum* but also presence of other species. Risk factors for males 10—20 years included sleeping outdoors during herding. Leveraging community knowledge on malaria transmission and community engagement in malaria control efforts tailored for nomadic pastoralists may be helpful in this context. Strengthening microscopy and continued monitoring of malaria species should be considered to improve malaria surveillance.

6412

MODELING RECURRENT MALARIA EPISODES OF MALARIA USING MARKOV MULTIPLE-STATE MODELS: A CASE STUDY FOR DANGASSA, MALI.

Fousseyni Kane[1], Mahamoudou Toure[1], Nafomon Sogoba[2], Moussa Keita[2], Daouda Sanogo[1], Sory Ibrahim Diawara[2], Drissa Konate[1], Soumba Keita[1], Alyssa Barry[3], Jeffey G. Shaffer[4], Mahamadou Diakite[1], Seydou Doumbia[1]
[1]University Clinical Research Center /ICERMali / USTTB, Bamako, Mali, [2]Malaria research training center/ICERMali / USTTB, Bamako, Mali, [3]School of Medicine, Deakin University, Bamako, Australia, [4]School of Public Health and Tropical Medicine, Tulane University, New Orleans, Bamako, LA, United States

In malaria-endemic areas, the incidence of clinical malaria reported from health centers may be overestimated because of recurrent events for individuals living in these areas. It is not well-established how the recurrence

of malaria cases affects malaria reporting and resource allocation. This analysis assesses the characteristics of malaria recurrence in Dangassa, Mali. Longitudinal cohort data from April 2018 to December 2023 were obtained from the Mali International Centers of Excellence for Malaria Research (ICEMR) and classified by age, season, prevalence of resistance molecular marker, and multiplicity of infection (MOI). Markovian multiple-state models were used to determine malaria incidence at the individual level while accounting for undetected parasitemia or confirmed malaria illness states. Analyses were performed to determine the patient's transition probabilities per state. A total of 10,688 visits with patients presenting malaria symptoms during the passive cases were included. Patients who reached a confirmed state more than three times a year ranged from 9.1% in 2018 to 24.0% in 2023. Significant variation was observed within patient age groups, with 11.0%, 48.0%, 25.0%, and 9.6% for patients aged under 5 years, 5 to 9 years, and 10 to 14 years, and 15 years old and over, respectively. The transition probabilities were 7.6% for the undetectable group, 10.69% for those transitioning to the confirmed state, 72.0% for those remaining in the confirmed state, and 9.6% for those returning to the undetected state. Younger age groups shoed a higher probability of moving from undetected to the confirmed state than older groups. Seasonal variation also significantly affected state transitions. Molecular marker prevalence and yearly MOI were strongly associated with remaining in a confirmed state. The results of this study showed changes in the probability of malaria recurrence in a patient depend on several factors. Adapting control methods to transmission intensity with appropriate community use and compliance with malaria treatment are required to efficiently control the malaria burden in endemic areas.

6413

EFFECT OF RAINFALL AND TEMPERATURE ANOMALIES ON MALARIA INCIDENCE IN THE DEMOCRATIC REPUBLIC OF THE CONGO

Donal Bisanzio[1], Carrie Ngongo[2], Brian Hutchinson[2], Karl Angendu Baki[3], Pierre Akilimali[3]

[1]RTI International, Washington, DC, United States, [2]RTI International, Seattle, WA, United States, [3]Institut National de Sante Publique, Kinshasa, Congo, Democratic Republic of the

Climate change is one of the main factors that could affect future trend of malaria cases in endemic countries. This research aimed to investigate the effect of weather indicators on malaria cases in DRC. Monthly data on population and reported malaria cases were gathered from the DRC's national health management information system (HMIS) for all health zones 2018 and 2023. Monthly temperature and precipitation were estimated using remote sensing data from MODIS and from the Global Precipitation Climatology Centre. Time series analyses were performed to capture trends of malaria and weather indicators. The relationship between malaria incidence and weather indicators was investigated using generalized additive random effect models. From January 2018 to December 2023, 116,835,122 malaria cases were reported in DRC's HMIS. Malaria cases increased from 156.1 cases per 1,000 people to 182.6 cases per 1,000 CU5 (16.9%) from 2018 to 2023. The fraction of severe malaria cases among reported cases declined by 12.3% from 2018 to 2023. Average temperatures increased (mean overall increase: +0.5C°, 95% CI= +0.4C°; +0.7C°) and were correlated with a decrease of monthly precipitation (mean decrease: +12mm; 95% CI: +9 mm; +15 mm). Malaria incidence was significantly associated with weather conditions occurring in the previous 5 months. The association between malaria incidence and rainfall was larger than with temperature. Understanding the relationship-between malaria and climate could help health systems to adapt their surveillance and intervention approaches and strategies to climate change.

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GENETIC DIVERSITY OF *PLASMODIUM VIVAX* DUFFY BINDING PROTEIN IN ETHIOPIA AND COMPARISON WITH OTHER GEOGRAPHICAL ISOLATES

Abnet Abebe, **Abnet Abebe**

Ethiopian Public Health Institute, Addis Ababa, Ethiopia

Plasmodium vivax Duffy binding protein (PvDBP) is a merozoite surface protein located in the micronemes of *P. vivax*. The invasion of human reticulocytes by *P. vivax* merozoites depends on the parasite DBP binding domain engaging Duffy Antigen Receptor for Chemokine (DARC) on these red blood cells (RBCs). PvDBPII shows high genetic diversity which is a major challenge to its use in the development of a vaccine against *vivax* malaria. A total of 58 blood samples confirmed positive for *P. vivax* by Polymerase Chain Reaction (PCR) were included in the study to determine PvDBPII genetic diversity. PvDBPII were amplified using primers designed from reference sequence of *P. vivax* Sal I. Alignment and phylogenetic tree constructions using MEGA version 10.1.1. Nucleotide diversity and haplotype diversity were analysed using DnaSP, and haplotype network was generated with PopART. The mean age of the participants was 25 years, 5 (8.6%) participants were Duffy negatives. From the 58 PvDBPII sequences, seven haplotypes based on nucleotide differences at 8 positions were identified. Nucleotide diversity and haplotype diversity were 0.00267 ± 0.00023 and 0.731 ±0.036, respectively. Globally, a total of 39 haplotypes were identified from 223 PvDBPII sequences representing different geographical isolates obtained from NCBI archive. The nucleotide and haplotype diversity were 0.00373 and 0.845 ±0.015, respectively. The haplotype prevalence ranged from 0.45% to 27.3%. Two haplotypes were shared among isolates from all geographical areas of the globe. PvDBPII of the Ethiopian *P. vivax* isolates showed low nucleotide but high haplotype diversity. Among the Ethiopian *P. vivax* isolates, almost half of the sequences were identical to the Sal-I reference sequence. However, there were unique haplotypes observed in the Ethiopian isolates, which does not share with isolates from other geographical areas. Categorizing population haplotype frequency can help to determine common haplotypes for designing an effective blood-stage vaccine which will have a significant role for the control and elimination of *P. vivax*.

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POPULATION GENOMICS OF *PLASMODIUM MALARIAE* FROM FOUR AFRICAN COUNTRIES

Zachary R. Popkin-Hall[1], Kelly Carey-Ewend[1], Eniyou C. Oriero[2], Misago D. Seth[3], Melchior M. Kashamuka[4], Billy Ngasala[5], Innocent M. Ali[6], Eric S. Mukomena[7], Celine I. Mandara[3], Rachel Sendor[1], Alfred Simkin[8], Alfred Amambua-Ngwa[2], Antoinette Tshefu[4], Abebe A. Fola[8], Jeffrey A. Bailey[8], Deus S. Ishengoma[3], Jonathan B. Parr[1], Jessica T. Lin[1], Jonathan J. Juliano[1]

[1]University of North Carolina, Chapel Hill, NC, United States, [2]MRC Unit The Gambia at LSHTM, Fajara, Gambia, [3]National Institute for Medical Research, Dar es Salaam, Tanzania, United Republic of, [4]Kinshasa School of Public Health, Kinshasa, Congo, Democratic Republic of the, [5]Muhimbili University of Health and Allied Sciences, Bagamoyo, Tanzania, United Republic of, [6]Université de Dschang, Dschang, Cameroon, [7]Programme National de Lutte contre le Paludisme, Kinshasa, Congo, Democratic Republic of the, [8]Brown University, Providence, RI, United States

Plasmodium malariae (*Pm*) is already prevalent in some African regions and may become more prevalent as *P. falciparum* (*Pf*) declines. *Pm* has been more frequently identified in West and Central Africa, while it is generally rare in East Africa. While *Pf* and both *P. ovale* species (*Po*) have shown evidence of population structure in Africa, a previous study of *Pm* using microsatellites found no clear geographic structure and high intrapopulation diversity. To better understand this neglected but widespread malaria pathogen, we completed the largest genomic study of *Pm* to-date by performing hybrid capture and sequencing of 77 *Pm* isolates collected from Cameroon (n = 7), the Democratic Republic of the Congo (n = 16), Nigeria (n = 4), and Tanzania (n = 50) between 2015 and 2021. In addition, we selected 76 publicly available *Pf* genomes to spatially match the origins

of our *Pm* isolates. We found no significant geographic separation by principal component analysis among these 77 *Pm* isolates. The majority of isolates (92.2%, n = 71/77) were monoclonal. When comparing 1-1 gene orthologs to geographically matched *Pf* samples, we identified significantly less nucleotide diversity and lower SNP density in *Pm* compared to *Pf*. Genome-wide scans of selection identified several regions under either balancing or directional selection. In contrast to studies in *Pf*, *Po* and *P. vivax*, Tajima's D scans for balancing selection identified no orthologous antigens to falciparum vaccine candidates as top hits, while nS_L scans for directional selection detected no putative antimalarial resistance genes as top hits. Using a candidate gene approach to identify signatures of selection at these loci, six putative drug resistance markers (CRT, DHFR, DHPS, MDR1, MRP1, and MRP2) show significant evidence of selective sweeps as determined by the DH test, a combination of Tajima's D and Fay and Wu's H that is more robust to demographic influences than either test alone. Our findings of low genetic diversity and no geographical population structure suggest that *Pm* is atypical compared to other human malaria species and may be subject to distinct evolutionary pressures.

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GENE EXPRESSION NETWORKS IN STAGE-CONTROLLED *PLASMODIUM VIVAX* INFECTIONS FROM NORTHERN THAILAND: A WEIGHTED GENE CO-EXPRESSION NETWORK ANALYSIS

Graham Ellis[1], Francis C. Motta[2], Suwanna Chaorattanakawee[3], Nichaphat Uthaimongkol[3], Worachet Kuntawunginn[3], Chadin Thongpiam[3], Chatchadaporn Thamnurak[3], Montri Arsanok[3], Mariusz Wojnarski[1], Pattaraporn Vanchayangkul[3], Nonlawat Boonyalai[3], Philip L. Smith[4], Michele D. Spring[5], Krisada Jongsakul[3], Ilin Chuang[6], Sidhartha Chaudhury[3], Jeffrey Livezey[3], Steven B. Haase[7]

[1]Walter Reed National Military Medical Center, Bethesda, MD, United States, [2]Florida Atlantic University, Boca Raton, FL, United States, [3]US-Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand, [4]U.S. Military HIV Research Program Walter Reed Army Institute of Research, Bethesda, MD, United States, [5]SUNY Upstate Medical University, Syracuse, NY, United States, [6]US Naval Medical Research Center-Asia, Singapore, Singapore, [7]Duke University, Durham, NC, United States

Genomic methods have identified virulence factors and antigenic targets in *falciparum* malaria; however, the virulence determinants of relapsing malaria, *Plasmodium vivax*, remain less well-known due in part to notorious challenges in culturing the parasite *in-vitro*. *P. vivax's* distinct ecology and propensity for recrudescent infection threatens future eradication efforts. We present the analysis of a weighted gene co-expression network for *P. vivax* from ten *ex-vivo* parasite stage-synchronized infections against various host clinical variables. *P. vivax* isolates, which were confirmed by microscopy to be in the predominantly early-trophozoite stage of bloodstream infection, were collected from ten patients in northeastern Thailand prior to treatment and cultured *ex-vivo* over a 48-hour period with sampling every 3 hours. Complete gene expression profiles were obtained using RNA-Seq. A co-expression network was constructed using the weighted gene co-expression network analysis (WGCNA) on the R statistical platform. Pairwise gene expression correlations across the cohort were used to develop a matrix of gene correlation coefficients. Using hierarchical clustering, the *P. vivax* genome was grouped into 22 gene modules which showed stability to resampling and at various soft-thresholding powers. Statistically significant correlations were identified between specific gene co-expression modules and clinical variables collected from patients, including baseline laboratory parameters, history of malarial infection, and sleep patterns. A functional enrichment analysis of these modules using PlasmoDB explored gene functions correlating with these clinical variables. Our identification of co-expressed gene modules, preserved across resampling and aligned to logical gene functions, in an unique parasite stage-controlled dataset, provides a platform for hypothesis-generation and further genome exploration of parasite virulence in *P. vivax* infections.

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USE OF GENETIC METRICS TO CHARACTERIZE MALARIA TRANSMISSION PATTERNS AND DISTINGUISH COTRANSMISSION FROM SUPERINFECTION IN BURKINA FASO

Issiaka Soulama[1], Emilie Badoum[2], Daouda Ouattara[3], Amidou Diarra[3], Wesley Wong[4], Maurice Ouattara[3], Shao Kairon[4], Anna Burkhard[4], Amidou Ouédraogo[3], Jean Sawadogo[3], Issa Nebié Ouédraogo[3], Alphonse Ouédraogo[3], Daouda Ndiaye[5], Sarah K. Volkman[4], Alfred B. Tiono[3], Dyann F. Wirth[4], Sodiomon B. Sirima[3]

[1]Institut de Recherche en Sciences de la Santé (IRSS), Ouagadougou, Burkina Faso, [2]2Groupe de Recherche Action en Santé (GRAS), Ouagadougou, Burkina Faso, [3]Groupe de Recherche Action en Santé (GRAS), Ouagadougou, Burkina Faso, [4]Harvard T.H. Chan School of Public Health (HSPH), Boston, MA, United States, [5]CIGASS, Université Cheikh-Anta-Diop, Dakar, Sénégal, Dakar, Senegal

Traditional malaria surveillance uses clinical information, rapid diagnostic tests (RDTs) or microscopy. These tools provide important information about clinical cases, but cannot provide comprehensive and detailed data on transmission dynamics or changes in the parasite population with intervention use. Molecular surveillance can provide more granular details about the modality of malaria transmission and promises to help guide intervention stratification and impact. Burkina Faso, with a high malaria burden, has recently introduced the RTS,S vaccine. Molecular strategies provide excellent tools for monitoring parasite population dynamics and threats to elimination including drug resistance. We conducted a pilot study at two National Malaria Control Program sentinel sites, Banfora and Kaya, with distinct malaria transmission contexts, and evaluated parasite population structure with a 24 single nucleotide polymorphism (SNP) genotyping tool for Plasmodium falciparum. The study objective was to describe basic transmission characteristics, to distinguish monogenomic from polygenomic infections, to quantify the relative cotransmission and superinfection levels using R_H, and to identify any clonal infections. We enrolled 937 febrile RDT+ individuals, 468 and 469 from Kaya and Banfora respectively. The results found a higher polygenomic fraction (0.61 [CI: 0.50, 0.72] in Kaya, compared to Banfora (polygenomic fraction of 0.46 [0.37, 0.56]). R_H analysis showed that the majority of the polygenomic samples (COI ≥ 2) from both Banfora (0.74, [0.62, 0.85]) and Kaya (0.76, [0.65, 0.88]) were the result of superinfection, and there was no evidence of clonal parasites, consistent with a high level of transmission. These baseline data provide critical information for evaluating intervention impact including malaria vaccination. Ongoing work will help generate a comprehensive picture of the genomic epidemiology landscape of parasites in Burkina Faso, and monitor changes to the parasite population structure as various interventions, including the RTS,S vaccine are being implemented.

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GENOME-WIDE ASSOCIATION STUDY OF GLOBAL *PLASMODIUM VIVAX* POPULATIONS PROVIDES INSIGHTS INTO THE EVOLUTION OF DRUG RESISTANCE

Gabrielle C. Ngwana-Joseph[1], Jody E. Phelan[1], Emilia Manko[1], Jamille G. Dombrowski[1], Simone da Silva Santos[2], Martha Suarez-Mutis[2], Ricardo L. Dantas Machado[3], Claudio R. F. Marinho[4], Debbie Nolder[1], Francois Nosten[5], Colin J. Sutherland[1], Susana Campino[1], Taane G. Clark[1]

[1]London School of Hygiene and Tropical Medicine, London, United Kingdom, [2]Instituto Oswaldo Cruz, Sao Paulo, Brazil, [3]Universidade Federal Fluminense, Rio de Janeiro, Brazil, [4]University of Sao Paulo, Sao Paulo, Brazil, [5]University of Oxford, Oxford, United Kingdom

Plasmodium vivax malaria is a serious global health concern, putting 3 billion people at risk each year. Resistance to chloroquine, the current front-line antimalarial drug for *P. vivax* treatment, has increased globally. Consequently, several countries, including Indonesia, which is the epicentre of chloroquine resistance (CQR), have now adopted dihydroartemisinin-piperaquine as a frontline treatment instead. Although several genes have been posited as putative determinants of CQR, including the multidrug resistance gene, *pvmdr1*, the evidence of this candidate in driving CQR is conflicting. Using a genome-wide approach, we perform a genomic analysis of 1,534 *P. vivax* isolates across 29 endemic countries, detailing population

structure, patterns of relatedness, selection, and resistance profiling, providing insight into putative drivers of CQR. Differential selection metrics applied between isolates from low-grade and high-grade CQR regions revealed sweeps in a locus proximal to *pvmdr1* and in transcriptional regulation genes, suggesting transcriptional control underpins CQR. Our investigation of the temporal dynamics of selective sweeps in 106 isolates from the high-grade CQR region Indonesian Papua between 2008-2017 revealed *pvmrp1* as an emerging candidate for piperaquine resistance. Overall, our work provides novel markers for resistance surveillance in candidate loci, supported by evidence of regions under recent directional selection in this continually evolving parasite.

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IMPACT OF SICKLE CELL GENOTYPES ON PEDIATRIC MALARIA OUTCOMES IN A HOLOENDEMIC *PLASMODIUM FALCIPARUM* TRANSMISSION REGION: INSIGHTS FROM A LONGITUDINAL STUDY

Nikita Jaiswal[1], Clinton Onyango[2], Qiuying Cheng[3], Ivy Hurwitz[3], Perez Olewe[4], Samuel Anyona[2], Evans Raballah[5], Collins Ouma[2], Kristan Schneider[3], Douglas J. Perkins[3]

[1]University of New Mexico School of Medicine, Albuquerque, NM, United States, [2]Maseno University, Maseno, Kenya, [3]University of New Mexico Health Sciences Center, Albuquerque, NM, United States, [4]University of New Mexico-Kenya Global Health Programs, Kisumu and Siaya, Kenya, [5]Masinde Muliro University of Science and Technology, Kakamega, Kenya

Malaria remains a major health challenge in the World Health Organization African region. Pediatric patients (<5 years of age) accounted for 80% of malaria-related deaths in the region due to naïve immunity. Malaria exerts a strong selective pressure, resulting in a high prevalence of sickle cell trait (HbAS) and sickle-cell disease (HbSS). We examined the impact of the hemoglobinopathy on clinical outcomes in a pediatric cohort (aged 2-38 mos., n=1,619) followed for 36 mos. (>16,000 visits) in a holoendemic *Plasmodium falciparum* transmission region (Siaya, Kenya). Sickle-cell status, determined by hemoglobin (Hb) electrophoresis, identified 1,358 HbAA (homozygous dominant), 243 HbAS, and 18 HbSS children. We employed three regression models (logistic, Poisson, and Cox regression) to determine the relationship between the sickle cell genotypes and malaria outcomes [malaria episodes, severe malarial anemia (SMA, Hb<5.0 g/dL), and all-cause mortality], controlling for the following malarial risk factors: age at first visit, HIV status, sex, G6PD deficiency, alpha thalassemia, and time of last visit. HbAS confered protection against malarial episodes (IRR=0.809, *P*=2.22E-8; HR=0.815, *P*=6.7E-8) and SMA episodes (OR=0.511, *P*=0.175E-3; IRR=0.446, *P*=7.439E-5, HR=2.54,*P*=4.31E-3). Although HbSS was protective against malarial episodes (IRR=0.453, *P*=1.575E-6, HR=0.496, *P*=2.21E-5), carriage of the trait increased the risk of developing SMA (OR=6.841, *P*=5.125E-6, IRR=2.267, *P*=1.10E-2, HR=2.54, *P*=4.31E-3), and was associated with a seven-fold increase in all-cause mortality (HR=7.05, *P*=1.94E-4). The prospective longitudinal study shows that HbAS confers protection against both acquiring malaria and developing SMA. In contrast, while HbSS carriage is protective against malarial episodes, once infected, they have a 2.5 times greater risk of developing SMA and 7 times greater risk of mortality. In conclusion, findings here underscore the protective and detrimental roles of sickle cell genotypes in pediatric malaria, highlighting the complex interplay between genetic factors and malaria outcomes in endemic regions.

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EVALUATING HOW THE MEANING OF IDENTICAL BY DESCENT VARIES WITH MUTATION AND RECOMBINATION RATES

Janeesh K. Bansal[1], Meng-Chun Chang[2], Robert Verity[3], Richard A. Nichols[1], John Wakeley[4], **Hsiao-Han Chang**[2]

[1]Queen Mary University of London, London, United Kingdom, [2]National Tsing Hua University, Hsinchu City, Taiwan, [3]Imperial College London, London, United Kingdom, [4]Harvard University, Cambridge, MA, United States

Identical by descent (IBD) describes the genetic similarity between individuals resulting from shared ancestry. In the field of malaria genetic epidemiology, a high degree of IBD between individuals indicates proximity within a transmission chain. When integrated with epidemiological data, such as age or geographic location, IBD can help identify risk factors for transmission. However, the exact criteria for IBD remain unclear, especially regarding how recent the shared ancestry should be to classify two individuals or two gene segments as IBD. In this study, we applied coalescent simulations to explore interpretations of IBD across varying mutation and recombination rates. Genetic data were generated, and the proportion of IBD was estimated using a hidden Markov model-based method. This was followed by determining the time cutoff that best explained the estimated proportion of IBD. Our results show that the implicit cutoff period for designating IBD depends on recombination and mutation rates. An increase in the mutation rate or a decrease in the recombination rate loosens the criteria for IBD, thus increasing the probability of classifying samples as IBD. This observed trend can be partially explained by the number of generations that have experienced recombination, a parameter co-estimated with IBD proportion in the hidden Markov model framework. This variability exposes an inconsistency in IBD definitions across various mutation and recombination rates, indicating a need for a cautious interpretation of IBD, particularly when making comparisons across different transmission settings where effective recombination rates differ. Moreover, the number of SNPs used in IBD estimation can significantly alter the results. We recommend conducting sensitivity analyses to refine the understanding of IBD. Our study clarifies the meaning of IBD and provides critical insights into the interpretation of IBD. The findings have implications beyond malaria genetic epidemiology, extending to broader research that employs IBD concepts.

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DYSREGULATION OF VASO-OCCLUSIVE AND VASOCONSTRICTIVE MOLECULAR PATHWAYS IN PEDIATRIC PATIENTS WITH SICKLE CELL ANEMIA AND SEVERE MALARIA ANEMIA

Shamim W. Osata[1], Sharley W. Wasena[2], Qiuying Cheng[3], Evans Raballah[4], Ivy Hurwitz[3], Clinton O. Onyango[2], Philip D. Seidenberg[3], Benjamin H. McMahon[5], Kristan A. Schneider[6], Collins Ouma[7], Samuel B. Anyona[7], Douglas J. Perkins[3]

[1]University of New Mexico-Kenya Global Health Programs, Kisumu and Siaya, Kenya, [2]Department of Biomedical Sciences and Technology, School of Public Health and Community Development, Maseno University, Maseno, Kenya, [3]Department of Internal Medicine, Center for Global Health, University of New Mexico, Albuquerque, NM, United States, [4]Department of Medical Laboratory Sciences, School of Public Health, Biomedical Sciences and Technology, Masinde Muliro University of Science and Technology,, Kakamega, Kenya, [5]Theoretical Biology and Biophysics Group, Theoretical Division, Los Alamos National Laboratory, Los Alamos, NM, United States, [6]Department Applied Computer and Bio-Sciences, University of Applied Sciences Mittweida, Mittweida, Germany, [7]Department of Medical Biochemistry, School of Medicine, Maseno University, Maseno, Kenya

Malaria and sickle cell anemia (SCA, HbSS) represent significant health burdens, particularly in holoendemic *Plasmodium falciparum* regions such as Siaya County, western Kenya. Understanding the molecular landscape in children with SCA is crucial for improved therapeutics. As such, we explored the entire expressed blood transcriptome in children with severe

malarial anemia [hemoglobin (Hb)<6.0 g/dL], stratified into SCA and non-SCA (i.e., HbAA and HbAS). There were 2,820 differentially expressed genes (DEGs, FDR<0.05) with 2,110 upregulated and 710 downregulated in SCA. Canonical pathway enrichment analyses using MetaCore ™ revealed that SMA patients with SCA had enhanced dysregulation in established molecular networks for SCA: Role of Cell Adhesion in Vaso-occlusion (CAVO; FDR=4.778E-8), Role of Red Blood Cell Adhesion to Endothelium in Vaso-occlusion (RBCEVO; FDR=1.624E-4), and Role of Endothelin-1 in Inflammation and Vasoconstriction (E1IVC; FDR=4.835E-2). The pathways involved in vaso-occulsion (CAVO and RBCEVO) revealed that SCA was defined by increased transcripts for BCAM (+3.17), GLPA (+2.93), TfR1 (+1.94), VCAM1 (+1.35), and IL-1 alpha (+1.16), suggesting enhanced dysregulation of cellular adhesion, metabolism, iron homeostasis, endothelial activation, and inflammation. In addition, the pathway involved in vasoconstriction (E1IVC) demonstrated downregulation of EDNRA (-4.00), EDNRB (-2.32), IL-8 (-1.98), VEGFR1 (-1.65), and MAPK14 (-1.32) indicating a compensatory mechanism for improved blood flow in the context of endothelial dysfunction, tissue hypoxia, and vascular complications. These findings provide novel insight into the molecular interactions in children with SCA who develop severe malaria and the importance of considering both conditions concurrently for improved therapeutic interventions. We are currently investigating specific molecular mechanisms driving these dysregulations to develop targeted interventions tailored to this vulnerable population.

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DECLINING POLYMORPHISM OF THE C-TERMINUS MEROZOITE SURFACE PROTEIN 1 AMIDST INCREASED *PLASMODIUM KNOWLESI* TRANSMISSION IN THAILAND

Parsakorn Tapaopong

Mahidol University, Bangkok, Thailand

Recent reports from Thailand indicate a substantial increase in *Plasmodium knowlesi* cases over the past decade, with more than an eightfold increase in incidence by 2023 compared to 2018. In this study, we aim to investigate temporal changes in genetic polymorphisms associated with the increased transmission intensity of knowlesi malaria observed in Thailand over the past two decades. Briefly, genomic DNA from 25 recent *P. knowlesi* samples collected in Thailand from 2018 to 2023 underwent sequencing for the 42-kDa region of *pkmsp1*. Comparative analysis was conducted with 24 former *P. knowlesi* samples obtained from 2000 to 2009. Overall, 7 unique haplotypes were identified in recent samples, compared to 15 in former samples. Nucleotide diversity and haplotype diversity were lower in the recent period ($\pi = 0.016$, Hd = 0.817) than in the former ($\pi = 0.018$, Hd = 0.942). A significantly higher synonymous substitution rate was observed in both periods ($d_S - d_N = 2.77$ and 2.43, $p < 0.05$). Analysis of the 42-kDa region of *pkmsp1* revealed a decrease in the genetic diversity of *P. knowlesi* in Thailand, which is associated with higher transmission intensity. Purifying selection was evident, indicating that parasites carrying deleterious mutations were less likely to survive, leading to reduced genetic diversity over time. The remaining parasites demonstrated fitness and adaptability, reflecting the increased transmission intensity in recent years compared to former years. Population differentiation based on fixation index (F_{st}) revealed high genetic differentiation between parasite populations in central and southern Thailand or Malaysia. Conversely, the relatively lower F_{st} value between southern Thailand and Malaysia indicates a closer genetic relationship, possibly reflecting historical gene flow and shared ancestry between these neighboring regions. In conclusion, our findings highlight a decline in genetic diversity and evidence of purifying selection among the current higher incidence of *P. knowlesi* populations in Thailand, indicating an adaptive response to increased transmission intensity.

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PREDICTING THE FUNCTIONAL IMPACT OF STRUCTURAL VARIATION AT A *PLASMODIUM FALCIPARUM* SICKLE-ASSOCIATED LOCUS

Annie J. Forster[1], James Docker[1], Anna Jeffreys[1], Richard Pearson[2], Katie Healey[1], Mariateresa de Cesare[1], Alex J. Mentzer[1], Jason Hendry[3], Gavin Band[1]

[1]University of Oxford, Oxford, United Kingdom, [2]Wellcome Sanger Institute, Cambridge, United Kingdom, [3]Max Planck Institute, Berlin, Germany

Malaria parasites impart a strong selective force on the human genome, but whether human resistance mechanisms in turn shape parasite variation is less well understood. However, it was recently discovered that protein-altering mutations in three regions of the malaria parasite genome - termed *Plasmodium falciparum sickle-associated (Pfsa)* 1-3 - are strongly associated with the human sickle haemoglobin (HbS) polymorphism, which is normally thought to confer significant resistance to malaria infection. A natural hypothesis is that these '*Pfsa*+' mutations enable parasites to infect, grow, and cause disease in HbS-carrying individuals (an 'HbS-protection escape' phenotype). Uncovering the biological function of these regions is therefore a research priority. Here, we focus on the *Pfsa3* region where structural variation has previously been identified, but the extent to which this variation contributes to the HbS-protection escape phenotype remains unclear. We develop statistical methods to call structural variation in short-read whole-genome sequence data from > 5,000 infections from the MalariaGEN Pf6 resource, and from severe infections from The Gambia and Kenya. Our analysis identifies a set of structural variants that segregate at this locus, including both deletions and a set of ancestrally-related complex duplication variants that are closely linked to the *Pfsa3*+ mutation. These variants are shared across African parasite populations, implying a single mutational origin of *Pfsa3*+, yet they vary widely in frequency. We confirm this structure by using long-read sequencing to assemble the haplotype of the Uganda Palo Alto (FUP-H) isolate, a lab-adapted isolate carrying all three *Pfsa*+ mutations, showing that it carries the longest duplication variant. Moreover, these duplications are predicted to carry novel gene segments which we confirm by analysing available RNA-seq data for FUP-H against this assembly. Whether these additional transcripts contribute to the HbS-protection escape phenotype is not yet known.

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DEVELOPMENT OF *PLASMODIUM FALCIPARUM* WHOLE GENOME SEQUENCING WORKFLOW USING OXFORD NANOPORE SEQUENCING TECHNOLOGY TO SUPPORT MALARIA MOLECULAR SURVEILLANCE IN TANZANIA

Catherine Bakari[1], Aurel Holzschuh[2], Pierre Schneeberger[2], Celine I. Mandara[1], Jeffrey A. Bailey[3], Jonathan J. Juliano[4], Pascal Mäser[2], Deus S. Ishengoma[1], Christian Nsanzabana[2]

[1]National Institute for Medical Research, Dar es Salaam, Tanzania, United Republic of, [2]Swiss Tropical and Public Health Institute, Basel, Switzerland, [3]Brown University, Providence, RI, United States, [4]University of North Carolina, Chapel Hill, NC, United States

Whole-genome sequencing (WGS) of *Plasmodium falciparum* is increasingly becoming important for malaria molecular surveillance (MMS) and studies of other pathogens. Currently, MMS data generation relies predominantly on using short-read amplicon and whole genome sequencing (WGS) technology despite their shortfalls; and this has limited studies of the complex genome of *P. falciparum.* This challenge is even greater in most of malaria-endemic countries with limited resources for MMS. This study aimed at developing and optimizing a long-read-based WGS assay with Oxford Nanopore Technology (ONT) to explore structural variations in *P. falciparum* genome. To reduce human DNA contamination, we focused on depleting human DNA and/or enrichment of *P. falciparum* DNA. Mock samples were created by mixing laboratory strains in different ratios with uninfected human whole blood. Three DNA enrichment approaches were explored: enzyme digestion (McrBc and MspJI), NEBNext Microbiome DNA Enrichment Kit (NMDEK) and selective whole genome amplification (sWGA).

Multiplex qPCR of single-copy *P. falciparum* and human genes were used to evaluate workflow performance. qPCR results showed a significant reduction of human DNA with NMDEK alone, as evidenced by an increase in cycle threshold (ct) value from 22 to 30. Additional use of sWGA reduced human DNA with ct value going from 30 to 35; and increased parasite DNA (ct values from 26 to 16). Sequencing quality, coverage, and depth were thoroughly examined; and showed that a combination of NMDEK and sWGA gave a high mean read quality of 21 (>99% accuracy), with over 90% coverage across the chromosomes, and 88% of reads mapping to *P. falciparum*. Additional validation with field samples is underway and the results will be available soon. This workflow effectively balanced sequencing quality, coverage, and depth, and showed high potential for deployment and use in MMS in malaria-endemic countries.

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SURVEILLING *PLASMODIUM FALCIPARUM* AT FIRST ANTENATAL CARE VISITS THROUGH GENOMICS IN MOZAMBIQUE

Glória Matambisso[1], Clemente Silva[1], Dário Tembisse[1], Simone Boene[1], Paulino da Costa da Costa[1], Henriques Mbeve[1], Nelo Ndimande[1], Eduard Rovira-Vallbona[2], Nanna Brokhattingen[3], Andrés Aranda-Díaz Aranda-Díaz[4], Bryan Greenhouse[4], Neide Canana[5], Bernadete Rafael[6], Sónia Enosse[5], Maria Rodriques[5], Baltazar Candrinho[7], Francisco Saúte[1], Alfredo Mayor[8]

[1]Centro de Investigação em Saúde de Manhiça (CISM), Manhica district, Mozambique, [2]ISGlobal, Barcelona Institute for Global Health (ISGlobal),, Barcelona, Spain, [3]Barcelona Institute for Global Health (ISGlobal), Barcelona, Spain, [4]EPPIcenter Research Program, Division of HIV, Infectious Disease and Global Medicine, Department of Medicine, University of California San Francisco, California San Francisco, CA, United States, [5]Malaria Consortium (MC), Maputo, Mozambique, [6]National Malaria Control Program (NMCP), Ministry of Health, Maputo, Mozambique, [7]National Malaria Control Program (NMCP), Maputo, Mozambique, [8]Centro de Investigação em Saúde de Manhiça (CISM); ISGlobal, Barcelona Institute for Global Health (ISGlobal); Spanish Consortium for Research in Epidemiology and Public Health (CIBERESP) and Department of Physiologic Sciences, Faculty of Medicine., Manhica district, Mozambique

Pregnant women at first antenatal care (ANC) visit constitute a promising sentinel group for gathering information about malaria transmission. With the aim of testing if ANC-derived transmission and genomic metrics are representative of the wider population, we recruited pregnant women during their initial ANC visit throughout eight provinces, characterized by both low and medium-to-high intensity of malaria transmission, from January to December 2022. Simultaneously, infection result by RDT form children aged two to ten at health facilities and DHIS2 were recorded. Finger-prick blood samples onto filter papers were collected. qPCR positive samples form a random selection of up to 100 RDT-positive and 100 RDT-negative samples per province from pregnant women (PW) and all RDT-positive samples from children was submitted to sequencing. A total of 3940 PW were recruited. *Pf* positivity rate at ANC by RDT was lowest in the southern region (5.8%[119/2045]) and highest in the northern (41.6%[571/1374]), p<0.001). The preliminary genomic results show that among PW, no validated or candidate mutations of artemisinin and chloroquine resistance and no validated marker to amodiaquine were observed. A high prevalence of the *pfdhps-pfdhfr* quintuple mutant was observed (91.8%). However, no *pfdhps* A581G mutations were observed in pregnant women, which has been associated with reduced effectiveness of sulfadoxine-pyrimethamine (SP) for chemoprevention during pregnancy. In ANC attendees, the proportion of polyclonal infections was 55.5%. On average, PW had a multiplicity of infection of 3.04. Effective multiplicity of infection, was lower at 1.6, while 1-Wright's inbreeding coefficient was 0.21. Among ANC users, population mean expected heterozygosity across the 165 microhaplotype loci ranged from <0.01 to 0.92, with a mean of 0.57 (95%CI:0.54-0.59). Additionally, genetic metrics will be compared between PW and children. This study provides genomic information for the validation of an ANC-based malaria surveillance approach, in order to improve the programmatic performance of malaria control and elimination activities in Mozambique.

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ANALYTICAL VALIDATION OF A CAPILLARY ELECTROPHORESIS METHOD TO GENOTYPE *PLASMODIUM FALCIPARUM* GENES *MSP1*, *MSP2*, AND THE NEUTRAL MICROSATELLITE MARKER *POLY-*α

Culzean Kennedy[1], Breanna Horton[1], Zhiyong Zhou[2], Samaly Souza[2], Dragan Ljolje[2], Ira Goldman[2], Jessica McCaffery[2], Rispah Abdallah[1], Amy Kong[2], Stephen Lindstrom[2], Molly M. Freeman[2]

[1]Oak Ridge Institute for Science and Education, Oak Ridge, TN, United States, [2]Laboratory Science and Diagnostics Branch, US Centers for Disease Control and Prevention, Atlanta, GA, United States

To assess antimalarial drug efficacy, therapeutic efficacy studies (TES) use genotyping methods to distinguish new *Plasmodium falciparum* (Pf) malaria infections from recrudescence. The World Health Organization (WHO) recommends utilizing capillary electrophoresis (CE) to genotype merozoite surface proteins 1 (*msp1*) and 2 (*msp2*) genes and a neutral microsatellite marker. This study describes the analytical performance and limitations of a CE assay to genotype *msp1*, *msp2*, and *Poly-*α. Analytical performance was measured using seven Pf control strains over a three-log concentration range. DNA was extracted from dried blood spots (DBS) and Pf was confirmed by a photoinduced electron transfer PCR (PET-PCR) assay. PCR amplification and CE conditions were optimized for three polymorphic loci: *msp1* gene families K1, MAD20, and RO33, *msp2* gene families FC27 and 3D7/IC, and *Poly-*α. The limit of detection (LoD), defined by the lowest number of parasites detected by all six assays, was 112 parasites per reaction. Inclusivity was demonstrated by testing Pf strains at a concentration near the LoD. Exclusivity was demonstrated by testing 15 non-falciparum Plasmodium samples and by BLAST analysis of PET-PCR primers against sequences to publicly available genetic sequence data. No false negative or positive reactions were detected, and no primer cross-reactivity was detected resulting in 100% analytical sensitivity and specificity. Reproducibility of the CE assay was determined by two operators testing six panels of eight samples. Of 48 available results, 48 were in agreement for 100% reproducibility. The analytical performance of the assay indicates that this PCR/CE assay is a sensitive, specific and reproducible method for genotyping *msp1*, *msp2* and *Poly-*α from DBS. This assay may be used to evaluate the performance of *msp1*, *msp2* and *Poly-*α genotypes to distinguish between new and recrudescent malaria infections in field TES samples.

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SURVEILLANCE OF *PFHRP2* GENE DELETIONS AND ASSESSMENT OF FALSE NEGATIVE RAPID DIAGNOSTIC TESTS OUTCOMES FOR MALARIA DIAGNOSTICS IN SENEGAL

Djiby SOW[1], Awa Dème[1], Ibrahima M. Ndiaye[1], Mamadou A. Diallo[1], Aita Sene[1], Amy Gaye[1], Mariama Toure[1], Mouhamad Sy[1], Tolla Ndiaye[1], Mamadou S. Yade[1], Yaye D. Ndiaye[1], Khadim Diongue[1], Jules F. Gomis[1], Mame C. Seck[1], Mouhamadou Ndiaye[1], Ndeye C. Diop[1], Nogaye Gadiaga[1], Elhadj M. Ba[1], Mamane N. Garba[1], Bassirou Ngom[1], Fatou F. Ba[2], Ibrahima Diallo[2], Medoune Ndiop[2], Sarah K. Volkman[3], Bronwyn MacInnis[4], Doudou Sene[2], Aida S. Badiane[1], Dyann F. Wirth[3], Daouda Ndiaye[1]

[1]International Center for Research and Training on Applied Genomics and Health Surveillance (CIGASS) - Cheikh Anta DIOP University (UCAD), Dakar, Senegal, [2]National Malaria Control Program, Dakar, Senegal, [3]Department of Immunology and Infectious Diseases, Harvard T.H. Chan School of Public Health, Boston, MA, United States, [4]Infectious Disease and Microbiome Program, Broad Institute, Cambridge, MA, United States

The reliability of rapid diagnostic tests (RDTs) based on the detection of the *Plasmodium falciparum* Histidine-Rich Protein 2 (PfHRP2) may be compromised by emerging *P. falciparum* strains with deletions in the *pfhrp2/3* genes. WHO recommends that countries assess the prevalence of these gene deletions to inform diagnostic strategies. Between 2021 & 2022, 3,144 febrile patients from Kolda, Kédougou, Kaolack, and Diourbel were screened during the malaria season using (1) an SD BIOLINE HRP2-based

RDT, (2) microscopy, & (3) PET-PCR for *P. falciparum* confirmation. Samples were further analyzed through a multiplex fluorescent magnetic bead-based antigen assay comparing *Pf*HRP2 & *Pf*LDH, & PCR to evaluate possible *Pfhrp2* deletion. From the total 3,144 samples analyzed, there were 1,800 that tested positive for malaria by RDTs. *P. falciparum* was detected by PET-PCR in 38 of the remaining 1,344 samples that tested negative by RDT (2.83% false negative). Of these 38 PET-PCR positive cases, microscopic analysis identified 6 cases with parasitemia levels above 200 parasites per microliter (detection threshold of the RDTs). The remaining 31 cases had parasite densities that fell below this threshold. Additionally, within the RDT-negative samples, we identified one case each of *P. ovale* and of *P. malariae* monoinfection. Notably, among the PET-PCR confirmed *P. falciparum* cases, *Pfhrp2* gene deletions were detected in these 6 cases using the One Step PCR protocol (CDC). These findings are being validated using long-range Oxford Nanopore Technology sequencing to both confirm the gene deletion, to evaluate if there is also deletion of the *Pfhrp3* locus, and to map the break points of the gene deletions. Given this preliminary data using the combined total of PET-PCR and RDT positive cases (n=1,838), the propotion of *Pfhrp2* gene deletions was 0.32% (6/1,838), which remains below the WHO's critical 5% threshold that suggests a reconsideration of the diagnostic strategy. While these results are undergoing validation, our preliminary findings underscore the critical need for continuous surveillance of HRP2-based RDTs in Senegal.

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GENOMIC EPIDEMIOLOGY OF MALARIA IN ZANZIBAR: DEFINING THE ROLE OF IMPORTATION AND LOCAL TRANSMISSION

Sean Connelly[1], Mohamed Ali[2], Billy E. Ngasala[3], Wahida Hassan[2], Bakari Mohamed[2], Safia Mohammed[2], Shija J. Shija[2], Abdallah Zacharia[3], Msolo C. Dominick[3], Rebecca Crudale[4], Varun Goel[5], Barbara B. Choloi[6], Anders Björkman[7], Jeffrey A. Bailey[4], Jessica T. Lin[6], Jonathan J. Juliano[6]

[1]MD-PhD Program, University of North Carolina, Chapel Hill, NC, United States, [2]Zanzibar Malaria Elimination Program (ZAMEP), Zanzibar, Tanzania, United Republic of, [3]Department of Parasitology and Medical Entomology, Muhimbili University of Health and Allied Sciences, Dar es Salaam, Tanzania, United Republic of, [4]Department of Pathology and Laboratory Medicine, Brown University, Providence, RI, United States, [5]Carolina Population Center, University of North Carolina, Chapel Hill, NC, United States, [6]Division of Infectious Diseases, Department of Medicine, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC, United States, [7]Department of Global Public Health, Karolinska Institutet, Stockholm, Sweden

Zanzibar achieved and maintained low malaria prevalence of less than 1% for 15 years, but malaria elimination has proven difficult. Recent studies support that importation from mainland Tanzania is a major contributor, but local transmission, particularly with the recent increase in cases, remains important. To expand our understanding of the factors that drive imported malaria and local outbreaks, we have launched the Zanzibar and Imported Malaria (ZIM) study. We collected samples from individuals with positive malaria rapid diagnostic tests at 100 clinics in Zanzibar and 29 clinics in mainland Tanzania. Individuals also provided clinical, behavioral, and household data. To date, we have enrolled approximately 3,723 patients in Zanzibar and 7,270 patients in mainland Tanzania. Leveraging a highly multiplexed genotyping assay [molecular invasion probes (MIPs)], we are genotyping single nucleotide polymorphisms throughout the genome and targeted known and candidate drug resistance polymorphisms. Leveraging inheritance through identity by descent (IBD) analysis, we are comparing the genetic relatedness of isolates. To date, we have extracted 3792 and 1008 samples from mainland Tanzania and Zanzibar. A portion of these were sequenced (1212 from mainland Tanzania and 490 from Zanzibar) and after filtering to high quality samples, 552 (mainland Tanzania) and 182 (Zanzibar) samples were analyzed. These isolates show highly related parasite pairs/clusters within and between regions, including parasite isolates that are highly related between mainland Tanzania and Zanzibar. Antimalarial resistance polymorphisms are similar between mainland Tanzania and Zanzibar, despite using different antimalarials, suggesting importation having a large impact on the parasite population. The ZIM study is the largest study to combine genomics and epidemiology to study how parasite migration and importation occurs across the country to lead to better strategies to interrupt malaria transmission.

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EVALUATION OF THE *MSP1*, *MSP2*, AND *POLY-*α METHOD FOR DISTINGUISHING NEW INFECTIONS FROM RECRUDESCENCE

Breanna Horton[1], Culzean Kennedy[1], Zhiyong Zhou[2], Samaly Souza[2], Dragan Ljolje[2], Ira Goldman[2], Jessica McCaffery[2], Rispah Abdallah[1], Mateusz Plucinski[3], Leah Moriarty[3], Stephen Lindstrom[2], Molly M. Freeman[2]

[1]Oak Ridge Institute for Science and Education, Oak Ridge, TN, United States, [2]Laboratory Science and Diagnostics Branch, Centers for Disease Control and Prevention, Atlanta, GA, United States, [3]President's Malaria Initiative, Centers for Disease Control and Prevention, Atlanta, GA, United States

Antimalarial drug efficacy studies often require genotyping of recurrent *Plasmodium falciparum* infections at the time of treatment and recurrent parasitemia to distinguish new infections (NI) from recrudescence (REC). In 2021, the World Health Organization (WHO) recommended the use of merozoite surface proteins 1 and 2 (*msp1* and *msp2*), and a neutral microsatellite (NMS) to classify NI and REC during therapeutic efficacy studies (TESs). This study compares the ability of two capillary electrophoresis (CE) genotyping methods, *msp1, msp2,* and *Poly-*α vs seven NMS (7NMS), to distinguish NI from REC. Amplification and fragment analysis was optimized for six polymorphic targets of the selected loci: *msp1* gene families K1, MAD20, and RO33, *msp2* gene families FC27 and 3D7/IC, and neutral microsatellite *Poly-*α. Fifty pairs of samples with recurrent infections, collected as dried blood spots (DBS) from four African TESs, were selected to investigate assay performance. Classification with *msp1, msp2,* and *Poly-*α match-counting was compared with 7NMS match-counting. In the match-counting approaches for both assays, REC was defined by the presence of a matching allele at all loci, NI was defined by the absence of matching alleles at any one locus, and inconclusive (INC) was defined by missing data at one or more markers and matched alleles at all amplified markers resulting in removal from further interpretation. Of the 50 pairs, 45 were classified as NI by 7NMS due to mismatch at one or more loci while 40 (88.9%; 95% CI: 76.1-95.6%) were classified as NI by *msp1*, *msp2*, and *Poly-*α match-counting. Four pairs were classified as REC by both methods (100%; 95% CI: 45.4-100%). Of the six remaining pairs, two were NI by 7NMS but REC by *msp1*, *msp2*, and *Poly-*α*,* three were NI by 7NMS but INC by *msp1*, *msp2*, and *Poly-*α due to *msp2* non-amplification*,* and one was INC by 7NMS due to *C2M34* non-amplification but REC by *msp1, msp2,* and *Poly-*α*.* The *msp1*, *msp2*, and *Poly-*α assay and match-counting method demonstrates high agreement (88%; 95% CI: 75.8-94.8%) with 7NMS match-counting and may be a valid approach to assess antimalarial drug efficacy in high malaria transmission areas.

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SEROLOGICAL BIOMARKERS FOR DETECTION OF ASYMPTOMATIC *PLASMODIUM VIVAX*-INFECTED INDIVIDUALS IN THE PERUVIAN AMAZON

Alonso Cruz-Echevarría[1], Elizabeth Villasis[1], Rhea Longley[2], Ramin Mazhari[2], Ivo Mueller[2], D. Herbert Opi[3], James Beeson[3], Joseph Vinetz[4], Dionicia Gamboa[5], Katherine Torres[1]

[1]Laboratorio de Malaria, Laboratorios de Investigación y Desarrollo, Facultad de Ciencias e Ingeniería, Universidad Peruana Cayetano Heredia, Lima, Peru, [2]Walter and Eliza Hall Institute of Medical Research Department of Population Health and Immunity, Parkville, VIC, 3052, Australia, [3]Burnet Institute, Melbourne 3004, Victoria, Australia, [4]Laboratorio ICEMR-Amazonia y Enfermedades Infecciosas Emergentes, Laboratorios de Investigación y Desarrollo, Facultad de Ciencias e Ingeniería, Universidad

Peruana Cayetano Heredia, Lima, Peru, [5]Instituto de Medicina Tropical Alexander von Humboldt, Universidad Peruana Cayetano Heredia, Lima, Peru

Loreto is a malaria-endemic region of the Amazon that maintains residual transmission of malaria via submicroscopic asymptomatic carriers. People with blood-stage *Plasmodium vivax* (*Pv*) parasitemia who do not feel sick (asymptomatic subjects) are thought to be clinically immune to malaria. We hypothesized that asymptomatic (Asym) *Pv*-infected subjects from the Peruvian Amazon induce different IgG antibody titers in an antigen (Ag)-dependent fashion compared to symptomatic (Sym) individuals. This study performed a bead-based multiplex assay in a Luminex platform to analyze IgG antibody levels against a 30-antigen panel of *Pv*. The study population consisted of a total of 108 individuals. Serum samples from healthy Australian negative controls with no previous history of malaria (n=20); endemic negative controls from Iquitos city (n=30) (no history of malaria in the past 3 years and no *Plasmodium* infection confirmed by microscopy and qPCR); and Asym (n=28) and Sym (n=30) Amazonian subjects with confirmed *Pv* infection were evaluated. RAU (relative antibody units) were compared between study groups for each antigen by permutation ANOVA. Asym relative to Sym subjects showed higher IgG antibody levels against *Pv* antigens: hypothetical protein (PVX_091710), PvMSP7, SIAP2, and CSP247. Interestingly, aside from PvMSP7, the other antigens corresponded to the sporozoite stage**.** These limited set of antigens are thus related to naturally acquired clinical immunity in Asym *Pv*-infected individuals from low transmission settings of the Peruvian Amazon.

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INVESTIGATING THE ROLE OF NON-VAR2CSA SPECIFIC ANTIBODIES IN PROTECTION FROM PLACENTAL MALARIA

Yvonne Dube[1], Wina Hasang[1], Mwayiwawo Madanitsa[2], Victor Mwapasa[3], Jonathan Kurtis[4], Morten Nielsen[5], Stephen Scally[6], D. Herbert Opi[7], Kamija Phiri[8], Feiko O ter Kuile[9], Elizabeth H. Aitken[1], Stephen J. Rogerson[1]

[1]The Peter Doherty Institute for Infection and Immunity, University of Melbourne, Melbourne, Australia, [2]Department of Clinical Sciences, Academy of Medical Sciences, Malawi University of Science and Technology, Thyolo, Malawi, [3]Department of Epidemiology and Biostatistics, School of Global and Public Health, Kamuzu University of Health Sciences, Blantyre, Malawi, [4]Department of Pathology and Laboratory Medicine, Brown University Medical School, Providence, RI, United States, [5]Department of International Health, Immunology and Microbiology, University of Copenhagen, Copenhagen, Denmark, [6]Walter and Eliza Hall Institute of medical Research, The University of Melbourne, Melbourne, Australia, [7]Burnet Institute, Melbourne, Australia, [8]Training and Research Unit of Excellence, Blantyre, Malawi, [9]Department of Clinical Sciences, Liverpool School of Tropical Medicine, Liverpool, United Kingdom

Placental malaria (PM) is caused by *Plasmodium falciparum*-infected erythrocytes (IEs) sequestration in placenta via chondroitin sulphate A, and antibodies to VAR2CSA have been associated with protection from PM and adverse pregnancy outcomes. There are no VAR2CSA specific vaccines available, whereas vaccines based on other parasite antigens have progressed to clinical trials. Their role in protection against PM is unclear. We investigated if antibodies to antigens other than VAR2CSA contributed to protection from PM. Plasma collected mid-pregnancy from Malawian infected pregnant women, with (n=75) or without evidence of PM (n=88) at delivery, was used to measure antigen-specific IgG, IgG1-4, IgA1, IgA2, IgM, interactions with Fc receptors and C1q to thirteen *P. falciparum* recombinant antigens. Phagocytosis of merozoites and placental binding IE by THP-1 cells and neutrophils, IgG binding to merozoites and to placental binding IE, and levels of adhesion-blocking Ig to placental binding IE, were also measured. Using univariate analysis, we observed twenty-seven antibody features were higher in women with PM ($P \leq 0.05$), with 19 being antibody features to merozoites (IgG to MSP3, PfRh5; IgG1 to AMA1, EBA175, MSP1-p19, MSP2, PfRh2a1; IgG3 to MSP3; FcγRIIA to AMA1; FcγRIIIA to AMA1; FcγRIIB to AMA1, MSP1-p19, MSP3, FcγRIIIB to AMA1, MSP3; IgA2 to AMA1, MSP1-p19; C1q to AMA1, PfRh2a1. Five antibody features were higher in women with no placental malaria ($P \leq 0.05$), including IgA1 antibodies to MSP2, MSP9, PfRh2a1, PfRh5 and C1q binding antibodies to PfRh5, as were antibodies to placental binding IE and adhesion-blocking Ig. Phagocytosis of opsonised IEs by THP-1 cells or of merozoites by neutrophils did not differ between groups. Data will be further analysed using logistic regression and principal components analysis, but little evidence that antibodies towards non-VAR2CSA proteins protect against PM was observed so far. The IgG binding and adhesion-blocking data suggest antibodies to VAR2CSA are protective. Antibodies to non-VAR2CSA proteins may be markers of exposure to PM rather than markers of protection.

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EVALUATE THE ROLE OF CYTOKINES AND CHEMOKINES IN THE DEVELOPMENT OF COMPLICATIONS IN MALARIA CAUSED BY *PLASMODIUM VIVAX*

Catalina Tovar Acero[1], Javier Ramírez-Montoya[2], María Camila Velasco[3], Paula Avilés-Vergara[1], Dina Ricardo-Caldera[1], Juan Rivera-Correa[4], Ana Rodríguez[5], María Fernanda Yasnot-Acosta[3]

[1]Universidad del Sinú, Montería, Colombia, [2]Universidad del Córdoba, Montería, Colombia, [3]Universidad de Córdoba, Montería, Colombia, [4]Autoimmunity and Inflammation Program, Hospital for Special Surgery, New York, USA; Department of Medicine, Weill Cornell Medicine, , New York, USA, NY, United States, [5]New York University School of Medicine, New York, NY, United States

Plasmodium vivax can cause complicated manifestations, the mechanisms that lead to this situation are not entirely clear. The presence of parasite and parasite-derived components triggers the inflammatory response, which is characterized by the production of pro- and anti-inflammatory molecules. These molecules may be responsible for the damage observed in different affected organs in complicated malaria. Evaluate the role of cytokines and chemokines in the development of complications in malaria caused by *P. vivax*. Thirteen cytokines and chemokines were quantified in 106 people with malaria (severe and not severe) and 50 controls, with bead-based multiplex assay. The study variables were analyzed by non-parametric tests were carried with Prima and R statistical software. Fitting models with interaction to study the complication probability, using Lasso Regression with readjustment of Gamlss models of binomial family. IL-10, IL-6 and IFNg had higher concentration in the severe malaria group (<0.0001) and lower concentration of TGF- β (<0.0001), compared with non-severe malaria group and control group. IL-10, IL-6, IFNg showed a negative correlation with platelet count in severe malaria, IL-6 and IFNg specifically with severe thrombocytopenia; and a positive correlation between IFNg and transaminases, and IL-2 and creatinine. Lasso regression model suggests that IL-4, IL-10, CCL2 and TGF-β might be developed as prognostic for severity in *P. vivax* malaria. The inflammatory response during *P. vivax* infection can mediate the development of hematological, renal, and hepatic complications. TGF- β to protect against the development of complicated forms of *P. vivax* malaria.

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IGG ANTIBODY-MEDIATED COMPLEMENT FIXATION AND ACTIVITY AND ITS ASSOCIATIONS WITH PROTECTION AGAINST SEVERE MALARIA

Jennifer Suurbaar[1], Nicaise T. G Ndam[2], Kwadwo A. Kusi[3], Micheal F. Ofori[3], Anja R. T Jensen[4]

[1]University for Development Studies, Tamale, Ghana, [2]Université de Paris, MERIT FR, France, [3]University of Ghana, Accra, Ghana, [4]University of Copenhagen, Copenhagen, Denmark

Plasmodium falciparum infected erythrocytes (IEs) sequestration leading severe malaria (SM) in children is primarily mediated by *P. falciparum* erythrocyte membrane protein 1 (PfEMP-1). The magnitude of Immunoglobulin G (IgG) to IEs has been associated with reduced risk of SM in children. IgG Antibodies mechanisms that might contribute to SM immunity include IgG that blocks IEs binding to cognate receptors or mediates IE opsonization for phagocytosis. Another IgG antibody mechanism is complement-dependent functional activities, however, can complement fix the problem of SM in children? To certain complement

mechanism in SM, a cohort of *P. falciparum* exposed children (n=84) from Benin was studied. We measured the ability of IgG antibodies to fix and activate complement using PfEMP-1 recombinant domains. We determined IgG antibody-mediated complement fixation and activity and its associations with protection against SM. Some children acquired IgG antibodies that effectively promoted complement fixation and activity on PfEMP-1 recombinant domains and complement fixation, C1q correlated with C3 activity. There was however limited evidence for membrane attack complex activity that is C5-9 activity on the PfEMP-1 recombinant domains. Importantly, a higher magnitude of complement C1q fixing antibodies was associated with reduced risk of SM for some domains. These findings provide new insights into mechanisms mediating immunity to SM in children and therefore, targeting the complement system on IEs could be a potential adjunctive therapy to address SM in children by modulating immune responses and enhancing protective immunity.

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MOSQUITO-*PLASMODIUM* IGG ANTIBODIES AND CLINICAL PRESENTATION OF MALARIA IN COLOMBIA

Julio A. Ventocilla[1], Gissella M. Vasquez[2], Hugo O. Valdivia[2], Alyssa Schwinn[3], Zoe Jacobs[3], Alberto Tobon[4], Danielle Pannebaker[2], Berlin Londono-Renteria[3]

[1]Vysnova Partners Inc., Lima, Peru, [2]U.S. Naval Medical Research Unit SOUTH, Lima, Peru, [3]Tulane University, New Orleans, LA, United States, [4]Antioquia University, Medellin, Colombia

Malaria remains the main vector-borne parasitic disease worldwide. Understanding mosquito vector biology during the malaria infection process is critical for the identification of novel markers of exposure which could be used to develop protective countermeasures against malaria infection. Recently, human immune responses against mosquito salivary protein (SP) have been used to measure mosquito bite intensity and disease risk. We previously identified *Anopheles darlingi* AdP4230 SP as immunogenic against sera from malaria infected populations in Colombia. Here, we evaluated IgG antibody levels measured against two novel mosquito peptides designed from An. darlingi AdP4230 SP to determine association with malaria clinical presentation. ELISA assays were performed to compare level of antibodies IgG by measure of optical density (OD) in serum samples from uninfected (n=50), malaria submicroscopic (n=70), and malaria microscopic (n=89) populations from Colombia during 2016-2018. In addition, an exposure malaria marker merozoite surface protein 1 (MSP1), was used to compare immunogenicity with novel mosquito peptides AdP4230. Our results showed that patterns of IgG antibody responses against the Ad4230 peptide 1 (AdP4230p1) and peptide 2 (AdP4230p2) are associated with clinical presentation of malaria. Higher median level of antibodies (OD:0.42) against AdP4230p1 were associated with symptomatic malaria and AdP4230p2 with both symptomatic (OD:0.25) / asymptomatic (OD:0.28) individuals, $p<0.05$. In addition, we observed a low positive correlation (Spearman Rho: 0.16-0.31, $p<0.05$) between levels of IgG antibodies against AdP4230p1 and AdP4230p2 versus age (median age: 21, IQR: 11-35), which was similar to those against MSP1. Our results showed that IgG responses against mosquito salivary peptides AdP4230p1 and AdP4230p2 could be use as biomarkers for malaria clinical presentation. These novel SG markers could be integrated with other malaria parasitic markers associated with exposure as part of improved malaria control strategies in civilian and military populations living in endemic areas.

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ASSOCIATION OF NOVEL IGG3 ALLELE WITH MALARIA IN CHILDREN FROM SEPIK REGION OF PAPUA NEW GUINEA

Maria Saeed[1], Elizabeth H. Aitken[2], Myo Naung[3], Caitlin Bourke[3], Rhea Longley[3], Amy W. Chung[4], Timon Damelang[4], Benson Kiniboro[5], D. Herbert Opi[6], James G. Beeson[7], Ivo Mueller[8], Stephen J. Rogerson[9]

[1]Department of Infectious Diseases, The Peter Doherty Institute for Infection and Immunity, University of Melbourne, Melbourne, Australia, [2]Department of Infectious Diseases and The Department of Microbiology and Immunology, The Peter Doherty Institute for Infection and Immunity, University of Melbourne, Melbourne, Australia, [3]Population Health and Immunity Division, Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia, [4]Department of Microbiology and Immunology, The Peter Doherty Institute for Infection and Immunity, University of Melbourne, Melbourne, Australia, [5]Papua New Guinea Institute of Medical Research, Madang, Papua New Guinea, [6]Disease Elimination Program, Burnet Institute, Melbourne, Australia, [7]Disease Elimination Program, Burnet Institute and Department of Medicine, Microbiology and Immunology, and Infectious Diseases, The University of Melbourne, Monash University, Melbourne, Australia, [8]Population Health and Immunity Division, Walter and Eliza Hall Institute of Medical Research and Department of Medical Biology, The University of Melbourne, Melbourne, Australia, [9]The Department of Infectious Diseases and The Department of Medicine, The Peter Doherty Institute fro Infection and Immunity, Melbourne, Australia

Malaria causes death and severe illness in children under five years of age in malaria endemic regions. Recent work has established the importance of malaria-specific IgG3 in malaria immunity. IgG3 via its Fc region binds to Fcγ-receptors (FcγRs) on immune cells to instigate immunological defense against malaria. Changes in the amino acid sequences due to single nucleotide polymorphisms (SNPs) in IgG3-Fc regions give rise to IgG3 allotypes which can modulate IgG3 functions. A novel IgG3 allele, G3m29, was recently reported in pregnant women from Sepik, Papua New Guinea, and was shown to have enhanced affinity to FcγRIIa. We hypothesized that the prevalence of G3m29 in this population was associated with protection from *Plasmodium* species infections in children. In a longitudinal study cohort of children aged 1-3 years (N=203), with multiple *Plasmodium* species infections from the Sepik region in Papua New Guinea (PNG), we amplified the Fc region of IgG3 genes by polymerase chain reaction (PCR) using heavy chain constant domains 2 and 3 specific primers. We then used Sanger sequencing to identify SNPs and compared to the reference alleles of immunogenetics (IMGT) database. We identified that 78% of children in the cohort were either heterozygous (n=82, 40%) or homozygous (n=77, 38%) for G3m29. There were significantly fewer *Plasmodium* species infections in children with the novel G3m29 allele compared to non-G3m29 allele carriers (β= -1.736, 95% CI [-3.39, -0.079], $p<0.05$) measured using linear regression. This effect was most pronounced for numbers of *P. vivax* asymptomatic infections (β=-1.06, 95% CI [-2.01, -0.12], $p<0.05$). Moreover, novel G3m29 allele carriers also had significantly lower levels of total IgG and IgG1 to several *Plasmodium vivax* vaccine candidate antigens but no difference in IgG3 levels. Of note, IgG1 alleles were not associated with protection from malaria. In conclusion, the G3m29 allele is highly prevalent in the Sepik region of PNG and might be involved in protection against *P. vivax* infections. G3m29 also seems to alter IgG subclass distribution.

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MATURATION AND DIVERSIFICATION OF THE B AND T CELL RECEPTOR REPERTOIRES OVER 9 YEARS OF REPEATED MALARIA INFECTIONS

Helen George[1], Heike Baum[2], Stephan Lorenzen[3], Aissata Ongoiba[4], Safiatou Doumbo[4], Didier Doumtabe[4], Shanping Li[5], Maren Sandkuhl[6], Thomas Jacobs[1], Kassoum Kayentao[4], Boubacar Traore[4], Peter D. Crompton[7], Martin Davey[8], Dániel Cadar[2], Maria Mackroth[6], Christine S. Hopp[1]

[1]Protozoa Immunology, Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany, [2]Bernhard Nocht Institute for Tropical Medicine, National Reference Centre for Tropical Infectious Diseases, Hamburg, Germany, [3]Bioinformatics, Bernhard Nocht Institute, Hamburg, Germany, [4]Malaria Research and Training Centre, Department of Epidemiology of Parasitic Diseases, International Center of Excellence in Research, University of Sciences, Technique and Technology of Bamako, Bamako, Mali, [5]Malaria Infection Biology and Immunity Section, Laboratory of Immunogenetics, NIAID, NIH, Rockville, MD, United States, [6]First Medical Department, Division of Infectious Diseases, University Medical Center Hamburg-Eppendorf, Hamburg, Germany, [7]Malaria Infection Biology and

Immunity Section, Laboratory of Immunogenetics, NIAID, NIH, Rockvielle, MD, United States, [8]Division of Biomedical Sciences, Warwick Medical School, University of Warwick, Coventry, United Kingdom

The adaptive immune system identifies antigens through a vast array of antibodies (B cell receptors, BCRs) and T cell receptors (TCRs), known as the immune repertoire. Understanding the immune repertoires is key to comprehending the adaptive immune response in disease and infection. In malaria, natural immunity develops slowly: children living in regions with intense *Plasmodium falciparum* (*Pf*) transmission develop immunity to severe malaria within the first 5 years of life, but immunity to uncomplicated febrile malaria is not acquired until early adulthood. In a cohort in a malaria endemic region of Mali, we are performing longitudinal analyses using PBMCs from children with malaria infections over the course of nine years, including samples from two annual cross-sectional blood-draws and samples taken shortly after febrile malaria episodes. Using this unique set of samples, we are analysing the evolving immune repertoires to assess the impact of repetitive acute malaria episodes. To this end, *Pf*-specific and bulk B cells, as well as follicular helper and bulk T cells are sorted for BCR/TCR sequencing, while acquired phenotypic flow cytometry data are used for an analysis of cell subset distribution. BCRseq will be performed using a newly established highly sensitive BCRseq pipeline: cDNA synthesis with isotype-specific primers that target the Ig constant regions is followed by multiplex PCR with a primer pool targeting the 5′ leader regions of V genes. Finally, indexes are introduced by PCR to prepare the library for Illumina sequencing. Sequencing of the TCR β chain is performed using the QIAseq Targeted RNA Panel Human TCR kit. We will be able to detect specific clones that expand after malaria episodes and track their maturation over years, generating key insights into the longevity of malaria-specific B and T cell clones. Overall, we are generating unprecedented longitudinal data of interest to the general immunological community, that reveal the impact of repeated infections on the co-evolution of the BCR and TCR repertoires, yielding important information on the development and maintenance of naturally acquired immunity to malaria.

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THE CD4⁺T CELL MEMORY IN *PLASMODIUM FALCIPARUM* MALARIA

Marie-Theres Thieme[1], Johannes Brandi[2], Maren Sandkuhl[1], Thomas Jacobs[2], Maria Sophia Mackroth[1]

[1]University Medical Center Hamburg-Eppendorf, Hamburg, Germany, [2]Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany

The development of natural immunity against *Plasmodium falciparum* (*Pf*) malaria takes many years and is primarily mediated by antibodies and a Th1 $CD4^+$ T cell response against the blood-stage. Data from endemic areas indicate that natural immunity is short-lived and requires regular exposure. In contrast, experimental *Pf* infections or vaccinations of volunteers in Europe induce stable T and B cell responses. This raises the question of whether natural *Pf* infections interfere with the acquisition of long-term immunity. Our study aims to identify mechanisms influencing the induction of long-lived $CD4^+$ T cell memory in malaria. We hypothesize that the massive induction of co-inhibitory molecules during acute malaria inhibits the development of a long-lived T cell memory response to malaria. We are longitudinally studying the *Pf*-specific immune response in patients with acute malaria in Hamburg for 12 months. We will compare the T cell response of malaria patients with high and low expression patterns of co-inhibitory molecules. In addition, we will compare those malaria patients to individuals from controlled human malaria infection trials. Currently, we perform a detailed analysis of the immune cell responses during acute infection. We designed and validated a comprehensive 36-color flow cytometry panel for deep phenotyping of immune cells during acute infection with a special focus on T cells and immune checkpoints. Our preliminary results show a strong upregulation of the checkpoint inhibitors CTLA-4 and PD-1, and a trend towards upregulation of LAG-3 on $CD4^+$ T cells in malaria patients compared to healthy controls. Furthermore, $CD4^+$ T cells show an upregulation of the co-stimulatory molecule ICOS. Secondly, we will investigate *Pf*-specific T cell responses over 12 months to identify immunologic predictors of a long-lasting memory response. Subsequent analysis will try to decipher potential correlations between acute phenotypes and the persistence of *Pf*-specific $CD4^+$ T cell responses.

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INFLUENCE OF CYTOKINE RATIO (IL-10: TNF- α) ON ANAEMIA STATUS OF MALARIOUS CHILDREN IN SOUTH EASTERN NIGERIA

Chinyere Ihuarulam Okoro[1], Oluchi Ijeoma Okoro[2], Kingsley Excell Dunga[3], Easter Godwin Nwokah[4]

[1]federal University Teaching Hospital, Owerri North, Nigeria, [2]Life streams laboratory, Asaba, Nigeria, [3]Rhema University Aba, Abia, Nigeria, [4]River State University, Portharcourt, Nigeria

Interleukin 10 (IL-10) production appears to be important in the induction and maintenance of immunity to *Plasmodium falciparum* in naturally exposed populations. Down regulation of TNF-α production and consequent resistance to severe malaria, has been linked to the ability to produce the immuno-regulatory cytokine (IL-10), while a relative deficiency in immuno-regulatory cytokine (IL-10) and lower ratios of IL-10 to TNF-α has been recorded in patients with severe malaria. Children aged 1-72 months who presents with fever or history of fever in the last 24 hours at the selected Outpatient's Department of the Health facilities were enrolled after obtaining Ethical approval from the Research, Ethical Committee of Federal University Teaching hospital Owerri Imo state. Blood samples were collected from respondents who consented for the diagnosis of malaria and anemia using outlined standard operating procedures (SOPs). Plasma/serum of all randomly selected children (both TEST and CONTROL) were freeze dried in aliquots of 100µl in cryovial tubes at –20°C until they were used for cytokine assays in accordance with the manufacturer's manual. The geometric mean parasite density of children positive by microscopy was 1764 parasites/µl of blood with a range of (12-220,000 000parsites/µl of blood). Anemia ranged from mild to moderate, there was no severe malaria anemia observed. A significant relationship was observed between anemia and fever ($p < 0.001$), febrile children had higher percentage of mild and moderate anemia than afebrile children (18.3% vs 15.0%) and (25.7% vs 15.0%). The geometric mean of IL10/TNF-α ratios of 2.8pg/ml, 2.1pg/ml and 1.7pg/ml were recorded for normal hemoglobin, mild and moderate anemia. The IL -10 and TNF concentrations increased respectively with advance in anemia while the IL-10/TNF ratio decreased as Anemia advances. Increased IL-10/TNF-α ratio is associated with increased hemoglobin concentrations in acute, uncomplicated *P. falciparum* malaria ($p<0.001$). Thus, lower levels of IL-10 over TNF- α may contribute to development of malaria complications such as anemia in addition to other factors.

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FICOLIN-1 IN PAEDIATRIC *PLASMODIUM FALCIPARUM* MALARIA AND ITS POSSIBLE ROLE IN PARASITE CLEARANCE AND ANAEMIA

Di Zheng[1], Natalie Ferrington[1], Dilini Rathnayake[1], Agersew Alemu[1], Visopo Harawa[2], Emily Karahalios[3], Wina Hasang[1], Evelyne Gout[4], Nicole Thielens[4], Karl Seydel[5], Terrie Taylor[5], Wilson Mandala[6], Stephen Rogerson[1], **Elizabeth Aitken**[1], Louise Randall[1]

[1]Peter Doherty Institute of Infection and Immunity, Melbourne, Australia, [2]Kamuzu Univeristy of Health Sciences, Blantyre, Malawi, [3]University of Melbourne, Melbourne, Australia, [4]University of Grenoble Alpes, Grenoble, France, [5]Blantyre Malaria Project, Blantyre, Malawi, [6]Kamuzu University of Health Sciences, Blantyre, Malawi

Plasmodium falciparum malaria causes significant disease, especially in children under five. A successful immune response to *P. falciparum* is a major determinant of clinical outcome. The ficolins are a family of lectins that act as Pattern Recognition Receptors that can activate the lectin complement pathway and may promote inflammation and facilitate opsonization and lysis of pathogens. Here, we have investigated the potential roles of ficolin-1 and ficolin-2 in the context of *P. falciparum* infection. We measured ficolin-1 and ficolin-2 concentrations in plasma from Malawian children (presenting with uncomplicated or cerebral malaria)

with and without P. falciparum malaria infection (healthy Controls) by ELISA. Using flow cytometry, we then assessed if ficolin-1 bound to infected red blood cells (iRBC) and whether it binds sialic acid on the iRBC, we also investigated whether ficolin-1 could promote lysis of iRBC in the presence of complete sera *in vitro*. Ficolin -1 and 2 plasma levels were measurable in children from all clinical groups. Compared to healthy controls Ficolin-1 concentrations were higher in children with uncomplicated (coef. (95% CI) 0.63, (0.22, 1.04)) and cerebral malaria (coef. (95% CI) 0.5 (0.01, 0.9)). Ficolin-1 levels were positively associated with peripheral blood monocyte (coef. (95% CI) 0.26 (0.02, 0.51)) and neutrophil counts (coef. (95% CI) 0.06, (0.00, 0.12)). Ficolin-2 was not associated with malaria infection or disease severity. Haemoglobin levels were negatively associated with ficolin-1 (coef. (95% CI) -0.38 (-0.68, -0.09)) and ficolin-2 plasma levels (coef. (95% CI) -0.36 (-0.68, -0.04)). Ficolin-1 bound more to iRBC compared to uninfected RBC and binding was reduced in a ficolin-1 mutant that does not bind to sialic acid. The addition of ficolin-1 to iRBC and uninfected RBC in the presence of serum was associated with increased RBC lysis. These results highlight a largely overlooked role for ficolin-1 in the immune response to *P. falciparum* infection and point to a potential role for lectins contributing to parasite clearance and anaemia by binding and promoting lysis of iRBC.

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ASSESSMENT FOR NEUTROPHIL EXTRACELLULAR TRAPS MARKERS IN *PLASMODIUM FALCIPARUM* MALARIA-INFECTED PREGNANT WOMEN IN A HIGH MALARIA BURDEN REGION

Rebecca Chukwuanukwu[1], Chioma Agu[1], Alfred Ehiaghe[1], Dorothy Ezeagwuna[2], Gerald Udigwe[3], Martin Herrmann[4]

[1]Nnamdi Azikiwe University, Awka, Awka, Nigeria, [2]Parasitology Unit, Nnamdi Azikiwe University Teaching Hospital, Nnewi, Nnewi, Nigeria, [3]Obstetrics & Gynaecology Department, Nnamdi Azikiwe University, Awka, Nnewi, Nigeria, [4]Friedrich-Alexander University, Erlangen -Nurnberg, Germany

Malaria infects millions worldwide and is particularly a problem in the vulnerable including pregnant women. Neutrophil extracellular traps (NETs) formation is an anti-microbial activity of neutrophils and NET structures are formed from nuclear and cytoplasmic components that can capture and kill pathogens. However, recent findings have shown that these NETs have a role in inflammation and may have serious consequences contributing to disease morbidity and mortality. What is the situation in malaria disease? This study was carried out to assess for markers of neutrophil activation and NETs in pregnancy and malaria infection. Ninety pregnant women aged between 18 and 40 years were recruited for this study. Blood and placenta samples were collected. The study population were grouped into two categories: 45 pregnant subjects infected with Plasmodium falciparum malaria and 45 apparently healthy pregnant subjects. Neutrophil elastase, myeloperoxidase, Citrulline H3, total white blood cell counts, white blood cell differential counts and haematocrit were assessed in blood. Sections of paraffin wax embedded tissues were stained with immunofluorescent dyes and examined for expression of NETs markers. Findings from this study show significantly different levels of neutrophil elastase and myeloperoxidase in malaria infected pregnant women (P>0.05). Total white blood cells, lymphocyte and neutrophil counts are significantly different as well when comparing the groups. There were differences in the expression of NET marker in the placenta from the malaria infected compared to the control group. Findings show interesting immunomodulatory effects, with possible implications for malaria disease pathogenesis and control in pregnancy.

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MEMORY CD8$^+$ T-CELLS SPECIFIC FOR CIRCUMSPOROZOITE PROTEIN EPITOPE SEQUENCE YLNKIQNSL RECOGNIZE AND KILL *PLASMODIUM FALCIPARUM* MALARIA INFECTED HEPATOCYTES

Roos van Schuijlenburg[1], Beatrice Winkel[1], Sascha Bezemer[1], Severine C. Chevalley-Maurel[1], Jeroen C. Sijtsma[1], Krista E. Meijgaarden[1], Els Baalbergen[1], Fiona Geurten[1], Felix M. Behr[2], Giampietro Corradin[3], Ramon Arens[2], Blandine Franke-Fayard[1], Meta Roestenberg[1]

[1]Leiden University Center for Infectious Diseases (LU-CID), Leiden University Medical Center, Leiden, Netherlands, [2]Department of Immunohematology and Blood Transfusion, Leiden University Medical Center, Leiden, Netherlands, [3]Institute of Biochemistry, University of Lausanne, Epalinges, Switzerland

Infected *Anopheles* mosquitoes transmit *Plasmodium falciparum* (*Pf*) sporozoites (SPZ) by inoculation in the skin. Only a few SPZ will migrate to the liver, where they infect roughly 1 out of 10^9 human hepatocytes and hide intracellularly. In the hepatocyte, the parasite will multiply to form ~30,000 merozoites within 7 days. In animal models, CD8$^+$ T-cells have been shown to play an important role in killing infected hepatocytes. However, the role of human CD8$^+$ T-cells protection against *Pf* liver stages remains incompletely understood. Elucidating this may crucially inform novel vaccine strategies. During hepatocyte invasion and the first days of replication, the *Pf* parasite expresses large quantities of circumsporozoite protein (CSP), a SPZ surface protein. Here we aimed to unravel if memory HLA-A*02 CD8$^+$ T-cells specific for the CSP epitope YLNKIQNSL can be activated *in vitro* to specifically recognize and kill malaria infected hepatocytes. We show that memory CSP YLNKIQNSL CD8$^+$ T-cells can be activated by co-culture with CSP-stimulated HLA-A*02 antigen presenting cells. Upon activation, CD8$^+$ T-cells show a 7-fold increase in activation markers CD137$^+$ and IFNγ^+. Furthermore, we demonstrate upregulation of granzyme B (p=0.03, mean 66%) and perforin (p=0.03, mean 19%). Subsequently, we find that these CD8$^+$ T-cells kill 98% of CSP epitope sequence YLNKI**Q**NSL stimulated hepatocytes (mean nucleus 230;SD 145) but do not kill when stimulated with aspecific epitope sequence YLNKI**K**NSL (mean nucleus 14200;SD 1200). Finally, we demonstrate that the CSP-specific CD8$^+$ T-cells kill 45% of exoerythrocytic forms (SPZ infected hepatocytes) when added 24 hours post infection. Killing increased to 55% when CSP CD8$^+$ T-cells were added 48 hours post infection. We thus provide unequivocal evidence that human CD8$^+$ T-cells specific for the CSP epitope YLNKIQNSL can specifically recognise and kill malaria infected hepatocytes. This finding underlines the importance of discovering novel T-cell epitopes expressed by *Pf* parasites in the liver stage to be included in the next generation of vaccines and provides a methodology to test killing capacity *in vitro.*

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BONE VOYAGE: HOW *PLASMODIUM* INFECTION DISRUPTS THE PLASMA CELL MICROENVIRONMENT IN THE BONE MARROW

Elizabeth Fusco, Alexander R. Maldeney, Layne Bower, Wei Luo, Nathan W. Schmidt

Indiana University School of Medicine, Indianapolis, IN, United States

Malaria is a disease caused by protozoan parasites in the genus *Plasmodium* . *Plasmodium* infection is cleared in an antibody dependent manner, and plasma cells (PCs) are a type of immune cell that secrete high levels of antibody in response to antigen. After generation, these PCs home to the bone marrow to differentiate into long-lived PCs (LLPCs) if they receive the proper survival signals from other bone marrow resident cells. Individuals that live in malaria endemic regions can be reinfected with *Plasmodium*, indicating that the immune memory response against *Plasmodium* is impaired. It is hypothesized that LLPCs are crucial in protecting against *Plasmodium* reinfection due to their ability to continuously secrete *Plasmodium* specific antibodies. However, we have discovered that there is poor accumulation of LLPCs in the bone marrow of *P. yoelii*

infected mice. We hypothesize that *P. yoelii* impacts the bone marrow microenvironment, affecting the ability of PCs to differentiate into LLPCs. To test this hypothesis, we compared the 'unhealthy' bone marrow microenvironment that develops during *P. yoelii* infection to a 'healthy' bone marrow microenvironment developed during immunization. We analyzed bone marrow resident cell populations using flow cytometry, and characterized the number of antigen-specific PCs present in the bone marrow using ELISpot. In addition, we used fluorescent microscopy to characterize the distribution of these bone marrow resident cells in the LLPC microenvironment during *P. yoelii* infection compared to immunization. We observed a decrease in the number of bone marrow resident cells involved in providing PCs with signals to differentiate into LLPCs during *P. yoelii* infection. There are also fewer of these cells present in the bone marrow of *P. yoelii* infected mice compared to immunized mice. *P. yoelii* infection is negatively impacting the composition of the bone marrow, which may contribute to the poor accumulation of LLPCs following *P. yoelii* infection.

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NOVEL ASSAY TO ASSESS THE SEROLOGICAL EQUIVALENCE OF VACCINE-INDUCED RESPONSES TO CRITICAL MONOCLONAL ANTIBODIES

Jessica S. Bolton[1], Randall S. MacGill[2], Emily Locke[2], Elke S. Bergmann-Leitner[1]

[1]Biologics Research & Development Branch, Walter Reed Army Institute of Research, Silver Spring, MD, United States, [2]Center for Vaccine Innovation and Access, PATH, Washington, DC, United States

Circumsporozoite protein (CSP), the primary surface antigen of the *Plasmodium falciparum* sporozoite and has been the target of many pre-erythrocytic malaria vaccine candidates. Due to the lack of confirmed immune correlates of protection induced by CSP-specific responses, assessing vaccine efficacy through surrogate immunological parameters is currently not possible. Controlled human malaria infection (CHMI) remains the only way to assess protective vaccine efficacy through clinical trials but comes with great cost and time. Since induced antibodies appear to mediate protection, we have developed a novel equivalence assay to improve our ability to down-select vaccine candidates. This multiplex assay, known as the CSP-based assay for serological quantification and equivalency (CBASQE), generates the CSP-epitope specific profile in preclinical and clinical samples (serum/plasma) and assesses the equivalence of these antibodies to functionally relevant monoclonal antibodies across key regions of CSP in a species-independent assay. Results inform the ability of vaccine formulations to induce epitope-specificities considered to be critical for protection. Here, we demonstrate the potential of the assay to accurately identify protected vs. non-protected recipients of CSP-based vaccine thus revealing the crucial role of specific CSP-regions/epitopes in mediating protection. Moreover, we report the evolution of these epitope-specific humoral responses throughout a three-dose vaccination regimen, answering the question whether booster vaccinations only quantitatively impact the immune response (e.g., expansion of specific clones) or also impact the quality of the response (e.g., changes in the epitope-specific profile). Lastly, we discuss how this method can be modified for other antigens and disease models if functionally relevant monoclonal antibodies or the respective protective antigen(s) are available in that model.

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BURKHOLDERIA GLADIOLI PRODUCTION OF ARSINOTHRICIN TO LIMIT TRANSMISSIBILITY OF *PLASMODIUM FALCIPARUM* WHEN INTRODUCED INTO THE *ANOPHELES GAMBIAE* MIDGUT

Ajay Bhatia, Tina Goble

Twin Valley Behavioral Health Services, Columbus, OH, United States

Burkholderia gladioli (GSRB05) is a bacterial rice rhizosphere microbe which produces arsenic-containing arsinothricin (AST) -- resulting in Bacterial Panicle Blight (BPB), a negative feedback loop of the rice life cycle. Here, evidence exists for GSRB05 production of AST, a Glutamine Synthetase I (GSI) inhibitor. Subsequently, NH4+ accumulates, causing a largely toxic local environment for the rice's soil, leading to rice growth limitations and BPB syndrome. This is a negative feedback loop that may mirror a naturally occurring process of inhibition of malarial transmission. Evidence exists for GSRB05's presence in the midgut the *Anopheles gambiae* (A.g.) mosquito, and that through production of AST creates a hypertoxic microenvironment with buildup of NH4+ thus preventing malarial transmission. Fundamentally, we have a naturally occurring microbe, with its concomitant broad spectrum arsenic containing antibiotic in GSRB05, and AST, respectively. The production of AST causes selective inhibition of prokaryotic GSI and lack of significant deleterious effect on eukaryotic (including human) GSII -- meaning that GSRB05 (and AST) exposure to humans is safe. In terms of an effective intervention, the critical step is introduction of GSRB05 into the midgut of the A.g in a manner that is effective, efficient, inexpensive, and practical on a broad scale. This has been attempted successfully in other analogous situations. AST is non-toxic to human cell lines. But arsenic is. Arsenic naturally exists in the soil in many environments where A.g. can proliferate. Two bottle necks for A.g. mosquito proliferation are: (a.) blood feeding and (b.) moist soil larvae growth. Introducing GSRB05 in the soil where Arsenic already exists could mitigate malarial transmissibility with no added potential risk to humans during (a.) because GSRB05 and AST are safe in humans; while simultaneously allowing maximal introduction of AST in the A.g. midgut in (b), creating a hypertoxic microenvironment for malaria there. Fundamentally this may lead to less need for anti-malarial pesticide, drug, or vaccine use while still minimizing malarial transmission.

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THE LONDON SCHOOL OF HYGIENE & TROPICAL MEDICINE (LSHTM) HUMAN MALARIA TRANSMISSION FACILITY: AN OPEN FACILITY FOR EXPERIMENTAL TRANSMISSION STUDIES OF *PLASMODIUM* PARASITES

Mojca Kristan, Harry Pollard, Lindsay Stewart, Luke Brandner-Garrod, Mufuliat T. Famodimu, Penny Sparkes, Eduardo Alves, Gisela Henriques, Chris J. Drakeley, Christiaan van Ooij, Michael Delves, Colin J. Sutherland

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

The Human Malaria Transmission Facility was established in December 2020 at the London School of Hygiene & Tropical Medicine (LSHTM), supported by a Biomedical Resources grant from the Wellcome Trust, to provide research groups worldwide with access to *Anopheles* spp. mosquitoes infected with human malaria parasites. The facility now boasts a history of successful transmission experiments through vigorous optimisation of methods. Our specialist team works with collaborators to design and execute studies relating to the transmission of *Plasmodium* parasites. Experiments have been carried out to investigate various stages of the malaria transmission cycle using *Plasmodium* spp. gametocytes grown *in vitro* in the laboratory or collected directly from clinical samples received in the UKHSA Malaria Reference Laboratory and fed to insectary-reared *Anopheles* mosquitoes via artificial membrane-feeding. A variety of experimental end points can be analysed, depending on the specific research question. This includes but is not limited to imaging of ookinetes, measuring prevalence and intensity of oocysts in the midgut lining, sporozoite positivity and intensity within the salivary glands of infectious mosquitoes, and oocyst genotyping. The facility supports studies of the impact of transgenic *P. falciparum* lines on transmission capabilities, studies of the relationships between mosquito microbiome and malaria transmission, xenomonitoring of parasite prevalence in non-vector blood-feeding insects, and investigations of the influence of parasite drug resistance on fitness for mosquito infection, as well as investigations of the effects of insecticides and endectocides on sporogony. Furthermore, we have been able to infect mosquitoes with clinical isolates with varying anti-malarial resistance phenotypes, providing insight into the effects of drug resistance and providing proof-of-principle that various *P. falciparum* strains,

of different origins, can be transmitted to mosquitoes in the facility. We will present examples of results obtained from studies with UK and international collaborators.

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ASSESSING THE IMPACT OF DRUG RESISTANCE ON MALARIA TRANSMISSION

Margarida Ruivo[1], Ines Marreiros[1], Ana Belen Garcia[1], Malhar Krushu[2], Selina Bopp[2], David Calvo[1], Lorena Cortes[1], Carmen Cuevas[1], Helena Garuti[1], Jose Luis Llergo[1], Noemi Magan[1], Sara Viera-Morilla[1], Dyann Wirth[3], **Maria Jose Lafuente-Monasterio**[1], Amanda Lukens[3]

[1]GSK, Global Health Medicines R&D, Tres Cantos, Madrid, Spain, [2]Harvard T.H. Chan School of Public Health, Boston, MA, MA, United States, [3]Broad Institute of MIT and Harvard, Cambridge, MA, MA, United States

The development of resistance to antimalarial drugs presents one of the greatest threats to malaria control and results in increased malaria morbidity and mortality. The emergence and propagation of drug-resistant *Plasmodium* spp. are intrinsically tied to the diverse forms assumed by the parasite and the variety of environments it traverses, from the mosquito midgut and salivary glands to human hepatocytes and erythrocytes. Changes in parasite fitness and transmissibility to mosquito vectors can affect the spread of resistance. Several studies have shown that the development of atovaquone-resistant parasites in mosquitoes is impaired or halted suggesting their transmission could be limited in the field. This data reflects that a fitness cost could be potentially associated with the bc1 genotype, which causes a sizeable reduction in the onward probability of infection relative to wild type parasites in the absence of atovaquone. In a series of independent experiments, we have carried out he selection of atovaquone and pyrimethamine resistant parasite mutants in the *Plasmodium berghei* mouse model using an inadequate therapeutic regimen dose to expand our knowledge on drug-resistant transmission. Mutational changes underlying the resistance were identified to be S110N in dihydrofolate reductase for pyrimethamine and M133I, Y268N and V284F in cytochrome b for atovaquone resistant parasites. We have evaluated the effect of these mutations on parasite transmission stage development, and we have conducted *in vivo* efficacy studies. In addition, we have included the evaluation of clinical isolates and we have monitored its growth and development in anopheles mosquitoes and its transmissibility to mice engrafted with human hepatocytes and erythrocytes.

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EXPERIMENTAL INFESTATION OF *ANOPHELES GAMBIAE* WITH *PLASMODIUM OVALE* ISOLATES FROM PATIENTS WITH UNCOMPLICATED MALARIA

Fatoumata I. Ballo, Laurent Dembélé, Dinkorma Ouologuem, Karim Sawadogo, Mohamed Touré, Yacouba N. Barré, Siaka M. Goïta, Alpha Seydou Yaro, Abdoulaye Djimdé

Malaria Research and Training Center, Bamako, Mali

Herein, we investigated *Plasmodium ovale* development dynamic within the mosquito vector *Anopheles gambiae* using fresh field isolate parasites from the locality of N'tjiba, Mali. We conducted an experimental study from April to December 2023 at the Malaria Research Training and Center laboratory in Bamako on samples from Faladjè, capital of the commune of N'tjiba. The study included all volunteers aged 12 months or older, presenting to the health center and having *P. ovale* infection by light microscopy as well have provided their informed consent/assent. Following determination of the parasite density and species, venous blood sample was taken and sent to Bamako in an incubator set at 37°C and dried blood spots (DBS) were made for molecular identification. The blood collected was used to feed adults *An. gambiae s.s,* Kisumu strain aged 3-5 days through an artificial membrane. The dissection was carried from the 3rd to the 14th day post feeding in search of oocysts. Positive midguts were fixed with 4% paraformaldehyde for morphological identification. For each carrier, at least 20 mosquitoes were dissected per dissection day. Oocysts were observed on the midguts from 3rd to 14th day post feeding. The prevalence of infected mosquitoes among positive samples varied between field isolates and days of dissection. It varied from 4.08% to 81.8% for days 3, 4, 6, 7, 8, 9, 10, 11, 12, 13 and 14 post feeding. The oocyst load was low for all on of the days of dissection, with an average varying from 1 to 7 oocysts per midgut (min = 1, max = 30). Until day 14 post feeding, the majority of oocysts were still intact. *P. ovale* oocysts were spherical with a well-defined rounded edge and the presence of parasitic pigments. *P. ovale* is efficiently transmitted by *An. gambiae s.s*, Kisumu strain in N'tjiba. The oocysts are clearly visible from day 7 until day 14 post feeding where the majority is still intact.

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PRELIMINARY CHARACTERIZATION OF *PLASMODIUM FALCIPARUM* SPLICING FACTOR 3A SUBUNIT 2 (SF3A2) GENE IN GAMETOCYTE DEVELOPMENT

Olatunbosun Olabanji Aringbangba, Camilla V. Pires, Prem Prakash, Shulin Xu, Min Zhang, Chengqi Wang, John Adams

University of South Florida, Tampa, FL, United States

The burden of malaria remains a major global public health problem especially among pregnant women and young children in sub-Saharan Africa. Development of transmission-blocking interventions and global eradication efforts rely on understanding the mechanisms underlying the development of the parasite's sexual forms, known as gametocytes, which is the only transmissible stage of the parasite to the mosquito vectors. Currently, limited knowledge exists regarding the genetic factors and mechanisms governing gametocyte development. Here, we investigate the role of pre mRNA metabolism required for gametocyte development of P. falciparum, studying the role of SF3A2 of the U2 SnRNP complex. SF3A2 of the spliceosome complex was previously identified as essential for gametocyte development in a large-scale genetic screen of piggyBac mutants. Although the SF3A2 piggyBac mutant had no observable defect in asexual intraerythrocytic development, this piggyBac mutant exhibited reduced abundance and incomplete development of gametocytes compared to the isogenic NF54 wild-type parent post induction for gametocyte culture. Further characterization of the P. falciparum SF3A2 mutant by scRNAseq identified aberrant transcription and splicing of canonical gametocyte genes. As part of the intranuclear U2 SnRNP spliceosome complex, the essential function of SF3A2 appears to be integral in regulating early gene expression during gametocyte development. These findings lay the groundwork for further investigations on the importance of nuclear RNA processes in the context of sexual stage development vital for mosquito infections and transmission of malaria parasites.

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MALARIA RISK STRATIFICATION: A CRITICAL TOOL FOR MALARIA CONTROL AND ELIMINATION IN HIGH BURDEN COUNTRY, CASE OF MALI

Mady Cissoko[1], Mahamadou Magassa[1], Assitan Dembélé[1], Seybou Coulibaly[1], Ibrahim A. Cissé[1], Daouda S. Samaké[1], Chaka Sanogo[1], Vincent Sanogo[1], Aissata Koné[1], Mamady Koné[2], Sylla Thiam[3], Seydou Fomba[1], Issaka Sagara[2]

[1]Programme National de Lutte contre le Paludisme, Bamako, Mali, [2]Malaria Research and Training Center, Bamako, Mali, [3]Sunu Santé Consulting, Hann Bel Air Dakar-Senegal, Dakar, Senegal

Malaria risk stratification is an approach for identifying different transmission zones and prioritizing the implementation of control measures. As part of the development of the new 2024-2028 strategic plan, the stratification was done to take into account World Health Organization (WHO) guidelines and to target interventions appropriately. Data collection covered all 75 health districts (HDs) in 2022. Data collected included malaria cases (confirmed and suspected), supplemented by national survey data and a literature review on malaria and entomology in Mali. Adjusted incidence was calculated by taking into account health data reporting, malaria

diagnostic positivity and health facility attendance rates at health district level. Mixed interventions were defined on the basis of adjusted incidence, prevalence, seasonality, vector resistance to pyrethrinoids and parasite distribution by region. Four strata have been defined according to the 2017 WHO Malaria Elimination Framework. The majority of HDs (54) are ranged in high and moderate transmission, i.e. 84% of the population (~18 million). *Plasmodium vivax* was identified in two regions with a prevalence ranging from 4% - 22%. In this regard, specific Pf/Pv RDTs will be used for diagnosis in these regions. Vaccination has been recommended for all HDs with high and moderate transmission. Seasonal Malaria Chemoprevention (SMC) is maintained in 57 HDs. Both Dual-insecticide ITNs and PBO ITNs will be used in high prevalence area on the basis of insecticide resistance distribution. For cIPTg, HDs with high and moderate transmission coverage rates have been targeted to implement this intervention. Finally, to delay drug resistance with Artemether Lumefatrine (AL), Dihydro-artemisinin-Piperaquine (DHA-PPQ) will be introduced as a first line treatment along with AL in 5 high burden regions. Malaria stratification is a critical for strategic planning and appropriate deployment of malaria control interventions. It requires a multi-disciplinary team as well as recent and reliable data, with periodic updates.

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ASSESSMENT OF MOSQUITO FEEDING ASSAYS TO MEASURE ENDPOINTS IN CHILDREN FOR FUTURE TRANSMISSION BLOCKING TRIALS

Daman Sylla[1], Adama Sacko[1], Heather Goodman[2], Mahamadoun H. Maiga[1], Abdrahamane Fofana[1], Boubacar Coulibaly[1], Moussa Diallo[1], Sale Sidibe[1], Yacouba Diarra[1], Amadou Berthe[1], Mohamed L. Diarra[1], Moridie Sidibe[1], Salifou Kone[1], Sekou Goita[1], Adama Coulibaly[1], Mariam Doumbia[1], Amadou Guindo[1], Ousmane Sacko[1], Yacouba Dembele[1], Amadou S. Traore[1], Issaka Sagara[1], Sara A. Healy[2], Alpha S. Yaro[1], Patrick E. Duffy[2], Jen C. Hume[2]

[1]Malaria Research and Training Center, Bamako, Mali, [2]LMIV/NIAID/NIH, Bethesda, MD, United States

A vaccine to interrupt malaria transmission will be a valuable tool for elimination and control of malaria. Recently, progress has been made in malaria vaccines for young children with the R21 and RTS,S vaccines now approved in several sub-Saharan African countries. However, additional control measures are needed to supplement existing vaccines and extend protection to all vulnerable individuals. Transmission blocking vaccines (TBV) that reduce the number of infected mosquitoes have shown promising results and Phase 1 studies to test the two vaccines in combination are planned in Mali this year. TBV are unique in their action by killing parasites in the mosquito vector rather than human host. To accurately measure their efficacy, TBV require specialized assays such as the direct skin feeding (DSF) assay and direct membrane feeding assay (DMFA). Here we investigate the performance of both feeding assays and determine the adverse events related to direct skin feeding in Bancoumana, Mali. From November 2023 to February 2024, we recruited 200 volunteers aged 5-17 years who completed monthly visits including a blood smear and RDT. All RDT positive participants completed a DSF along with 10 participants who were RDT negative on each calendar day that feeds were performed. Additionally, the first 20 RDT positive individuals completed a DMFA, as well as 2 participants who were RDT negative. In total 252 DSF were conducted - 92 from RDT positive individuals. Of the RDT positive individuals, 8 feeds (8.7%) yielded a positive infection with a total of 67 positive mosquitoes recorded (1.9%). 124 DMFA were performed of which 4 out of the 92 RDT positive individuals (4.3%) yielded a positive infection with a total of 12 positives mosquitoes (0.34%). No positive feeds (either DSF or DMFA) were recorded in RDT negative individuals. We will discuss mosquito feeding parameters such as: mosquito feeding rate, survival rate, infection rate and feed positivity rates. We will also discuss the safety of the DSF procedure which yielded one Grade 1 adverse event overall. Data from this study support DSF as a surrogate assay for measuring the efficacy of TBV in future field trials.

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COMMUNITY ACCEPTABILITY OF ATTRACTIVE TARGETED SUGAR BAITS IN A CLUSTER RANDOMIZED CONTROLLED TRIAL IN WESTERN KENYA

Caroline Ogwang[1], Teresa Bange[1], Omollo Mevis[1], Dominic Ouma[1], Brian Seda[1], Julia M. Janssen[2], Jonathan S. Schultz[3], Julie R. Gutman[2], Aaron M. Samuels[3], Simon Kariuki[1], Feiko ter Kuile[1], Sarah G. Staedke[4], George Okello[4]

[1]Kenya Medical Research Institute, Centre for Global Health Research, Kisumu, Kenya, [2]Division of Parasitic Diseases and Malaria, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, GA, United States, [3]Malaria Branch, National Center for Emerging and Zoonotic Infectious Diseases, US Centers for Disease Control and Prevention, Kisumu, Kenya, [4]Liverpool School of Tropical Medicine, Liverpool, Liverpool, United Kingdom

Attractive Targeted Sugar Baits (ATSBs) are a novel malaria vector control intervention that aim to attract and kill mosquitoes. We conducted a qualitative evaluation of community perceptions of ATSBs alongside an ongoing cluster-randomised trial in western Kenya evaluating ATSB impact on malaria incidence. ATSB were deployed in March 2022, with 2 ATSB stations hung outside on walls and replaced every 6 months over 2 years. Between May and October 2023, 12 in-depth interviews (IDIs) and 30 focus group discussions (FGDs) were conducted with 303 community members aged 13 to 83 purposively selected from intervention clusters. Interviews were conducted in Dholuo, Kiswahili, or English, recorded, and transcribed verbatim to English. The interview guides, conceptual framework, and evaluation objectives were used to create predetermined codes for the domains of potential barriers and facilitators to ATSB acceptability and high coverage. Data were coded and organized using NVivo 12, and data within each code were assessed. Patterns among themes and across types of respondents were identified and interpreted. Initially, most participants were unsatisfied that ATSBs did not eradicate mosquitoes. By the second year of deployment, most participants associated ATSBs with positive experiences, such as reduced mosquitoes and malaria; and compensation and free treatment in some sub-studies, which increased acceptability. Some participants were still unsatisfied with ATSBs due to perceived ineffectiveness but opted to have them for the benefit of doubt and their trust in the research implementors. Other barriers to ATSB acceptability included perceived bias in the selection of participants for sub-studies and activities, religious ideologies and ATSB leakage. Periodic community engagement to understand and address community concerns greatly improved the acceptability of ATSBs. Notably, high ATSB coverage of over 98% was maintained throughout the study period with minimal consent withdrawal. The information on factors influencing community acceptance of ATSBs can guide future sensitization and education of communities about ATSBs.

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A SYSTEMATIC REVIEW OF THE COST OF DELIVERING SEASONAL MALARIA CHEMOPREVENTION

Anika Ruisch, Miranda Heilweil, Ishani Mathur, Sara Harris, Colin Gilmartin

Management Sciences for Health, Medford, MA, United States

Seasonal malaria chemoprevention (SMC) is a cost-effective intervention for preventing malaria in children. SMC includes monthly doses of antimalarials during high transmission periods and has been implemented in 17 countries. To inform future planning and resource allocation, there is a need to understand the cost of SMC delivery, especially as new malaria preventive technologies are being adopted. This systematic review aims to determine the financial and economic costs of SMC delivery from provider and patient perspectives. Six databases were reviewed in accordance with the PRISMA guidelines. We included studies published between 2012 and 2023 which focused on the cost of SMC delivery and included a defined costing methodology. Two authors reviewed each study to ensure criteria were met and extracted relevant cost data. Results were adjusted for

inflation and study quality was assessed using the CHEERS checklist. We identified 4,034 studies, of which six met the inclusion criteria, including results from nine countries. The number of children targeted for SMC varied from 104,225 to 2,020,597. The total financial cost per child covered by SMC ranged from $1.71 to $12.46 and the total economic cost from $2.11 to $29.06. The Incremental cost-effectiveness ratio (ICER) per clinical case averted ranged from $5.41 to $138.03, ICER per DALY averted from $24.51 to $182.88, and ICER per death averted from $688.86 to $18,418.81. While some components (e.g. training, drugs and supplies) were universally costed, others (e.g. transport, per diem) were only incorporated into some calculations, leading to discrepancies in aggregate cost estimates for SMC delivery. Costing methodologies, reporting, and the intervention scale varied by study, limiting comparability. As most studies only captured SMC costs from a provider perspective, important household costs may have been overlooked. Given emerging malaria preventive technologies (e.g. monoclonal antibodies and malaria vaccines), understanding SMC cost implications is critical to ensure evidence-based resource allocations and improved efficiency.

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EQUITY IN ACCESS TO IPTP3+ AMONG WOMEN WHO ATTENDED ANC4 IN 12 SUB-SAHARAN COUNTRIES, BEFORE AND AFTER WHO RECOMMENDATION CHANGES

Ashley Malpass[1], Susan Youll[1], Ashley Garley[1], Cameron Taylor[2]
[1]U.S. President's Malaria Initiative, Washington, DC, United States, [2]The DHS Program, ICF, Rockville, MD, United States

Intermittent preventive treatment in pregnancy (IPTp) with sulfadoxine-pyrimethamine (SP) is a proven intervention for preventing malaria in pregnancy. The World Health Organization (WHO) initially recommended that pregnant women receive at least two doses of IPTp in 2004 and revised this recommendation in 2012 to three or more doses (IPTp3+). Although this recommendation has been in place for over 10 years, no country has met their target of 80% coverage of IPTp3+. The primary platform for delivering IPTp is through antenatal care (ANC), however despite women attending four or more ANC visits (ANC4+), they often do not receive the minimum three doses of IPTp. To understand how IPTp coverage changed for different demographics of pregnant women since the 2012 recommendation, a secondary analysis of women who attended ANC4+ was conducted using data from 12 countries with population-based surveys completed before and after countries adopted the 2012 WHO recommendation. If multiple surveys were available, the most recent were used. IPTp3+ coverage for women who attended at least four ANC visits was compared against demographic indicators including wealth, education, urbanicity, and age. While 8 of the 12 countries showed better access to IPTp3+ among the lowest wealth quintile (pro-poor) before the revised recommendation and more equitable access after, no demographic indicator was significantly associated with an increased likelihood of receiving IPTp3+ across the twelve countries included in this analysis. In the pooled analysis, IPTp3+ access was pro-poor in earlier surveys [concentration index (CI): -0.03] and moved to being more equitable between high and low wealth quintiles in later surveys (CI: 0.01). There are many known barriers to ANC attendance, but the barriers to receiving SP while attending ANC are less well documented. This analysis found that demographic indicators often associated with health inequity are not associated with reduced access to IPTp3+ among women who attended four or more ANC visits. Further analysis is needed to understand key factors associated with low IPTp coverage among pregnant women attending ANC.

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COMMUNITY-BASED STRATEGIES TO INCREASE UPTAKE OF INTERMITTENT PREVENTIVE TREATMENT IN PREGNANCY WITH SULFADOXINE-PYRIMETHAMINE IN SUB-SAHARAN AFRICA: A SYSTEMATIC REVIEW, META-ANALYSIS, META-ETHNOGRAPHY, AND ECONOMIC ASSESSMENT

Kadiatou Koita[1], Kassoum Kayentao[2], Eve Worrall[1], Anna Maria Van Eijk[1], Jenny Hill[1]
[1]Liverpool School of Tropical Medicale, Liverpool, United Kingdom, [2]Malaria Research and Training Center, University of Sciences, Techniques, and Technologies of Bamako, Bamako, Mali

Community-based approaches may increase uptake of intermittent preventive treatment in pregnancy with sulfadoxine-pyrimethamine (IPTp-SP). We assessed the effects of community-based approaches on IPTp-SP and antenatal care (ANC) coverage, and barriers and facilitators to implementation in sub-Saharan Africa. We undertook a meta-analysis and meta-ethnography. We searched online databases for trials, mixed-method, qualitative and cost-effectiveness studies evaluating community health worker (CHW) promotion of ANC and/or IPTp-SP delivery (cIPTp) with no language restriction up to March 20, 2024. Information on interventions, IPTp doses, ANC visits, and barriers/facilitators were extracted. Meta-analysis (random effects) was conducted comparing effects on two-or-more (IPTp2+), three-or-more IPTp doses (IPTp3+), one-or-more (ANC1+) and four-or-more ANC visits (ANC4+). We followed Noblit and Hare's method of meta-ethnography to synthesize qualitative findings, using reciprocal translation and line-of-argument synthesis. A theory for increased cIPTp uptake was developed. A summary of cost and cost-effectiveness studies was done. Of 4755 records screened, 23 reporting on 15 studies were included. CHW involvement was associated with an increase in IPTp2+ (pooled RR [pRR] 1·48, 95% CI 1·24-1·75, 12 sub-studies, I^2 94·7%) and IPTp3+ (pRR 1·73, 95% CI 1·19-2·50, 10 sub-studies, I^2 97·5%), with no decrease in ANC4+ (pRR 1·17, 95% CI 1·00-1·36, 13 sub-studies, I^2 90·3%). Barriers to cIPTp included women's fear of side effects, lack of knowledge, lack of trust in CHWs, and sociocultural factors. Community sensitization, engagement of husbands, pre-established CHW networks and trained and supported CHWs facilitated cIPTp. Incremental cost-effectiveness ratios ranged from $1·1 to $543 per DALY averted. Community-based approaches increased IPTp coverage and may have a positive impact on ANC visits in addition to being cost-effective, though there was high heterogeneity among studies. Community sensitization and engagement in addition to established, trained, and supported CHWs can facilitate cIPTp delivery and uptake.

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ASSESSMENT OF EPIDEMIOLOGIC IMPACT ON MALARIA FOLLOWING DRONE-BASED LARVICIDING WITH *BACILLUS THURIGIENSIS ISRAELENSIS IN* TWO DISTRICTS OF MADAGASCAR, 2022

Anna B. Bowen[1], Sarah Zohdy[2], Jean-Desire Rakotoson[3], Laurent Kapesa[4], Solofo Razakamiadana[5], Omega Raobela[6]
[1]CDC - PMI, Antananarivo, Madagascar, [2]CDC, ATLANTA, GA, United States, [3]ABT Associates, PMI EVOLVE, Antananarivo, Madagascar, [4]USAID-PMI, Antananarivo, Madagascar, [5]USAID - PMI, Antananarivo, Madagascar, [6]National Malaria Program, Antananarivo, Madagascar

In Madagascar, rice is cultivated throughout the country near human housing. Rice paddies are key habitats for the larvae of the *Anopheles* mosquito, which transmits malaria. Malaria burden in Madagascar is heterogeneous, with persistently high or increasing incidence in some areas despite repeated national distributions of insecticide-treated bednets and years of support for malaria case management. In 2022, we conducted a feasibility study of drone-delivered *Bacillus thurigiensis israelensis* (Bti), a bacterial larvicide, as a possible adjunct intervention in two districts with a high density of rice paddies. Bti was applied every two weeks to paddies within one km of human housing in a subset of villages within 3 of 15

communes in Ankazobe District and 3 of 9 communes in Morombe District from February to June 2022, which is typically within the malaria high-burden season. Using commune-level data from DHIS2, we used malaria case counts from 2021 and 2022 to compare communes that received larviciding (Bti) and those that did not (comparison) in a random intercept model, adjusted for district, to explore the difference-in-difference (DID). Median case counts per commune in 2022 were lower in both arms in both districts (Ankazobe: Bti = 2451 [interquartile range (IQR) 2,155, 3,716] in 2021 and 331 [IQR 247, 1160] in 2022; comparison= 1,614 [IQR 816, 2,737] in 2021 and 358 [IQR 123, 903] in 2022. Morombe: Bti= 1,970 [IQR 1,908, 3,383] in 2021 and 1,289 [IQR 1,249, 2,548] in 2022; comparison= 2,254 [IQR 1,804, 2,847] in 2021 and 1,384 [IQR 902, 2,018] in 2022). The DID was not significant (p=0.3). Although the intervention was not designed to fully cover all villages in each commune, and the number of communes available for analysis was small, biweekly larviciding with Bti throughout the high-transmission season was not associated with reductions in malaria burden that could be measured at the commune level using routine data. Future assessments of the epidemiologic impacts of larviciding would benefit from access to more geographically granular health data, more complete intervention coverage, or larger sample sizes.

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THE EFFECT OF INTERMITTENT PREVENTIVE TREATMENT OF MALARIA IN PREGNANCY (IPTP) ON THE MATERNAL INTESTINAL MICROBIOME AND ITS RELATIONSHIP WITH FETAL GROWTH

Patricia J. Hunter[1], Dagmar G. Alber[1], Jemima A. Hair[1], Lily Gates[1], Godwin Tembo[2], Crispin Mukerebe[3], Mwayiwawo Madanitsa[4], Hellen C. Barsosio[5], Daniel T. Minja[6], John P. Lusingu[6], Eric D. Onyango[5], Jenny Hill[7], James Dodd[7], Ulla Ashorn[8], Julie R. Gutman[9], Nigel Klein[1], Feiko O. ter Kuile[5], Raymund M. Chico[10]

[1]UCL Great Ormond Street Institute of Child Health, London, United Kingdom, [2]Malawi-Liverpool-Wellcome Programme, Blantyre, Malawi, [3]National Institute of Health Research, Mwanza, Tanzania, United Republic of, [4]Malawi University of Science and Technology, Thyolo, Malawi, [5]Kenya Medical Research Institute, Kisumu, Kenya, [6]National Institute of Medical Research, Tanga, Tanzania, United Republic of, [7]Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [8]Centre for Child, Adolescent and Maternal Health Research, Tampere University, Tampere, Finland, [9]Centers for Disease Control and Prevention, Atlanta, GA, United States, [10]London School of Hygiene & Tropical Medicine, London, United Kingdom

The IMPROVE trial was a double-blinded randomized controlled trial in Kenya, Tanzania, and Malawi that compared monthly intermittent preventive treatment of malaria in pregnancy (IPTp) with sulfadoxine-pyrimethamine (SP) vs monthly IPTp with dihydroartemisinin-piperaquine (DP), alone or combined with a single course of azithromycin (DP+AZT). IPTp with SP was superior to DP and DP+AZT at improving maternal weight gain and fetal growth despite demonstrating inferior antimalarial activity relative to DP. The mechanism by which SP protects against low birthweight may involve changing the maternal intestinal microbiome to support increased release of nutrients and energy. For example, expanding the Prevotellaceae family of intestinal bacteria has been shown to increase mono- and di-saccharide release from dietary glycans such as mannan and galactan leading to improved weight gain in children with undernutrition. We are characterizing the intestinal microbiomes of 152 trial participants at enrolment (16 - 28 weeks gestation) and, again, near delivery (32 - 35 weeks). DNA was extracted from stool samples and the V3-V4 region of 16S rRNA genes from an initial 19 sample pairs were amplified and sequenced using Illumina MiSeq. Analysis of the first 19 pairs revealed diverse and dynamic microbiomes. The Prevotella component of the microbiome expanded between enrolment and delivery in women who received SP (11.1% +/- 9.4% at enrolment compared with 31.0% +/- 29.3% near delivery) with 6 out of 7 sample pairs showing an increase. In contrast, the size of the Prevotella compartment in the DP and DP+AZT groups remained the same (17.3% +/-21.5% at enrolment compared with 19.8% +/- 22.8% near delivery) with only 5 out of 12 sample pairs showing an increase (P=0.04). These data support Prevotella expansion as a possible non-malarial mechanism through which SP increases birthweight and is in line with data from other studies demonstrating improved weight gain in children. We will present full length 16S rRNA gene sequencing and metagenomics for the remaining 133 sample pairs to confirm this finding and identify other components associated with fetal growth.

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ANTIPLASMODIAL AND INSECTICIDAL ACTIVITIES OF THIRD GENERATION IVERMECTIN HYBRIDS

Diana Fontinha[1], Parth Juneja[2], Sofia Santana[1], Catarina Rôla[1], Carla Bastos Oliveira[1], Miguel Prudêncio[1], Kamaljit Singh[2]

[1]Instituto de Medicina Molecular Joao Lobo Antunes, Lisbon, Portugal, [2]Guru Nanak Dev University, Amritsar, India

Current antimalarial therapeutic and vector control strategies are threatened by the development of drug resistance. Hence, new tools to control this infectious disease, particularly drugs that can act throughout the complex life cycle of *Plasmodium* parasites, malaria's etiologic agents, are needed. Ivermectin (IVM), an endectocide used in mass drug administration to treat neglected tropical diseases, has shown promising potential for malaria control, either indirectly, by killing the mosquito vector, or directly, by impairing the parasite's development. Although IVM was shown to impact the liver stage bottleneck of infection in the mammalian host, its effect on the subsequent, symptomatic blood stage of infection remains controversial. Aiming to develop compounds that could efficaciously tackle both the liver and blood stages of *Plasmodium* infection, we have previously designed two generations of IVM hybrid conjugates in which the IVM macrolide is covalently linked to antimalarial pharmacophores, yielding compounds with increased antiplasmodial potency, relative to IVM. However, one of the most potent antiplasmodial compounds lacked the insecticidal activity displayed by the parental IVM molecule. Seeking to recover this crucial feature of IVM, while improving its antiplasmodial activity, and stemming from our previous structure-activity relationships, we have designed a third-generation of hybrid IVM molecules. Here, we present the evaluation of the liver stage, blood stage, and insecticidal activities of these newly developed compounds. We further assess, for the first time, the blood stage activity of the most potent hybrids *in vivo*, in a rodent model of *Plasmodium* infection. Collectively, our results provide key structure-activity relationships to guide the rational design of new molecules, supporting the subsequent pursuit of the clinical development of IVM hybrids as potential antiplasmodial drugs.

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ASSESSING THE 2023 SCHOOL-BASED INSECTICIDE-TREATED NET DISTRIBUTION IN KONO DISTRICT, SIERRA LEONE

Keith Esch[1], Prince Owusu[2], Frederick Yamba[3], Raymond Sudoi[4], Malia Skjefte[5], Musa Sillah-Kanu[3], Jenny Carlson[6], Temitayo Labor[7], Kevin Opondo[8], Djenam Jacob[9], Charlene Youssef[10], Prince Nallo[10], Elisabeth Tyler[1], Stephen Poyer[11]

[1]PMI Evolve Project, Population Services International, Washington, DC, United States, [2]PMI Evolve Project, Population Services International, Accra, Ghana, [3]National Malaria Control Program, Freetown, Sierra Leone, [4]PMI Evolve Project, Population Services International, Nairobi, Kenya, [5]Population Services International, Washington, DC, United States, [6]U.S. President's Malaria Initiative, USAID, Washington, DC, United States, [7]U.S. President's Malaria Initiative, USAID, Freetown, Sierra Leone, [8]PMI Evolve Project, Abt Associates, Freetown, Sierra Leone, [9]PMI Evolve Project, Abt Associates, Rockville, MD, United States, [10]Nest Builders International, Freetown, Sierra Leone, [11]PMI Evolve Project, Tropical Health LLP, Bristol, United Kingdom

Insecticide-treated nets (ITNs) are a key malaria prevention and control tool. In Sierra Leone, the National Malaria Control Program (NMCP) targets universal ITN access through triennial mass distribution campaigns and continuous distribution through routine channels (such as antenatal care visits, etc). The 2021 Malaria Indicator Survey reported higher national malaria prevalence in children ages five through nine than in children under five. To cover this at-risk population, in 2023 PMI Evolve supported

the NMCP and the Ministry of Basic and Senior Secondary Education to conduct a school-based distribution (SBD) of piperonyl butoxide-synergist ITNs in 531 schools across Kono District, reaching about 89,000 students in classes 1, 3 and 5. A cluster random sample household survey, stratified by presence of a child in a targeted class, was conducted at 950 households to assess ITN use and access one month after the SBD. Key informant interviews (KIIs) were also conducted with 26 donor, government (national and sub-national) and other stakeholders. SBD was successful and led to significant increases in ITN access and use. Population access in intervention households (those with at least one child in class 1, 3 or 5) was significantly higher than in non-intervention households (69% vs. 46%, p<0.001). Household ownership of at least one ITN was also significantly higher in intervention households (93% vs. 65%, p<0.001), as was ownership of one ITN per two people (40% vs. 21%, p<0.001) and population-level ITN use (71% vs. 49%, p<0.001). KIIs highlighted funding dispersion delays and a need for more social behavior change communication. They also showed that rather than staggering recipient classes, the SBD could target consecutive classes (e.g. classes 1-3). Staggering is the global standard. However, while pilot SBD ITN uptake was high, unincluded community members expressed dissatisfaction. The consecutive approach may be more acceptable while reducing operational costs. To strengthen the scale up of SBD nationally, the NMCP and its partners should decide which roll out strategy to pursue and resolve other identified challenges.

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KNOWLEDGE, ATTITUDES, PRACTICES AND SATISFACTION 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 Tambacounda, Tambacounda, Senegal

Digitizing the payment of healthcare workers could improve their well-being, that of patients and the performance of the healthcare system. The aim of this study is to identify factors associated with satisfaction with digital payment by applicators of the intra-domestic insecticide spraying campaign against malaria in the Koumpentoum health district. This is a descriptive and analytical cross-sectional study. Data collection took place from January 16 to 28, 2023. Sampling was exhaustive. Data were collected via a questionnaire from applicators of the 2022 indoor insecticide spraying campaign. We performed a descriptive, bi-variate analysis. Logistic regression was used to identify factors associated with satisfaction. Data were analyzed using R software version 4.2.2. A total of 159 applicators were interviewed. The proportion of men was 67.92%, 61.01% were married and 58.49% had a monthly income of less than 50,000 CFA francs. The average age was 26.96 +/- 5 years, and 94.07% had studied in French. Health workers represent only 15.09% of the working population. Almost all (97.48%) were familiar with the definition of digital payment and its use. Acceptability of digital payment in healthcare was 92.43%. Satisfaction was 94.41%, the main reasons being speed (92.59%), security (85.93%), convenience (84.44%), ease of use (83.7%), traceability (80%) and anonymity (77.04%). In multiple logistic regression, only perceptions of safety and speed were predictive of applicator satisfaction. Digitizing the payment of healthcare workers is a major challenge for the healthcare system. It should be taken into account in mass public health campaigns to improve performance.

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LEVERAGING COMMUNITY HEALTH WORKERS TO SUSTAIN UNIVERSAL BED NET COVERAGE IN RURAL UGANDA: A PILOT FEASIBILITY STUDY

Annika K. Gunderson[1], Rapheal Mbusa[2], Emmanuel Baguma[2], Emmanuel Ayebare[2], John Barber[1], Moses Ntaro[2], Edgar M. Mulogo[2], Ross M. Boyce[1]

[1]University of North Carolina at Chapel Hill, Chapel Hill, NC, United States, [2]Mbarara University of Science & Technology, Mbarara, Uganda

Long-lasting insecticidal nets (LLIN) are a cornerstone of malaria control programs but sustaining target coverage levels, defined as 1 LLIN per 2 household members, between mass distribution campaigns is challenging. We evaluated the feasibility of a novel distribution strategy leveraging community health workers (CHW) operating in an integrated community case management (iCCM) program. We conducted cross-sectional household surveys in two villages in Kasese District to assess (i) baseline and (ii) post-intervention LLIN coverage and parasite prevalence (PfPR). The intervention consisted of an open-label, pilot feasibility trial in which CHWs in both villages were trained to measure household LLIN coverage when a child is RDT positive. CHWs in the intervention village also distributed LLINs to households below target levels. At baseline 92 (28%) of households had at least 1 LLIN with 20 (6%) meeting universal coverage. Parasitemia $PfPR_{2-10}$ was 36% in the control and 9% in the intervention villages, respectively. Between July and September 2023, CHWs evaluated 311 children <5 years of age with 61% of those tested have a positive RDT in each village. In the intervention village, all 100 LLINs were distributed with the median number of 3 LLINs required to meet universal coverage by each household. Post-intervention, 12 (8%) of households in the intervention village reached universal coverage and only 2 (2%) in the control village. The percentage of children <5 years old who slept under a LLIN increased from 16% to 32% compared to a decrease from 29% to 11% in each village, respectively. In interviews, CHWs conveyed favorable opinions of the distribution method. Our results demonstrate low proportions of universal LLIN coverage near the end of the 3-year distribution cycle. LLIN distribution through iCCM appears feasible and may supplement mass distribution campaigns, with marked trends improvements in children sleeping under LLINs in the intervention village.

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IMPLEMENTATION PERFORMANCE OF INSECTICIDE TREATED NET (ITN) DISTRIBUTION THROUGH THE HEALTH FACILITIES IN TANZANIA: FIVE YEARS OF EXPERIENCE (2018-2022)

Mponeja Gitanya[1], Samuel Lazaro[1], Charles Dismasi[1], Hannah Koenker[2], Matt Worges[3], Benjamin Kamala[4], Ruth Msolla[4], Naomi Serbantez[5], Israel P. Nyarubeli[6], Heavenlight A. Paulo[6], William Kisinza[7], Johnson Matowo[8]

[1]Tanzania National Malaria Control Program, Ministry of Health, Dodoma, Tanzania, United Republic of, [2]Tropical Health LLP, Baltimore, MD, United States, [3]Tropical Health LLP, London, United Kingdom, [4]PMI Vector Control Project, Johns Hopkins University Center for Communication Programs, Dar es Salaam, Tanzania, United Republic of, [5]U.S. President's Malaria Initiative, U.S. Agency for International Development, Dar es Salaam, Tanzania, United Republic of, [6]School of Public Health and Social Sciences, Muhimbili University of Health and Allied Sciences, Dar es Salaam, Tanzania, United Republic of, [7]Amani Research Centre of the National Institute for Medical Research, Tanga, Tanzania, Tanga, Tanzania, United Republic of, [8]Kilimanjaro Christian Medical University College, Kilimanjaro, Tanzania, United Republic of

Routine distribution of insecticide-treated nets (ITNs) to pregnant women at antenatal care (ANC) and to infants through the Expanded Programme for Immunization (EPI) has been a primary mechanism of maintaining population access to ITNs. As the implementation performance of these channels has not been assessed, we conducted a historical analysis of bed net issuing rates through ANC and EPI channels over five years (2018-2022). The team used monthly data from District Health Information

System 2 (DHIS2) on ITN distribution across 6,790 health facilities in 26 regions of mainland Tanzania. Descriptive analyses were conducted to separately assess the performance of ITN issuing rates through ANC and EPI across time, region, health facility, ownership (public/private), setting (urban/rural), and malaria transmission strata. Performance was categorized as "good" if all women or infants attending ANC or EPI received nets in a given month. Across all years and facilities, ANC outperformed EPI with "good" issuing rates of 83% and 70%, respectively ($p<0.001$). Issuing rates started low, about 44% and 34% in 2018, and increased to 93% and 80% in 2022, for ANC and EPI, respectively. Public facilities performed better for both ANC (89%) and EPI (76%) compared to private facilities (53% and 42%, respectively) ($p<0.001$). Dispensaries and health centers performed better than hospitals and clinics for both ANC and EPI issuance ($p<0.001$). Ruvuma region had the best performance, and, in most regions, performance improved markedly in the first year but then increased at a very slow rate. Rural areas had higher performance (72%) during ANC visits compared to urban areas (51%) ($p<0.001$). Across epidemiological strata, ITN issuing rates during EPI activities were notably higher in high (79%) and moderate (76%) transmission epidemic strata compared to low (54%) and very low (65%) ($p<0.001$) strata. By 2022, Tanzania substantially improved ITN issuance via ANC and EPI compared to 2018 levels. Exploration of the reasons for differences among analyzed variables in issuing rates are recommended to understand additional characteristics of high performing facilities.

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COMBINING SEASONAL MALARIA CHEMOPREVENTION WITH A MULTI-STAGE PRODUCT FOR MALARIA PREVENTION: A MATHEMATICAL MODELLING STUDY

Lydia Braunack-Mayer[1], Josephine Malinga[2], Narimane Nekkab[1], Sherrie L. Kelly[1], Melissa A. Penny[2]

[1]Swiss TPH, Allschwil, Switzerland, [2]Telethon Kids Institute, Nedlands, Australia

In recent years, research and development programs for vaccines, monoclonal antibodies, and small molecule drugs have led to significant advancements in preventing Plasmodium falciparum malaria. Innovations include products that target multiple stages of the parasite life cycle that, if approved, might be deployed as part of a comprehensive prevention strategy. To effectively prioritize vaccine and drug candidates for clinical development, evidence is needed to understand the benefits of combining both single- and multi-stage tools with malaria chemoprevention. We used an individual-based malaria transmission model to estimate the impact of combining seasonal deployment of a novel therapeutic with seasonal malaria chemoprevention (SMC) in children under five. Our model combined emulator-based methods with explicit models of intervention dynamics for vaccines, monoclonal antibodies, and long-acting injectable drugs with both pre-erythrocytic and blood stage activity. We estimated reductions in the cumulative incidence of uncomplicated and severe malaria throughout childhood relative to SMC alone. Results indicated that, when a pre-erythrocytic product was combined with SMC, lasting protection was needed to limit the impact of delayed malaria after children were no longer eligible for interventions. Depending on prevalence setting and SMC deployment, a protection half-life of more than 230 days was required for a pre-erythrocytic product with 50% initial efficacy to achieve a more than 5% reduction in cumulative incidence of severe malaria by ten years old. Higher duration and efficacy were required when SMC's deployment was optimized. Adding blood stage activity increased impact on cumulative severe malaria throughout childhood. Our modelling quantifies the benefits of combining multi-stage therapeutics for malaria prevention with SMC by estimating their possible impact on malaria burden throughout childhood. This evidence articulates the need for a novel tool to fill an existing gap in malaria prevention tools, thus informing the selection of multi-stage therapeutics for malaria prevention.

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IDENTIFICATION AND MAPPING AREAS WITH AN INCREASED RISK O MALARIA TRANSMISSION AMONG HARD-TO-REACH HIGH-RISK GROUPS IN RWANDA

Epaphrodite Habanabakize

Rwanda Biomedical Centre, Kigali, Rwanda

Malaria continues to be a significant public health concern in Rwanda, posing a risk of infection to the entire population. Understanding the spatial distribution of high-risk populations is crucial for targeted interventions. Malaria high-risk groups as hard-to-reach people are estimated at 3.4% (413,367) of the Rwandan population from mapping assessment of 2022. This research focuses on mapping malaria hotspots among hard-to-reach high-risk groups in Rwanda, aiming to identify and map areas with an increased risk of malaria transmission. A mixed-methods approach study integrates Matchbox assessment of 262 participants, comprising 22 individual interviews and 240 for focus group discussions (FGDs) and rapid assessment data (455participants for FGD). Integration of geospatial data, demographic information, and malaria incidence records into Geographic Information System (GIS) analysis illustrated the precision of hotspots mapping of high-risk groups.The findings reveal that 17.6% (80/455) reported fever cases in the past two weeks, with 22% (100/455) experiencing malaria in the last nine months (2 episodes). Of those, 68% (309/455) sought care at health centers, 12% (37/309) bought malaria drugs without a diagnostic test, and 7% (7/100) received community-level treatment. Female Sex Workers (FSWs) had the highest malaria incidence (30.5%; 30/95), followed by seasonal workers (28.8%; 19/66), bicyclists (20%; 15/75), People with Disabilities (PWD) (18.8%; 9/48), and cross-border traders (18.4%; 7/38). Reported barriers included a lack of repellent (80%; 356/455), insufficient knowledge (70%; 317/455), outdoor activity delays (69%; 312/455), lack of updated information (60%; 273/455), and limited access to care (9%; 42/455). This study successfully mapped and identified malaria hotspots among hard-to-reach high-risk groups in Rwanda, providing a valuable tool for precision public health interventions. This strategic approach enhances resource allocation for Rwanda's malaria elimination efforts allowing optimization of the impact of malaria control objectives.

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INSECTICIDE TREATED NET (ITN) TARGETED MASS CAMPAIGN (TMC) FOR MALARIA PREVENTION IN THE KAGERA REGION, TANZANIA: IMPLEMENTATION PROCESSES, OUTCOMES AND CHALLENGES

Benjamin Kamala[1], Peter Gitanya[2], Naomi Serbantez[3], Lulu Msangi[3], Dismass Charles Mwalimu[2], Samuel Lazaro Nhiga[2], David Dadi[1], Dana Loll[4], Ruth Msolla[1]

[1]PMI Tanzania Vector Control Project, Johns Hopkins Centre for Communication Programs, Dar es Salaam, Tanzania, United Republic of, [2]Tanzania National Malaria Control Program, Ministry of Health, Dodoma, Tanzania, United Republic of, [3]U.S. President's Malaria Initiative, US Agency for International Development, Dar es Salaam, Tanzania, United Republic of, [4]PMI Tanzania Vector Control Project, Johns Hopkins Centre for Communication Programs, Baltimore, MD, United States

Kagera region, Tanzania, has been receiving continuous distribution of insecticide-treated nets (ITNs) through antenatal clinics and school net programs over the past 10 years. However, in 2023, a Targeted Mass Campaign (TMC) in Kagera was conducted in response to high malaria prevalence (18%), low population access to ITNs (62% vs. 80% national target), and confirmed partial artemisinin resistance. We aimed to document the process, outcomes, and challenges of TMC implementation in Kagera. We quantified the estimated number of required ITNs using the standard formula, population (from national census data 2022) divided by 1.8. Planning and advocacy meetings were held at the regional and council levels. Training was conducted to orient volunteers, village leaders, and ward leaders on using the TMC Management Information System (TMC-MIS), household registration, ITN issuance , data management,

and accountability. Targeted supportive supervision and mentorship were informed by TMC-MIS data based on low registration and issuance rates at different levels. A total of 190 ward executive officers, 790 village leaders, and 2,476 volunteers were trained from 192 wards in all six councils of the Kagera region. We quantified a need of 1,713,815 ITNs, of which 1,693,318 (98.8%) were issued through 2,104 issuing points, covering an estimated 3,094,278 people. TMC-MIS challenges included poor internet connectivity in hard-to-reach villages that slowed registrant data uploads and TMC-MIS server failure due to data overload, preventing data upload. We conducted a total of 287 and 217 targeted supervision visits at the village level during household registration and ITNs issuance respectively contributing to the timely mitigation of observed challenges. Real-time digital ITN data entry, monitoring, and intervention by trained volunteers, leaders, and supervision teams aided in identification and prompt mitigation of challenges during TMC ITN distribution.

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PREGNANCY DESIRES AND MALARIA PREVENTION IN SUB-SAHARAN AFRICA

Bolanle Olapeju[1], Michael Bride[2]

[1]Uniformed Services University of the Health Sciences, Bethesda, MD, United States, [2]Johns Hopkins University Center for Communication Programs, Baltimore, MD, United States

Trends in antenatal (ANC) attendance and malaria in pregnancy prevention are suboptimal in sub-Saharan Africa. It is unclear to what extent pregnancy desires influence these outcomes. This study explores the relationships among women aged 15 - 49 years old in 19 sub-Saharan African countries. Secondary data analysis of recent (2018-2023) Demographic and Health Surveys was conducted among women who had a live birth in the past year preceding the survey and stated whether the index child was wanted then, later or not at all (N= 45,200). Other outcomes included early ANC defined as the first visit within the first trimester, ANC retention (at least four (ANC4) or eight (ANC8) contacts), mosquito net use and intermittent presumptive treatment in pregnancy (IPTp). Control variables included age, residence, religion, education, household wealth, parity, country and year of survey. Overall, 25% of women did not want the index pregnancy when it happened had early ANC with intercountry variation ranging from 13% in Madagascar to 48% in Gabon. Not wanting a pregnancy was associated with reduced odds of early ANC (aOR: 0.67, 95% CI: 0.63, 0.71), ANC4 (aOR: 0.68; 95% CI: 0.64, 0.73), ANC8 (aOR: 0.83; 95% CI: 0.73, 0.94), mosquito net use (aOR: 0.88; 95% CI: 0.82, 0.94) and IPTp (aOR: 0.88; 95% CI: 0.83, 0.95). Women who did not want the pregnancy were more likely to be single, 15 - 24 years or older than 34 years old, urban, non-Christian, with a secondary education, from poorer households and have two or more children less than five years old. Women's pregnancy desires influence their uptake of care services and subsequent malaria prevention behaviors. Study findings corroborate the need for a multi-sectoral approach to optimizing malaria in pregnancy outcomes. Integration of reproductive health services, malaria service delivery and behavior change interventions can help to improve pregnancy intentions and outcomes in order to ensure healthier pregnancies, women and children.

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ASSESSING MISSED OPPORTUNITIES IN ROUTINE LONG-LASTING INSECTICIDE-TREATED NETS DISTRIBUTION AMONG PREGNANT WOMEN ATTENDING PUBLIC HEALTH FACILITIES IN TARGETED COUNTIES IN KENYA

Robert M. Mwaganu[1], Fredrick O. Odhiambo[2], Emma Nyandigisi[1], Beatrice K. Machini[1], James N. Kiarie[1]

[1]National Malaria Control Program, Ministry of Health, Nairobi, Kenya, [2]National Public Health Institute, Nairobi, Kenya

To prevent malaria in pregnancy (MIP), the World Health Organization recommends universal coverage of at-risk pregnant women with long-lasting insecticide-treated nets (LLINs), which are routinely accessed through antenatal care (ANC) clinics. The Kenya Malaria Strategy aims to protect 100% of at-risk individuals in the lake endemic zone (LEZ), coastal endemic zone (CEZ), and highland epidemic-prone zone (HEZ) through LLINs. Biannual malaria commodities review meetings facilitate inter-county discussions on malaria commodity management best practices and identify gaps. LLINs issued to pregnant women are recorded in the ANC register, summarized in the integrated summary report (MOH 711), and replicated in the malaria commodities report (MOH 743) monthly. This information is uploaded to the Kenya Health Information System (KHIS). At the time of the review meeting, the number of new ANC attendance and LLINs dispensed recorded in MOH 711 for September 2023 was downloaded from KHIS. The total number of LLINs dispensed and recorded in the two summary tools was compared. The overall reporting rate for MOH 711 and 743 was 98% of the total no. of reports expected, with 2% over-reporting for LLINs dispensed in MOH 743. The median months of stock for LLINs was 5 months, with none of the counties reporting stock out. Notably, about 9,200 (10%) of the pregnant women attending the first ANC clinic did not receive ITNs despite the availability of the commodity within the county. The HEZ achieved the least coverage at 85%, while the LEZ achieved 98%. Data discordance indicated over-reporting on the commodities reporting tool, and data quality audits should be carried out to improve reporting. The Ministry of Health should intensify the development of health workers' capacity in reporting and social behavior change in communities as some counties reported pregnancy stigma and negative cultural perceptions of pregnancy among teenage and older (over 35 years) mothers, resulting in poor ANC and LLIN uptake.

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DETERMINANTS OF NON-USE OF LONG-LASTING INSECTICIDE-TREATED NETS (LLIN) AMONG MOTHERS OF CHILDREN UNDER 5 YEARS OLD: A SECONDARY ANALYSIS OF DATA FROM THE USAID NOTRE SANTÉ KNOWLEDGE ATTITUDES AND PRACTICES SURVEY

Datolo Kone[1], Elizabeth Fitch[2], Richard Reithinger[3], Aguima Tankoano[1], Mohamed Keita Sitan[1], Lamine Bangoura[4], Eliane Mbounga[4], Alioune Camara[5], Sylvestre Sandouno[1], Souleymane Berete[1], Diallo Abdoulaye[6]

[1]RTI International, Conakry, Guinea, [2]RTI International, RTP, NC, United States, [3]RTI International, Washington, DC, United States, [4]President's Malaria Initiative, USAID, Conakry, Guinea, [5]National Malaria Control Program, Conakry, Guinea, [6]Johns Hopkins CCP, Conakry, Guinea

Malaria remains a major public health challenge in Guinea, negatively impacting the economy and particularly affecting children under 5 years old (CU5) and pregnant women. In October 2023, the USAID Notre Santé Activity, implemented by RTI International, undertook an in-depth study of the knowledge, attitudes, and practices (KAP) related to malaria prevention and treatment of women with CU5 in the Conakry, Kindia, Boké, and Labé regions. Based on the hypothesis that socio-demographic factors, level of malaria knowledge, and disease attitudes play a role in the use of LLINs in these households, a secondary analysis was undertaken to study those factors. Statistical approaches included descriptive statistics, Chi Square and t tests, and logistic regression to pinpoint factors associated with LLIN non-use. The study included 1460 women of whom 31.4% did not sleep under a LLIN the previous night. Household dialogue about malaria, positive attitude towards LLIN repair, self-efficacy and favorable social norms were associated with increased LLIN use (p less than 0.05). The impact of ethnicity (p less than 0.001) and marital status (OR-1.85, p=0.03) on non-use of LLINs highlights the need to integrate cultural and relational issues in malaria control programs. The positive association between household size and non-use of LLINs (OR=1.07, p less than 0.001) raises questions about net use in the contexts of limited or shared resources. Contrary to expectations, women with higher levels of education were less likely to use LLINs (OR=1.19, p less than 0.001). Comparison with studies in other African regions reveal that while some dynamics, such as the impact of household size, appear to be universal, other factors vary, such as the influence of education or access to electricity, highlighting the importance

of local context in developing malaria prevention strategies. This research is part of a broader approach aimed at identifying more effective prevention and treatment strategies, accounting for local context and needs.

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CHALLENGES IN ESTIMATING COVERAGE INDICATORS FOR PERENNIAL MALARIA CHEMOPREVENTION (PMC) WHEN COMBINING STANDARD ROLLOUT PLUS CATCH-UP APPROACHES: LESSONS LEARNED FROM PILOT IMPLEMENTATION IN DEMOCRATIC REPUBLIC OF CONGO (DRC)

Aline Maliwani[1], Mvuama Mazangama[2], Nono Koka[3], Packy Mukanya[1], Gloire Mbaka Onya[2], Rova Ratsimandisa[2], Michael Hainsworth[4], Arantxa Roca Feltrer[5], Caterina Guinovart[6], Eric Mukomena[1], Henry Ntuku[7]

[1]National Malaria Control Program, Kinshasa, Congo, Democratic Republic of the, [2]PATH, Kinshasa, Congo, Democratic Republic of the, [3]Provincial Malaria Control Program, Kongo Centrale, Congo, Democratic Republic of the, [4]PATH, Seattle, WA, United States, [5]PATH, Maputo, Mozambique, [6]The Barcelona Institute for Global Health, Barcelona, Spain, [7]PATH, Geneva, Switzerland

The DRC recently introduced PMC targeting children under 2. Implementation began in December 2023 in 4 health zones in Kongo Central province. DRC opted for a model based on the administration of Sulfadoxine-Pyrimethamine (SP) at six contacts aligned with the expanded immunization program (EPI) and Vitamin A (Vit A) calendar, i.e. at 10 and 14 weeks of age, 6, 9, 12 and 15 months. This corresponds to the timing of DTP2, DTP3, Vit. A at 6 months, Measles1, Vit A at 12 months and Measles2. A monitoring and evaluation plan was developed and defined various indicators to be tracked, including coverage indicators. The monthly coverage of each PMC dose is estimated the same way as the corresponding EPI antigens or Vit A using the same expected monthly population. To maximize coverage during the rollout phase, all children between 10 weeks and 15 months of age were targeted. Children received their first dose of PMC anytime between the age of 10 weeks and 15 months. With this approach, the monthly coverage of each PMC dose cannot be reliably compared with the corresponding EPI antigen or Vit A. In February, there were 4,591 doses of PMC 1 for 2,223 doses of DTP 2 delivered. This discrepancy will likely continue until the cohort is composed solely of children who received their first PMC dose at 10 weeks. The length of time will depend on the country specific schedule, and in DRC this will take 12 months. An overall PMC coverage indicator is used to track the performance of PMC against corresponding EPI antigens and Vit A during the initial rollout phase and guide decision making for improvement. This indicator compares the total number of PMC doses administered during the period (i.e 7,297 in January and 7,963 in February), regardless of age, to the total number of children expected for the 6 EPI and Vit A contacts, as well as the total number of children who received any EPI antigen or Vit A (7,152 in January and 7,302 in February) during the 6 contacts in the same period. This indicator showed better correspondence and suggested an increase in use of health services with an increase in the EPI/Vit A performance, probably boosted by the enthusiasm generated by the introduction of PMC.

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QUALITY AND PERFORMANCE OF COMMUNITY-OWNED RESOURCE PERSONS FOR MALARIA COMMUNITY CASE MANAGEMENT (MCCM) IN HARD-TO-REACH COMMUNITIES IN TANZANIA, 2023

Kanuth Dimoso[1], Abdallah Lusasi[2], Jacquiline Tungaraza[1], Francis Levira[1], Sarah-Blythe Ballard[3], Albert Ikonje[4], Lulu Msangi[4], Naomi Serbantez[4], Chonge Kitojo[4], Patrick Gulinja[1], Mwaka Kakolwa[1], Dunstan Bishanga[1]

[1]Ifakara Health Institute-PMI Shinda (Defeat) Malaria Project, Dar-es-salaam, Tanzania, United Republic of, [2]National Malaria Control Program, Ministry of Health, Dodoma, Tanzania, United Republic of, [3]U.S. President's Malaria Initiative, US Centers for Disease Control and Prevention, Dar-es-salaam, Tanzania, United Republic of, [4]U.S. President's Malaria Initiative, US Agency for International Development, Dar-es-salaam, Tanzania, United Republic of

In 2022, because policy prohibited malaria testing by community health workers, Mainland Tanzania introduced community case management of malaria (mCCM) by retired health professionals called Community-Owned Resource Persons (CORPs) to enhance the equity and reach of malaria service delivery in remote, high burden communities. To assess CORPs mCCM service delivery, we analyzed data collected by Council Health Management Teams (CHMT) during the two initial rounds of supportive supervision (SS). Training, equipment, and monthly stipends were provided to 33 CORPs providing mCCM services in 36 villages in Nsimbo and Tanganyika districts in Katavi region. Local government provided artemether-lumefantrine (AL), malaria rapid diagnostic tests (mRDTs), reporting tools, and SS. CHMTs used a standard checklist to assess service delivery, including patient volume, commodity management, community leader engagement, and overall competency. CHMTs assessed 33 (100.0%) CORPs in round 1 and 28 (84.8%) in round 2. From March to September 2023, CORPs conducted 15,437 mRDTs. Of these, 4,795 (31.1%) tested positive for malaria. Of these, all were diagnosed with uncomplicated malaria, and all were treated with AL. During stockouts of AL (24.3% round 1, 14.0% round 2) and mRDTs (28.3% round 1, 7.4% round 2), CORPs did not provide mCCM. Across both rounds, 70% of CORPs engaged community leaders for community mCCM sensitization. CORPs meeting overall competency standards was 54% in round 1 and 92% in round 2. Most improved competency areas included: stockout reduction, tool use (e.g., proper OPD summary filing [39% to 96%]), and mRDT quality control (e.g., recording start/reading time [83% to 96%]). CORP-administered mCCM improved over two SS rounds but was limited by stockouts. To improve service delivery, commodity availability should be paired with continued SS and other evidence-based supports. These findings are likely to be generalizable to future cadres of CHWs implementing the test and treat approach to mCCM.

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THE ECONOMIC BENEFITS OF INDOOR RESIDUAL SPRAYING IN RWAMAGANA DISTRICT, EASTERN PROVINCE, RWANDA

Michee Semugunzu Kabera[1], Dunia Munyakanage[1], Kaendi Munguti[2], Aimable Mbituyumuremyi[1], Emmanuel Hakizimana[1]

[1]Rwanda Biomedical Center, Kigali, Rwanda, [2]US President's Malaria Initiative, United States Agency for International Development, Kigali, Rwanda, Kigali, Rwanda

Malaria continues to be a major public health concern in Africa and particularly in Rwanda. Malaria reduces labor productivity as it affects people's ability to work, children misses school. Indoor residual spraying is one of the key interventions used to control malaria in Rwanda; however, few studies have evaluated its economic outcomes. This study aims to investigate the economic returns from investment in malaria control using IRS with Rwamagana district with 484,953 inhabitant. Malaria morbidity data were retrospectively collected through Rwanda Health Management Information System. Projected malaria cases in the absence of IRS intervention was done using the linear regression in Epi Info and adjusted for seasonal effects. The economic benefits were estimated using the minimum average cost of USD 8.67 for treating a simple episode of malaria case which includes direct cost and indirect costs. The IRS has been implemented for the first time in Rwamagana from June 2019. It is conducted on annual round and with more than 98% coverage. Two types of insecticides have been used on rotation every two years, Pirimiphos methyl 300 CS and Fludora Fusion 56.25 WP. Malaria cases were reduced by 95 percent from 235,320 cases in 2018 before IRS to 11,067 cases in 2023 while the incidence dropped from 959 to 23 cases per 1000 persons respectively. From June 2019 to December 2023, the projected malaria cases in absence of IRS are estimated to 4,431,401 and 239,08 reported malaria cases. The estimated averted malaria cases are 4,192,320 cases. The total cost for conducting IRS was USD 9,385,661 including both Insecticides and operation costs. The total estimated cost to treat malaria

cases in absence of IRS is 36,347,412 USD. The benefit due to averted outpatient malaria cases is estimated to 26,961,751 USD with benefit-cost ratios of 2.9 and the average IRS cost per Capita is 19.4 USD. IRS in Rwamagana district have high economic returns, its cost is much lower than benefits related to the averted malaria cases. Funding Malaria Control using IRS have good return on investment and in preventing malaria and saving lives.

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ASSESSING PATTERNS IN BEDNET USE USING ACCELEROMETER-BASED MONITORING IN COTE D'IVOIRE

Paul Krezanoski[1], Soro Dramane[2], Laurence Yao[2], Benjamin Koudou[2]

[1]University of California, San Francisco, San Francisco, CA, United States, [2]Centre Suisse de Recherches Scientifiques in Côte d'Ivoire, Abidjan, Côte D'Ivoire

Malaria has not declined despite the wide-scale distribution of long-lasting insecticide treated bednets. One explanation could be that the timing of use may not correspond with the timing of vector exposure. Surveys about bednet use are prone to recall and social desirability bias and only give a snapshot of bednet use the previous night. Remote monitors of bednet use can provide objective measurements of whether a bednet is in use or not over time. This observational study deployed accelerometer-based bednet monitors in 3 regions of Cote d'Ivoire representing different malaria transmission settings: urban Yamoussoukro, peri-urban Tiassalé and rural Korhogo. The objective was to characterize patterns in bednet use and assess differences between regions. Bednet use monitors were attached to the side of one bednet in each household. Accelerometer data was classified using a previously trained random-forest machine learning algorithm (Koudou et al. 2022). Mixed-effects regression models were employed to account for multiple measures per household. Logistic regression models were used for binary outcomes and generalized linear models for continuous outcomes. Fifty households were followed for a mean of 115 nights each (5,749 total nights). Overall, bednets were unfurled at 8:26 pm (95% CI: 8:20 pm - 8:39 pm) and folded up at 6:13am (95% CI: 6:03 am - 6:23am). Mean bednet use was 10.3 hours per night (95% CI: 9.8 - 10.9). Both Yamoussoukro (-1.3 hours; 95% CI: 0.5 to 2.5; p=0.042) and Korhogo households (-1.5; 95% CI: -0.0 to 3.0; p=0.054) used their bednets less compared to Tiassalé. Overall, households did not use their bednets on 4.8% of nights (95% CI: 2.2% - 7.4%), but there were no significant differences between regions. Within regions, there was substantial heterogeneity along both of these metrics and evidence of outlier households. These findings suggest that remote bednet monitoring could be utilized by National Malaria Control Programs to identify the behaviors, households and regions with sub-optimal prevention practices, thus allowing behavior change campaigns to be tailored to ensure maximum impact in improving malaria prevention.

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ANALYZING THE IMPACT OF MALARIA PREVENTIVE INTERVENTION ON MALARIA TEST POSITIVITY RATES: A FOUR YEAR STUDY IN ADAMAWA STATE NIGERIA

Chinedu J. Chukwu[1], **Victoria C. Erinle**[1], Bravo B. Otohabru[1], Jerry Agulehi[1], Isaac Adejo[1], Thomas A. Hall[2], Sonachi S. Ezeiru[3], Emmanuel U. Obi[3], Orezi S. Adhekoyibo[3], Frank Oronsaye[3], Nnenna N. Ogbulafor[4], Chukwu Okoronkwo[4], Fatima B. Ali[4], Mary E. Esema[4], Godwin N. Ntadom[4], James Ssekitooleko[5]

[1]Management Sciences for Health, Abuja, Nigeria, [2]Management Sciences for Health, Arlington, VA, United States, [3]Catholic Relief Services, Abuja, Nigeria, [4]National Malaria Elimination Program, Abuja, Nigeria, [5]Global Fund, Geneva, Switzerland

This study analyzes malaria testing and positivity rates in Adamawa State, Nigeria, from January 2020 to December 2023. The data include information on Rapid Diagnostic Tests (RDT) and Microscopy methods. The study considers the demographic characteristics of the state, including its diverse population and urban or rural settings. In 2023, Management Sciences for Health conducted a large-scale campaign for Insecticide Treated Nets (ITNs) and supported the Seasonal Malaria Chemoprevention campaign. The study aims to analyze the effectiveness of these campaigns in reducing malaria incidence. This data-driven exploration will inform future malaria elimination strategies and form a foundation for evidence-based decision-making in malaria prevention and elimination programs. The data used is from Nigeria DHIS2 to calculate Test Positivity Rates (TPR) for both RDT and Microscopy, analyzing monthly fever cases, malaria-positive cases, and those tested by RDT and Microscopy. The results reveal average dropping trends in malaria testing positivity rates over four years of SMC and ITN implementation in Adamawa State from 80% to 77%, 76%, and 75% in 2020, 2021, 2022, and 2023, respectively. While the overall average yearly number of persons presenting with fever continues to increase progressively from 59,567 in 2020 to 68,475 in 2023, the TPR demonstrated positive variations, providing insights into the efficacy and effectiveness of prevention and vector control strategies in the state, because we recorded malaria incidence rate declined from 7% (January) to 3% (December) in 2023. The analysis highlights the need for continuous monitoring and assessment of malaria testing processes and reporting in Adamawa State to optimize elimination vector control interventions. The study recommends regular evaluations of testing methodologies, and targeted interventions in high malaria positivity regions. In conclusion, monthly and yearly variances in malaria case patterns are determined, highlighting the impact of SMC and ITN interventions on the rate of malaria incidence in the states.

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RECONSIDERING INDOOR RESIDUAL SPRAYING COVERAGE TARGETS: LEVERAGING HIGH-RESOLUTION OBSERVATIONAL DATA FROM BIOKO ISLAND TO ESTIMATE THE DOSE-RESPONSE CURVE

David S. Sanders[1], Carlos A. Guerra[2], Liberato Motobe Vaz[1], Lucas Ondo Nze[1], Jeremias Nzamio Mba Eyono[1], Restituto Mba Nguema Avue[1], Teresa Ayingono Ondo Mfumu[1], Wonder P. Phiri[1], David L. Smith[3], Guillermo A. Garcia[2]

[1]MCD Global Health, Malabo, Equatorial Guinea, [2]MCD Global Health, Silver Spring, MD, United States, [3]University of Washington, Institute of Health Metrics and Evaluation, Seattle, WA, United States

Indoor residual spraying (IRS) has long been one of the cornerstones of malaria vector control, and high to extremely high coverage levels (at least 80-85%) have historically been recommended as necessary to ensure a community effect. This claim has little empirical evidence due to the difficulty of conducting high-quality studies. Here we present results from a retrospective analysis of five years (2016-2020) of programmatic IRS and annual malaria indicator survey (MIS) data from Bioko Island, estimating how IRS effect size varied with coverage. The first 15 years of IRS implementation on Bioko targeted the entire island, but the years analyzed targeted high-risk areas based on *P. falciparum* prevalence (PfPR) in the previous year. The implementation of a spatial decision support system on Bioko links household-level IRS and MIS data to map sectors (100m x 100m grid cells), which we use as the primary unit of analysis. Despite the observational nature of the data and non-random selection of areas to target, this period provides a sensible setting for the application of causal inference techniques, given the increased variation in achieved coverage and the relatively simple and well-understood mechanism for assigning treatment. In a causal inference framework, we define multiple coverage variables using different spatial scales (the map sector and 100m, 200m, and 300m buffers around sectors), and use MIS covariates to debias the relationship between coverage and PfPR. The analysis suggests a possible threshold for community protection at much lower coverage levels than previously thought, though extremely high coverages may provide additional benefit. This casts some doubt on the necessity of 80-85% coverage targets, and more importantly, points to the need for more research on the relevant coverage thresholds for IRS, the potential mechanisms causing the observed behavior, and other drivers of IRS effect size heterogeneity.

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SEASONAL MALARIA CHEMOPREVENTION COVERAGE SURVEY STRATEGIES TO SUSTAIN HIGH COVERAGE THROUGHOUT CAMPAIGN ARE NEEDED BENIN 2023

Sakariahou Kpanou[1], William Hindéwé Houndjo[1], Cyriaque Dossou Affoukou[1], Julien Codjo Aissan[1], Achille Achille Batonon[1], Virgile Gnanguenon[2], Raoul Chaffa Raoul Oloukoi[2], Djebo Goumanon[3], John Bernon[2], Catherine Dentinger[4]

[1]Ministry of Health, National Malaria Control Program, COTONOU, Benin, [2]U.S. President's Malaria Initiative, USAID, Cotonou, Benin, [3]U.S. President's Malaria Initiative, Malaria Branch, US Centers for Disease Control and Prevention, Atlanta, GA, USA, GA, United States, [4]U.S. President's Malaria Initiative, Malaria Branch, US Centers for Disease Control and Prevention, Atlanta, GU, United States

Benin assessed 2023 seasonal malaria chemoprevention (SMC) coverage of four monthly SMC cycles consisting of three daily medicine doses [sulfadoxine-pyrimethamine + amodiaquine (SPAQ) on day one; AQ on days two and three] given to a target of 432,710 children aged 3-59 months. A weighted, cluster-randomized sample of SMC-receiving households (sample size, 3403 children) from 15 communes was surveyed four weeks post-campaign. In six communes, distributors gave day-one doses; education to caregivers included AQ dose administration for days two and three (DOT1). In nine communes, distributors gave all doses each cycle (DOT3) along with SMC education. A questionnaire regarding one randomly selected, age-eligible child per household was administered to caregivers; data including demographics, number of doses per cycle, reasons for refusal, and education provided by the distributor were recorded using Survey Solutions software on a smartphone and analyzed with SPSS. Caregivers of 3573 children (1932 [54%] male; mean age 29 months) completed surveys; 452 (13%) had no SMC for diverse reasons (e.g., refusal, absent). In DOT1 and DOT3 communes, 1565/1663 (94%) and 1556/1910 (81%) children received at least one SPAQ dose, respectively. For DOT1, three SMC doses were taken by 1531 (92%), 1503 (90%), 1469 (88%) and 1106 (67%) children, for cycles one to four, respectively; for DOT3, these doses were 1486 (78%), 1415 (74%), 1306 (68%) and 1114 (58%), respectively. In total, 2137 (60%) children took all 12 doses. Reasons given by 65 caregivers who refused SMC for their child included father's absence for permission (n=24, 40%), and medicine safety concerns (n=27, 42%). Of 3121 caregivers, 2684 (86%) reported that distributors taught them about SMC benefits and 1092 (35%) were educated to manage adverse events. Coverage with at least one SPAQ dose was high; however, SMC coverage declined with each successive cycle. Lower coverage in DOT3 sites may reflect challenges finding children and caregivers at each visit. Areas to improve include uptake of all SMC doses, particularly in later cycles, and effectively teaching key information to caregivers.

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ENHANCING OFFLINE DATA COLLECTION SYSTEMS THROUGH HYBRID DATA MANAGEMENT: INSIGHTS FROM THE BOHEMIA CLINICAL TRIAL

Vegovito Antonio Mario Vegove[1], Eldo Elobolobo[2], Isaiah Omondi[3], Jamal Salim[3], Shadrack Karisa[3], Esther Yaa[3], Paula Ruiz-Castillo[1], Mercy Kariuki[3], Patricia Nicolas[1], Mary Mael[1], Francisco Saute[2], Regina Rabinovich[1], Carlos Chaccour[1], Marta Maia[3]

[1]ISGLOBAL, Barcelona, Spain, [2]CISM, Manhica, Mozambique, [3]Kemri, Kwale, Kenya

The BOHEMIA clinical trial, conducted in Mozambique and Kenya, investigated the impact of mass ivermectin administration on malaria control. Utilizing a comprehensive database of over 600,000 entries collected via Open Data Kit (ODK) on tablets, the trial showcased the benefits and challenges of electronic data capture in remote settings. Despite its efficiency, maintaining data integrity posed significant challenges due to digital literacy gaps and connectivity issues, necessitating innovative solutions for data quality assurance.The trial's data management strategy highlighted several hurdles, including data errors such as typographical mistakes, geolocation inaccuracies, and duplication. To mitigate these issues, the data collection system was enhanced with validation checks and warnings, complemented by a dynamic analysis script for identifying and addressing anomalies. This multi-tiered approach, bolstered by independent data reviews, ensured the robustness of the cleaning process. Crucially, the trial underscored the importance of human oversight alongside technological solutions. Direct feedback mechanisms and continuous communication between data managers, field supervisors, and collectors were vital in refining the data cleaning methodology. This process not only improved data accuracy but also fostered a transparent and replicable system, essential for the trial's success.This mixed-methods approach—combining technological tools with human insight—proved effective in overcoming the challenges of data collection in resource-limited settings. The BOHEMIA trial's experiences offer valuable lessons for future global health research, emphasizing the need for adaptable and resilient data management strategies in the face of connectivity and literacy barriers.>

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EVALUATING A PROGRAMMATIC MALARIA MASS DRUG ADMINISTRATION IN MOZAMBIQUE: MIXED-METHODS ANALYSIS OF OPERATIONAL PERFORMANCE

Laura de la Fuente[1], Maria Tusell[1], Jacopo Vecchio[1], Amâncio Nhangave[2], Khalid Bapú[3], Christina Riley[4], Mercia Dimene[5], Samira Sibindy[5], Baltazar Candrinho[5], Pedro Aide[3], Caterina Guinovart[1]

[1]ISGlobal, Barcelona, Spain, [2]Gaza Provincial Directorate of Health, Xai-Xai, Mozambique, [3]CISM, Manhiça, Mozambique, [4]Akros, Lusaka, Zambia, [5]National Malaria Control Program (NMCP), Ministry of Health, Maputo, Mozambique

The World Health Organization (WHO) recommends Mass Drug Administration (MDA) to reduce malaria transmission in low-transmission settings, with a target coverage of at least 80%. Developing effective and sustainable programmatic delivery strategies is key to achieving high impact.From Dec 2022 to Feb 2023, two rounds of programmatic MDA (pMDA) were conducted in Chidenguele (Gaza province), Mozambique, targeting an estimated population of 59,271. Door-to-door distribution using a satellite imagery-based mapping tool was conducted. To evaluate its operational performance (including acceptability, adoption, fidelity, feasibility, and appropriateness), we conducted a mixed-methods evaluation with a community household survey (HHS, n=770), a health facility survey (HFS, n=28), and field observations (FO, n=149). Out of the HHS participants (HHSp) eligible to receive medication, 96.0% (568/592) accepted taking the medication. All HFS participants (HFSp) and 91.3% (703/770) of HHSp agreed that taking antimalarials even if not sick was acceptable as a prevention strategy. Similarly, all HFSp and 84.1% (648/770) of HHSp agreed that the intervention was appropriate to decrease malaria transmission in the community. Programmatic coverage (% of survey participants treated), a proxy of feasibility, was 73.9% (569/770) and operational coverage (% of individuals treated among participants present during the pMDA) was 90.2% (569/631). In 74.5% (111/149) of the FO, the distribution team correctly informed family members about the purpose of the visit, in 86.6% (129/149) asked for verbal consent to conduct the visit and treat them, and in 60.4% (90/149) explained the importance of completing the full treatment (fidelity). In rounds 1 and 2, 86.0% (24/28) and 96.0% (27/28) of HFSp were very willing to adopt the campaign procedures respectively.Despite the pMDA delivery strategy being implemented with good operational performance and being well accepted by the community and implementers, achieving 80% coverage is challenging and requires high engagement from partners, community, and policymakers.

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GENETIC LINKAGE OF DRUG RESISTANCE GENOTYPES TO CONTINENTS USING *PFS47* AND *PFCPMP* FOR TRAVEL-ASSOCIATED *PLASMODIUM FALCIPARUM* MALARIA CASES WITH AN UNREPORTED TRAVEL HISTORY (USA, 2018-2021)

Edwin Pierre-Louis[1], Julia Kelley[2], Dhruviben Patel[3], Dragan Ljolje[2], Christina Carlson[2], Eldin Talundzic[4], David Jacobson[2], Joel Barratt[2]

[1]Laboratory Science and Diagnostics Branch, Division of Parasitic Diseases and Malaria, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention--Oak Ridge Institute for Science and Education, Atlanta, GA, United States, [2]Laboratory Science and Diagnostics Branch, Division of Parasitic Diseases and Malaria, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, GA, United States, [3]Laboratory Science and Diagnostics Branch, Division of Parasitic Diseases and Malaria, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention--Williams Consulting LLC, Atlanta, GA, United States, [4]Office of Associate Director of Laboratory Science, Global Health Center, Centers for Disease Control and Prevention, Atlanta, GA, United States

Travel-related malaria cases are regularly encountered in the United States where the Centers for Disease Control and Prevention (CDC) performs national surveillance through characterization of *Plasmodium falciparum* drug-resistance genotypes. An important aspect of antimalarial resistance surveillance is understanding its geographic distribution. However, CDC often receives malaria case data lacking a travel history. To complement drug-resistance surveillance at CDC, in addition to sequencing loci associated with resistance, amplicons of *Pfs47* and *Pfcpmp* are sequenced as markers of geographic origin. Here, we use these markers to classify *P. falciparum* to a continent and compare sequence-based classifications to travel histories provided for a subset of U.S. travel-associated malaria cases reported to CDC from 2018-2021 (n=380). We built 10 classification models by training a Naïve Bayes classifier using *Pfs47* and *Pfcpmp* sequences from >10,000 publicly available (MalariaGen) *P. falciparum* genomes of known origin to predict an isolate's continental origin. The classifier's performance was evaluated by comparing predictions obtained for 10 sets of 627 MalariaGen genomes (excluded from the 10 training datasets to test the trained models) from Africa (n=267), Asia-Oceania (n=338), and Latin America (n=22), plus 243 CDC cases with travel histories; 234 obtained sufficient sequencing coverage; 231 reported travel to Africa, one to India, one to Costa Rica, and one to the Dominican Republic. The classifier was 98% accurate, where incongruent classifications included a Costa Rican travel case classified to Africa, a Sierra Leone case classified to Asia, and on average, 16 Asian MalariaGen samples were misclassified to Africa. Of the CDC cases with unknown travel histories (n=137), one obtained insufficient sequencing coverage, 135 samples were classified to Africa, and one to Latin America. Given the importance of understanding the geographic distribution of drug-resistance, this work will improve domestic malaria surveillance efforts by linking geographic information to more cases.

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EFFECTS OF FACILITY BASED MALARIA SURVEILLANCE MONITORING AND EVALUATION MENTORSHIP MODEL ON DATA QUALITY IN KAKAMEGA COUNTY, KENYA

Faustinah Lukelesia Sakari

County Government of Kakamega, Kakamega, Kenya

In Kenya malaria data is reported through routine health information systems. There are data quality challenges in this system. Enhancing healthcare workers' knowledge and skills is one way of enhancing malaria surveillance monitoring and evaluation and improving the availability of quality data for decision making. This paper describes the impact of the facility based malaria surveillance mentorship model on data quality in Kakamega County, Kenya. This retrospective study analysed data collected during routine malaria data quality assessments before and after implementing the facility based malaria surveillance mentorship program. Data quality indicators. A total of 35 mentors were trained, 1,403 healthcare workers mentored in all 225 targeted health facilities. There were significant improvements in data completeness, timeliness, and accuracy following the mentorship program. Timely reporting increased from 96% to 99%, completeness of reports from 96% to 100%. Data accuracy improved for several key malaria indicators. Cross checks revealed discrepancies between baseline and round two assessments, with reduced accuracy in cross checks between laboratory and pharmacy registers. There were documented positive effects of facility based mentorship on malaria data quality through improvements in the data quality aspects. There is need for a routine capacity building of healthcare workers on best practices in data management and reporting, with emphasis on the newly recruited staff and ensuring continuity of data quality efforts.

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ANTI-MALARIAL DRUG RESISTANCE INTELLIGENT ADAPTIVE GEOSPATIAL SURVEILLANCE SYSTEM

Dr. Apoorv Gupta[1], Lucinda E. Harrison[2], Minu Nain[1], Sauman Singh Phulgenda[3], Rutuja Chhajed[3], Manju Rahi[4], Philippe Guerin[3], Mehul Dhorda[3], Jennifer A. Flegg[2], Praveen K. Bharti[1]

[1]NIMR-ICMR, Sector-8, Dwarka,, Delhi, India, [2]School of Mathematics and Statistics, University of Melbourne, Melbourne, Australia, [3]The Infectious Diseases Data Observatory (IDDO), Oxford Population Health, Oxford, United Kingdom, [4]ICMR - Vector Control Research Centre (VCRC), Puducherry, Puducherry, India

Disease monitoring resources are limited, so improvements are required to achieve comprehensive geographic mapping and real-time situational awareness. This optimization would help to improve resource allocation and reduce logistics and other costs. Countries with vast differences in population density and access to healthcare, as well as vast geography and terrain, use multiple distinct Antimalarial Drug (AD) treatments. All of these complications make mapping of AD resistance a challenging task. Despite the challenges, this study employs a novel approach to identify potential study sites for AD medication monitoring. The methodology also overcomes the limitations in existing data on the geographical distribution of AD resistance in *Plasmodium Falciparum* (Pf). We sought to identify locations with high median resistance marker prevalence and uncertainty. As a test case, we use updated WWARN molecular surveyor from India and neighbouring countries to examine the prevalence of resistance-conferring mutations in pfdhfr/pfdhps for sulfadoxine/pyrimethamine and pfk13 for artemisinin derivatives. This information was then used to develop geostatistical models to identify districts with the greatest potential for reducing predicted uncertainty while also verifying the expected high median resistance marker prevalence. A dynamic dashboard was also created using RShiny to make the site selection process more dynamic. The dashboard classifies districts as having high, medium, or low malaria endemicity. Sites in these areas can then be chosen based on practicality. This ensures that decision-making is supported by quantitative data derived from geographic models, making it easier to select appropriate locations for potential AD resistance surveillance. We demonstrated the efficacy of our technique in identifying areas for medication resistance surveillance in Pf malaria in India. With minor modifications, this technique could be used to improve the efficacy of surveillance initiatives in any other part of the world with a small dataset.

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GUIDING LOCALIZED SEASONAL MALARIA CHEMOPREVENTION STRATEGIES WITH ANTENATAL CARE-BASED MALARIA SURVEILLANCE IN TANZANIA

Joseph T. Hicks[1], Frank Chacky[2], Sijenunu Aaron[2], Samwel L. Nhiga[2], Julie R. Gutman[3], Patrick GT Walker[1]

[1]Imperial College London, London, United Kingdom, [2]National Malaria Control Programme, Ministry of Health, Dodoma, Tanzania, United Republic of, [3]Centers for Disease Control and Prevention, Atlanta, GA, United States

As of 2022, 17 countries in sub-Saharan Africa have implemented seasonal malaria chemoprevention (SMC), providing over 49 million children with at least one dose that year. WHO recommends using clinical case reports or rainfall data to inform SMC implementation, but clinical case reports are influenced by treatment seeking behaviour and only detect symptomatic infections. Outside the Sahel, correlations between rainfall and malaria burden are not well characterised. Malaria surveillance at antenatal care (ANC), which has been conducted nationally in Tanzania since 2014, could provide an alternate means to estimate seasonal malaria burden. Here, we fit an established mathematical malaria model within a Bayesian framework to estimate unobserved indicators of malaria transmission and simulate the impact of SMC strategies in candidate locations identified by the Tanzania National Malaria Control Programme. Monthly reports of malaria testing and positivity among pregnant women at first ANC between 2016 and 2022 were obtained from the Tanzania Ministry of Health. We used a particle Markov Chain Monte Carlo algorithm to fit the *malariasimulation* model to ANC prevalence. Estimated trends in adult mosquito emergence rates were then used to simulate counterfactual scenarios with varying SMC timing and rounds across 23 councils. Percent reduction in annual cases was calculated for each scenario. Overall, our results suggest that SMC could be effective. However, we also identified substantial variation in seasonal trends in malaria prevalence at ANC across candidate councils despite similar rainfall patterns. For example, in a hypothetical four round SMC campaign beginning in January, annual cases in Itigi district council, Singida Region, were reduced on average by 72.3% (95% confidence interval [CI]: 65.4-81.1%) compared with only a 37.8% (95%CI: 37.5-38.0%) reduction in Malinyi district council in Morogoro Region. ANC-based malaria surveillance data are a powerful tool for not only identifying ideal locations for SMC intervention, but also to adapt SMC timing and frequency to the local context.

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FALSE ALARM ON A MALARIA "OUTBREAK" LINKED TO IRREGULARITIES IN MALARIA DIAGNOSTIC SUPPLY: A CALL TO STRENGTHEN SUPPLY CHAIN MANAGEMENT — SIERRA LEONE, MAY-AUGUST 2023

Timothy N. DeVita[1], Aminata Kabia[2], James A.M. Khobi[2], Mugagga Malimbo[3], Samba Kamara[4], Bridget Magoba[2], Gebrekrstos Gebru[2], Anna Jammeh[2], John Painter[1], Thomas Ansumana[5], Musa Sillah-Kanu[5], David Schnabel[6]

[1]Malaria Branch, United States Centers for Disease Control & Prevention, Atlanta, GA, United States, [2]African Field Epidemiology Network, Freetown, Sierra Leone, [3]World Health Organization, Freetown, Sierra Leone, [4]United States President's Malaria Initiative, United States Agency for International Development, Freetown, Sierra Leone, [5]National Malaria Control Programme, Ministry of Health, Freetown, Sierra Leone, [6]United States President's Malaria Initiative, Centers for Disease Control and Prevention, Freetown, Sierra Leone

Malaria transmission in Sierra Leone is intense and perennial, accounting for 40% of clinical consultations. Medical workers diagnose suspected malaria cases using rapid diagnostic tests (RDT) and microscopy, with results reported to the Health Management Information System (HMIS) as monthly aggregates. We investigated a striking increase in confirmed malaria cases reported to HMIS, impacting all ages and districts during May-August 2023 and peaking in June. We first analyzed national and health facility HMIS data to assess RDT stocks, testing rates, and confirmed case counts. To control for transmission variations, 2023 data was compared to the same month's average from 2019 to 2022. We then visited four facilities in two districts with concurrent elevations in malaria cases to identify root causes through analysis of case registers and staff interviews. Investigation revealed inconsistent RDT distribution to and use by facilities. National RDT distribution spiked in May 2023, when 551,888 RDT test kits were delivered compared to the May 2019-2022 mean of 53,121. This was the largest single-month distribution on electronic record. Subsequently in June 2023, 386,343 tests were performed compared to the June 2019-2022 mean of 282,656 (37% increase); tests performed by community health workers increased to 92,817 from a mean of 25,899 (260% increase); and confirmed cases increased to 273,835 from a mean of 187,527 (46% increase). The four visited facilities reflected national trends: June 2023 had 1,910 confirmed cases compared to a 2019-2022 mean of 483 (300% increase). Staff reported recurrent RDT stockouts, with all facilities reporting a greater than 1-month RDT stockout immediately preceding their spike in cases. In conclusion, the 2023 spike in reported malaria cases was likely related to increased testing following an unusually large distribution of RDTs. Fluctuations in RDT availability impede the ability to recognize true case variations. Sierra Leone and its partners can strengthen supply chain logistics and health commodity stock tracking to ensure a consistent supply of RDTs and improve interpretation of surveillance data.

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QUALITY OF MALARIA ROUTINE SURVEILLANCE DATA IN GHANA, 2023

Benjamin Oteng, Wahjib Mohammed, Mohammed Abdul Gafaru, Dora Dadzie, Paul Boateng, George Adu, Ivy Forson, Stephen Appiah, Nana Yaw Peprah, Keziah Lawrencia Malm

National Malaria Elimination Programme, Accra, Ghana

Ghana changed its focus from malaria control to elimination in 2023. Accurate and reliable surveillance data play a pivotal role in shaping public health interventions and strategies. Recognizing the critical importance of data quality in informing evidence-based decision-making, a comprehensive assessment of routine malaria data was conducted to assess the accuracy of reported malaria data in Ghana. We conducted a descriptive cross-sectional assessment using records reviews across 28 health facilities in 10 regions of Ghana selected using a multistage approach. Source documents reviewed included patient folders, monthly statements of inpatient, and monthly summaries. Data was also extracted from the DHIMS-2. Indicators extracted were confirmed malaria cases and admissions. The accuracy of malaria data was assessed using the Malaria Routine Data Quality Assessment Tool. Verification Factor (VF) was calculated as the number of cases counted from the source documents divided by the reported value in DHIMS-2. VF between 0.9 and 1.1 indicated an acceptable accuracy level, VF of >1.1 indicated over-reporting and VF <0.9 indicated under-reporting. Malaria admissions were over-reported in seven out of the 10 regions. Only data reported in Greater Accra Region (VF = 0.92) and Central Region (0.90) were within acceptable level. Only facilities using paper-based (VF = 0.95), Phone Book (VF = 1.06), and Medlink (VF = 0.91) recorded acceptable levels. Government-owned facilities (VF = 2.43) had the highest level of poor data quality followed by quasi-governmental (VF = 2.02). Malaria admissions were thrice over-reported in the period evaluated. Health facilities using Smile and LHIMS software had the highest over-reporting. Coaching on data validation was organized for health information officers in the facilities assessed. The Ghana Health Service needs to strengthen data validation and verification activities in all health facilities across the country.

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TOWARD ZERO MALARIA IN THE DOMINICAN REPUBLIC: INTEGRATING IMPORTED INFECTIONS INTO SURVEILLANCE STRATEGIES

Luca Nelli[1], Keyla Keyla Ureña[2], Juan Mena Lapaix[3], Jose L. Cruz Raposo[2], Miguel De la Cruz Marrero[2], Luccène Désir[4], Greg Noland[4], Jason Matthiopoulos[1], Karen Hamre[4], Gilian Stresman[5]

[1]University of Glasgow, Glasgow, United Kingdom, [2]Ministerio de Salud Pública, Gobierno de la República Dominicana, Santo Domingo, Dominican

Republic, [3]University of Glasgow, The Carter Center, GA, United States, [4]The Carter Center, Atlanta, GA, United States, [5]University of South Florida, Tampa, FL, United States

Confirming malaria elimination requires evidence of the sustained absence of locally acquired cases. If infections are imported, they do not reflect local transmission and provide a critical piece of information to accurately determine between local elimination and ongoing transmission risk. Additionally, a health system's ability to confirm and report imported infections indicates its strength and functionality. The aim was to enhance the precision of system sensitivity and the probability of an area being malaria-free in the Dominican Republic by integrating data on imported malaria cases into the Freedom From Infection (FFI) model. This novel statistical framework, designed to probabilistically demonstrate the absence of malaria using routinely collected health system data, was adapted to incorporate information on imported infections. The study analysed detailed health facility data, including routine malaria surveillance data and questionnaires on health system factors from 48 facilities between 2019 and 2023. Initial analysis of the standard FFI model indicated that, out of 2688 facility-months observed, 387 months had a high probability of being free from infection, with 7 out of 48 facilities maintaining this probability in the last 36 months. However, the revised model, which included imported cases, showed an increase to 714 months and 12 facilities, respectively. By adjusting for the detection of imported infections and their impact on local transmission and surveillance effectiveness, the model offers a refined approach to evaluating elimination status. This improvement in reflecting reality enables public health officials to formulate intervention strategies and allocate resources more effectively, ensuring decisions are grounded in accurate assessments of malaria status. This method's significance extends beyond the Dominican Republic, providing a crucial strategy for regions similarly aiming to differentiate between imported and indigenous cases of malaria, thereby guiding more strategic and impactful public health actions.

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ASSESSMENT OF THE MALARIA SURVEILLANCE SYSTEM IN ELIMINATION-TARGETED NORTH BANK REGIONS, THE GAMBIA

Momodou Kalleh[1], Ousman Njie[2], Adama Jagne Sonko[3], Yankuba Fabureh[1], Balla Gibba[1], Arantxa Feltrer[4], Smita Das[5]

[1]National Malaria Control Programme, Banjul, Gambia, [2]PATH, Banjul, Gambia, [3]PATH, Dakar, Senegal, [4]PATH, Maputo, Mozambique, [5]PATH, Seattle, WA, United States

A strong malaria surveillance system is essential for achieving malaria elimination. In 2023, case-based surveillance (CBS) began in North Bank Regions (NBR), The Gambia, providing a unique opportunity to assess the malaria surveillance system in an elimination setting. Using the WHO Malaria Surveillance Assessment Toolkit, a rapid assessment comprised of a desk review and quantitative interviews with regional (n=5) and health facility (HF; n=29) staff was conducted from January-February 2024. The routine and CBS systems in NBR are functional with several strengths and areas for improvement. NBR HF reporting and participation rates were high: 100% and 92%, respectively, in the routine system and 100% for both rates in the CBS system. Private sector reporting and care-seeking were low, 62% and 16%, respectively. The community level does not participate in CBS, and foci investigation and response are not yet operational. Data for decision-making are used at national and regional levels but are limited at NBR HFs—40% do not use data and case classification rates varied, 64%-77%. Routine quality assurance activities are not done regularly at all health levels. The DHIS2 Tracker for CBS is considered accessible, flexible, stable, and has visualization capacities, but its integration capability has not been implemented and there is no master facility list. The national surveillance guideline is available at NBR regional health directorates (100%) and HFs (97%), but there is no supervision guideline. Despite half of planned trainings being conducted, all NBR regional and most HF staff said they received training in 2023. In consultation with country and partner stakeholders, recommendations were developed to improve the representativeness of routine and CBS systems, ensure availability and access to resources, strengthen data integration and linkage across systems, strengthen data analysis and use, and improve processes for ensuring high quality data. These recommendations will form the basis for a roadmap to guide prioritization of activities among stakeholders that will accelerate and expand malaria elimination efforts in The Gambia.

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PREGNANT WOMEN AS A SENTINEL POPULATION FOR GENETIC SURVEILLANCE OF MALARIA IN THE DEMOCRATIC REPUBLIC OF CONGO

Varanya Wasakul[1], Marie Onyamboko[2], Sarah Benie Bakomba[2], Daddy Kalala Kayembe[2], Bejos Kifakiou Nzambiwishe[2], Pascal Epe Ekombolo[2], Benjamen Basara Badjanga[2], Jean-Robert Moke Mayindombe[2], Jephte Ndundu Ngavuka[2], Eleonor Drury[3], Cristina Ariani[3], Sonia Goncalves[3], Naomi Waithira[1], Vanapol Chamsukhee[1], Tess Verschuuren[1], Sue J. Lee[1], Olivo Miotto[4], Caterina Fanello[4]

[1]Mahidol-Oxford Tropical Medicine Research Unit (MORU), Bangkok, Thailand, [2]Kinshasa-Oxford Medical Research Unit (KIMORU), Kinshasa, Congo, Democratic Republic of the, [3]Wellcome Sanger Institute, Cambridge, United Kingdom, [4]Centre for Tropical Medicine and Global Health, Nuffield Department of Medicine, University of Oxford, Oxford, United Kingdom

Genomic surveillance is a valuable tool for detecting changes in the drug susceptibility of malaria parasites, allowing rapid modification of preventive and curative treatment strategies when needed. To address some of the challenges of implementing surveillance in the most fragile settings, we investigated whether pregnant women attending antenatal care (ANC) services could serve as a more pragmatic sentinel population than the conventional target of children. We conducted a prospective malariometric survey in Kinshasa, Democratic Republic of Congo (DRC), between 2021 and 2023. The study recruited pregnant women attending ANCs regardless of age or trimester of pregnancy, as well as children under 14 years old living in the same area. A total of 4,001 women and 2,794 children participated in the study. While most enrolled women were asymptomatic regardless of parasitaemia, 19% tested positive by rapid diagnostic test (RDT). In contrast, 49% of children tested positive by RDT, with 71% reporting malaria-like symptoms in previous days. Blood samples were taken from positive cases to characterise and compare the genomes of *Plasmodium falciparum* isolates using the SpotMalaria genotyping platform. Comparison of allele frequencies in a 100-SNP barcode revealed a strong correlation between the parasite populations in pregnant women and children ($r=0.99$, $p<0.001$), with overlapping confidence interval at 98 out of 100 SNPs. This indicates that parasite populations were minimally differentiated between the two cohorts. Furthermore, the comparison of antimalarial resistance marker frequencies showed minimal or non-significant differences in variants within *dhfr*, *dhps*, *crt*, *mdr1* and *kelch13* genes. These findings suggest that, in a malaria endemic fragile context, the genomic surveillance of antimalarial drug resistance in pregnant women attending ANC services, can provide comparable results to those of children, with logistical and ethical advantages in implementation.

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IMPROVING THE APPROACH TO MONITOR AND REPORT ON COVERAGE OF MALARIA INTERMITTENT PREVENTIVE TREATMENT IN PREGNANCY: TIME FOR A RETHINK

Donal Bisanzio[1], Raquel González[2], Cristina Enguita[2], Clara Menedez[2], **Richard Reithinger**[1]

[1]RTI International, Washington, DC, United States, [2]ISGlobal, Hospital Clínic - Universitat de Barcelona, Barcelona, Spain

Malaria in pregnancy (MIP) is a maternal and neonatal health issue that continues to result in an estimated 10,000 women and 100,000 infants dying each year. With bednets and prompt case management, intermittent preventive treatment during pregnancy (IPTp) administered during antenatal care (ANC) contacts is the main intervention to prevent and mitigate against MIP effects. For it to be effective at a population level, WHO recommends 80% coverage of at least 3 doses of IPTp (IPTp3). However, as of 2024, no country has reached the 80% target. Indeed, of all malariometric indicators,

MIP is the indicator where endemic countries probably lag most. While this is due to a myriad of factors (e.g., low ANC coverage, SP shortages) we believe that this is also due to limitations in the monitoring and reporting of MIP indicators, specifically IPTp. We posit that the ratio of ANC contact to IPTp coverage, specifically ANC1+ (at least one ANC clinic visit) /IPTp1+ and ANC4+/IPTp3+, should be an added mandatory programmatic indicator for National Malaria Control Programs to monitor MIP efforts. In theory, if pregnant women attending ANC services were to receive IPTp at every ANC visit, the number of IPTp doses administered should track closely with the number of ANC visits, ideally in a 1:1 ratio. Publicly available data from nationally representative surveys conducted in 17 countries in the past five years were analyzed. Findings indicate that while many countries are close to meeting recommended ANC4+ targets, IPTp3 coverage is lagging behind. Thus, while the ANC1+/IPTp1+ ratio is tracking well (i.e., is close to 1), IPTp coverage diverges from ANC coverage with increasing number of ANC visits. Also, the ratio between ANC and IPTp at national level also often differs from the ratio at subnational levels, highlighting geographic areas ripe for programmatic intervention. Identifying countries where the differential in ANC and IPTp coverage is wide, both nationally and sub-nationally, may help identify barriers and facilitators in reaching the recommended IPTp3 coverage targets, as well as in the design of approaches that maximize the impact of MIP control efforts.

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MALARIA EPIDEMICS IN LOW AND VERY LOW BURDEN AREAS OF TANZANIA AND ALERT THRESHOLD SENSITIVITY FOR DISTRICT-LEVEL EPIDEMIC DETECTION, 2022-2023

Francis Levira[1], Frank Chacky[2], Dunstan Bishanga[1], Joseph J. Joseph[1], Kanuth Dimoso[1], Sarah-Blythe Ballard[3], Albert Ikonje[4], Naomi Serbantez[4], Lulu Msangi[4], Chonge Kitojo[4], Samwel Lazaro[5], Mwaka Kakolwa[1]

[1]Ifakara Health Institute-PMI Shinda (Defeat) Malaria Project, Dar-es-salaam, Tanzania, United Republic of, [2]National Malaria Control Program, Ministry of Health, Tanzania, Dodoma, Tanzania, United Republic of, [3]U.S. President's Malaria Initiative, US Centers for Disease Control and Prevention, Dar-es-salaam, Tanzania, United Republic of, [4]U.S President's Malaria Initiative, US Agency for International Development, Dar-es-salaam, Tanzania, United Republic of, [5]National Malaria Control Program, Ministry of Health, Dodoma, Dodoma, Tanzania, United Republic of

Malaria epidemics are associated with increased morbidity, mortality, and health system strain. Epidemic alerts and thresholds can provide context to routine data reviews and guide pre-emptive programmatic action. In 2023, the National Malaria Control Program in Tanzania has established weekly 50th and 75th percentile administrative area-specific thresholds for epidemic alerts and epidemics, respectively, from District Health Surveillance System 2 (DHIS2)-based outpatient department (OPD) case counts for the previous five years. We used the World Health Organization definition of an epidemic as weekly malaria cases exceeding the epidemic thresholds for two consecutive weeks. Using established weekly thresholds, we described the number of district-level epidemics that occurred in 2022 and 2023 in 56 low and very low malaria transmission districts and investigated the sensitivity and positive predictive values of the alert threshold for detecting malaria epidemics during the four weeks before the epidemic. All 56 district councils reported 52 weekly data points, creating a total of 5,824 district-weeks under analysis for 2022 and 2023. During 2023, annual OPD cases were 175,083 with median weekly cases of 18 (range: 0=39) cases and a weekly 75th percentile epidemic threshold of 35 (range: 17-55) cases. Over the two-year period, malaria epidemics occurred in 1,014/5,824 district-weeks (17.4%). At least one epidemic occurred in 49 (87%) districts. Of 1,014 district-weeks with epidemics, 838 reached the alert threshold four weeks before the actual epidemic (82.6% sensitivity), while 176 epidemics occurred without reaching the alert threshold (17.4% false negative). There were 2,236 epidemic alerts: 838 (37.5%) true and 1,398 (62.5%) false. Current epidemic detection algorithms have low sensitivity and very low positive predictive value, indicating inefficiency. Upstream data quality issues and lack of field data to confirm outbreaks indicate more work must be done before threshold-based programmatic actions can be recommended.

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MALARIA SURVEILLANCE DATA ANALYSIS, GA EAST MUNICIPALITY, GHANA, 2023

Benedict Adzogble[1], Grace A. Ocansey[1], Seth Baffoe[1], Charles L. Noora[1], Donne Ameme[1], Richael O. Mills[2], Ernest Kenu[1]

[1]University of Ghana, Accra, Ghana, [2]University of Cape Coast, Cape Coast, Ghana

Malaria remains a significant cause of morbidity and mortality in Ghana. In the Ga East municipality, the case fatality rate increased from 0.006 in 2018 to 0.015 in 2022. The malaria surveillance data in Ga East municipality was analysed to determine the burden, distribution, and gauge the effectiveness of malaria prevention and control initiatives and compare current reporting to the expected levels over a five-year period. An analysis was conducted on malaria surveillance data reported between 2018 and 2022 in Ga East municipality from the DHIMS 2 database. Data was extracted between February 2023 and April 2023. Summary descriptive analysis was performed on the data to generate frequencies, proportions, rates, and thresholds. The findings were presented in tables, charts, and maps.Out of the 261,588 malaria cases suspected, 98.8% (258,475/261,588) were tested, of which 22.1% (57,155/258,475) were positive. Females accounted for 58% (33,144/57,138) of the morbidity. Females in age group 20-34 years recorded the highest proportion of malaria, 18% (10,322/57,138) of cases in the municipality. The overall case fatality rate was 0.003% (4/57,155). Ashongman recorded the highest prevalence of 305 per 1,000 population, and the least prevalence of 82 per 1,000 population was recorded at Taifa. An overall malaria mortality rate of 0.14 per 10,000 population was recorded. Malaria prevalence varied by age and sex in the municipality, with females in 20-34 age groups having the highest incidence of malaria cases. The municipality missed two outbreaks in Abokobi and Haatso sub districts. Dome sub district recorded the highest under-5 years positivity rate. Malaria incidence declined gradually throughout the evaluation period whiles case fatality rate increased. The municipality achieved the WHO targets throughout the evaluation period. The Public Health unit of the health directorate and the NMCP should focus on case management training for all sub-districts.

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QUANTIFYING THE SUITABILITY OF WATERSHED-BASED AREAL UNITS FOR MALARIA MODELING IN THE PERUVIAN AMAZON REGION

Edson J. Ascencio, Antony Barja, Jazmin Qquellon, Gabriel Carrasco-Escobar

Health Innovation Laboratory - Institute of Tropical Medicine 'Alexander von Humboldt', Universidad Peruana Cayetano Heredia, Lima, Peru

The Amazon rainforest is one of the most important malaria hotspots worldwide. In Latin America, and particularly in Peru, the highest historical cases of malaria are concentrated in the Loreto department. The relationship between malaria and meteorological factors has often been modeled using administrative boundaries, which limits the natural geographic distribution of boundaries. We leveraged village-level data on *Plasmodium vivax* and *P. falciparum* cases (2009 - 2023) in the Peruvian Amazon rainforest to assess the performance of multiple spatial aggregation strategies (administrative and watershed-based) for malaria spatio-temporal models. After geolocating malaria cases, we used an Integrated Nested Laplace (INLA) approximation to build the spatio-temporal models for each malaria species, considering multiple components related to climate, vegetation, and bodies of water as predictors. Overall, 316,566 malaria cases were reported and geolocated, of which 79.2% were caused by *P. vivax* and 20.85% by *P. falciparum*. The model based on a hydrographic basin unit (level 6 of hydrobasin) showed the best prediction performance of *P. falciparum* and *P. vivax* cases, compared to traditional administrative

boundaries, evaluated using the Deviance Information Criterion (DIC) and Watanabe-Akaike Information Criterion (WAIC). However, it was observed that the best combination of variables for *P. falciparum* was associated with vegetation and bodies of water, while *P. vivax* showed a better association with climatic components, vegetation, and bodies of water, with Root Mean Squared Error (RMSE) values of 54.1 and 140.1 and Residual Standard Error (RSE) values of 0.56 and 0.40, respectively. These new hydrographic basin-based units could be useful tools for improving the specifications of future models and early warning systems.

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CONSISTENT POST MARKETING SURVEILLANCE ASSURES QUALITY OF ANTIMALARIAL MEDICINES IN KENYA

Edward Abwao[1], James Kimotho[2], Karim Wanga[3], Evans Sagwa[1], Mildred Shieshia[4], Anthony Toroitich[3], Emmah M. Nyandigisi[5], Welby Chimwani[5], Daniel Karimi[1]

[1]USAID's PQM+ Program, Nairobi, Kenya, [2]KEMRI, Nairobi, Kenya, [3]Pharmacy and Poisons Board, Nairobi, Kenya, [4]U.S. President's Malaria Initiative, USAID, Nairobi, Kenya, [5]Division of National Malaria Programme, Nairobi, Kenya

Malaria accounts for 13-15% of outpatient consultations in Kenya, with 4,000 lives lost annually. Access to quality assured antimalarials is crucial for effective malaria prevention and treatment. Kenya's medicines regulatory agency, with the national malaria program, implements a structured risk-based quality surveillance system to monitor the quality of antimalarials. The quality of artemisinin-based, quinine and sulfadoxine -pyrimethamine (SP) antimalarials sampled across the supply chain from both public and private health care facilities across Kenya were analyzed for the period between 2010-2023. Screening and compendial testing showed continuous improvement in the antimalarial product quality; from 84% in 2010 to 100% in 2019 and has been sustained onwards. Registered antimalarials were 49% less likely to fail quality screening as compared to non-registered ones. For compendial testing, 488 out of 622 samples (95%) passed lab tests. Based on the compendial testing results, several regulatory actions like product recalls and legal prosecution were implemented. Routine risk-based post-marketing quality surveillance of medical products is critical in assuring quality and mitigating risks from substandard and falsified products. Consistent investment in monitoring the quality of antimalarials ensures safe, quality treatment, thereby contributing to reducing malaria-related morbidity and mortality. Regulatory enforcement of antimalarial quality standards has helped to significantly reduce the presence of poor quality antimalarials in Kenya.

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IMPROVEMENTS IN INTERMITTENT PREVENTIVE TREATMENT FOR MALARIA IN PREGNANCY (IPTP) STOCK AND COVERAGE INDICATORS FOLLOWING DECENTRALIZATION OF SULFADOXINE-PYRIMETHAMINE (SP) PROCUREMENTS, TANZANIA, 2020-2023

Mwaka Kakolwa[1], Francis Levira[1], Frank Chacky[2], Kanuth Dimoso[1], Sara-Blythe Ballard[3], Albert Ikonje[4], Lulu Msangi[4], Naomi Serbantez[4], Chonge Kitojo[4], Samwel Lazaro[5], Dunstan Bishanga[1]

[1]Ifakara Health Institute, Dar es salaam, Tanzania, United Republic of, [2]National Malaria Control Program, Ministry of Health, Dodoma, Tanzania, United Republic of, [3]U.S. President's Malaria Initiative, US Centers for Disease Control and Prevention, Dar es salaam, Tanzania, United Republic of, [4]U.S. President's Malaria Initiative, US Agency for International Development, Dar es salaam, Tanzania, United Republic of, [5]National Malaria Control Program, Ministry of Health, Dar es salaam, Tanzania, United Republic of

Malaria in pregnancy (MiP) is associated with adverse health outcomes. Intermittent preventive treatment for malaria in pregnancy (IPTp) with Sulfadoxine-Pyrimethamine (SP) is a key MIP intervention. In 2020, Tanzania decentralized SP procurement, requiring facilities to procure SP. We examined SP availability and determinants of IPTp dose 3 (IPTp3+) coverage in Tanzania following this change from 2020 through 2023. IPTp3+ coverage determinants were assessed using standardized Malaria Services and Data Quality Improvement (MSDQI) supportive supervision (SS) checklists. IPTp3+ coverage was obtained from monthly reports of pregnant women receiving IPTp3+ out of all expected (number received 1st ANC). We modeled determinants of IPTp3+ using the Poisson regression with the dependent variable being the monthly number of women receiving IPTp3+ at health facilities offset by the number expected to receive IPTp (1st ANC). Of the assessed 7,559 facilities, 86% had the recommended RCH staffing; 85% had sufficient RCH equipment/supplies; 57% displayed current information, education, and communication (IEC) materials; and 54% had RCH reference materials available on average during the study period. Over the study period, SP stockouts were 13%; 16.5% in 2020 and 8.4% in 2023. Overall IPTp3+ coverage was 67%; 62% in 2020 and 72% in 2023. SP stockouts were associated with a 47% decrease in IPTp3+ coverage (incidence rate ratio=0.53, 95% confidence interval: 0.52-0.54%). Other significant IPTp3+ coverage determinants were: reference material availability, equipment and supply availability, displayed IEC/SBC materials, urban residency, high transmission stratum, and no heavy rains at the time of SS. SP availability at the facility is the strongest determinant of IPTp3+ uptake. Availability and coverage of IPTp improved over the three years following decentralization of SP procurement to health facilities, and it was higher on average than the average of 63% IPTp3+ coverage reported over 2018—2019, the two years before decentralization. This suggests IPTp3+ coverage gains can be made in the context of decentralized SP procurement

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TRENDS OF MALARIA BURDEN IN KENYA: MAPPING INCIDENCE TO TARGET INTERVENTIONS, 2019-2023

Dr. Beatrice K. Machini[1], Dr. Kibor K. Keitany[1], James N. Kiarie[1], Stephen Aricha[1], Dr. Joash Auka[2], Robert M. Mwaura[1]

[1]National Malaria Control Program, Nairobi, Kenya, [2]Ministry of Health, Nairobi, Kenya

Malaria remains a substantial public health concern in Kenya, despite a decline in prevalence from 11% in 2010 to 6% in 2020. Disruptions in health service delivery due to COVID-19 pandemic and climate change have impacted on malaria control efforts. We determined the geographical distribution and trends of malaria burden in Kenya. We analyzed retrospective malaria data extracted from the Kenya Health Information Systems, 2019-2023 by assessing the key malaria indicators. Malaria incidence rate was calculated as malaria cases per 1,000 population at risk. Confirmed malaria cases were done using both microscopy and RDTs. The annual blood examination rate (ABER) was calculated as the number of examinations of blood slides for malaria by microscopy per 100 population. Malaria case fatality rate (CFR) was assessed as a percentage based on malaria deaths per malaria admissions. Geospatial maps were developed in QGIS to visualize incidence patterns at sub-national levels. The confirmed malaria cases reduced from 4.7 in 2019 to 4.0 million in 2020, subsequently, the cases increased to 5.0 million in 2023. Malaria incidence increased from 97 in 2019 to 105 in 2023. The ABER reduced from 25 tests in 2019 to 22 tests in 2021 and later increased to 27 tests per 100 population in 2023. The malaria admissions decreased from 36,766 in 2019 to 11,555 in 2021 and deaths decreased from 1,043 in 2019 to 769 in 2021. In 2022 the malaria admissions increased to 29,941 as the deaths declined further to 219. The CFR increased from 2.8% in 2019 to 6.7% in 2021 and reduced to 0.7% in 2022. The incidence maps showed increased malaria transmission intensity overtime especially in the Lake endemic and Turkana counties. The malaria indicators assessed demonstrate a reverse trend for the achievements gained in malaria control. Despite decrease in malaria admissions and deaths, the CFR increased due to delayed care seeking by the community and the scale up of community case management improved access to malaria services. The incidence maps can be used to refocus targeted interventions.

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FINANCIAL AND ECONOMIC COSTS OF CARE FOR FEBRILE ILLNESS IN A MALARIA ENDEMIC REGION OF WESTERN KENYA - FINDINGS FROM A CROSS-SECTIONAL COMMUNITY SURVEY, 2022 - 2023

Victoria Seffren[1], Walter Ochieng[1], Oliver Towett[2], Brian Seda[2], Laura C. Steinhardt[1], Daniel P. McDermott[3], Feiko ter Kuile[3], Simon Kariuki[2], Julie R. Gutman[1]

[1]US Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Kenya Medical Research Institute, Kisumu, Kenya, [3]Liverpool School of Tropical Medicine, Liverpool, United Kingdom

In Kenya, malaria accounts for an estimated 13-15% of outpatient department consultations. Quantifying the costs associated with care seeking is important to understand the burden of health seeking and malaria care. We used data from a continuous cross-sectional household survey conducted between August 2022 - July 2023 in Rarieda sub-county, western Kenya to describe the costs of seeking care for febrile illness and to assess factors associated with financial (transport, registration/ consult, or laboratory) and economic (travel and wait time) costs. Of 6,708 consented participants, all ages, 5.8% (387/6,708) reported a fever in the two weeks prior. Of those, 59.4% (230/387) reported seeking care and 44.8% (103/230) of care seekers tested positive for malaria. Most (82.2%, 189/230) sought care at only one location. Care was sought at least once, 52.6% of the time in the formal public sector (facilities or community health volunteers), 32.6% of the time in the informal sector (pharmacies, retail outlets, or traditional medicine), and 17.0% of the time in the formal private sector. Financial costs were reported by 59.6% (137/230), with a median cost of $1.21 USD (IQR: $0.80 - $2.99) consisting of 48% transport, 41% consult, and 12% lab costs. Economic costs were reported by 41.3% (95/230), and median travel and waiting time was 2 hours (IQR: 1 - 3). In an adjusted Tweedie generalized linear regression model, household wealth, age, medical insurance, public or informal sector visit were not related to financial or economic costs; visiting a private facility was associated with $5.78 USD (95% CI: $0.69 - $10.88) higher financial costs ($p<0.05$). The majority (94.8%, 218/230) reported taking drugs for their illness. The median cost of treatment was $0.24 USD (IQR: 0 - 1.21) and did not differ significantly by malaria test positivity. Our findings provide an overview of the costs associated with care seeking for febrile illness in western Kenya. Financial and economic costs were low, but higher in the private sector. Two-part models (cost vs. no-cost and cost distribution) will be explored to account for high proportion of persons reporting zero-costs.

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EXTERNAL VALIDITY OF BED NET INDICATOR ESTIMATES FROM RANDOM DIGIT DIAL MOBILE PHONE SURVEYS CONDUCTED IN TANZANIA

Matt Worges[1], Ruth Ashton[2], Janna Wisniewski[2], Paul Hutchinson[2], Hannah Koenker[3], Tory Taylor[4], Hannah Metcalfe[5], Ester Elisaria[6], Josh Yukich[2]

[1]Tropical Health, LLC, North Harrow, United Kingdom, [2]Tulane University School of Public Health and Tropical Medicine, New Orleans, LA, United States, [3]PATH, Washington, DC, United States, [4]University of North Carolina, Chapel Hill, NC, United States, [5]Viamo, Dar es Salaam, Tanzania, United Republic of, [6]Ifakara Health Institute, Dar es Salaam, Tanzania, United Republic of

Malaria is a leading cause of morbidity and mortality among children under-5 and pregnant women in Tanzania. The national malaria programs (NMP) in Tanzania mainland and Zanzibar recommend the distribution and use of bed nets as the primary intervention for malaria prevention. Net coverage indicators help guide NMP strategies; however, the usual source of such indicators, national-scale household surveys, are expensive and occur infrequently. To rapidly estimate bed net coverage across Tanzania, two mobile phone surveys were conducted in 2017 and 2022. Regional-level bed net indicator estimates from these two random digit dial (RDD), interactive voice response surveys were compared against the 2017 Tanzania Malaria Indicator Survey (MIS) and the 2022 Tanzania Demographic and Health Survey (DHS) (both population-based household surveys), respectively, to assess their external validity. The core malaria indicators estimated via the RDD surveys were 1) households with at least one bed net of any type, 2) households with at least one bed net of any type per two de facto household population, and 3) de facto population access to a bed net of any type. When compared to the regional-level estimates similarly calculated from the 2017 and 2022 household surveys, the RDD estimates exhibited an average bias of -9.67 and -9.91 for indicator one, -3.39 and -5.29 for indicator two, and -10.63 and -11.97 for indicator three. Adjusted R-squared values were small for all three indicators, ranging between 7.9-40.5%, suggesting poor model fit for the simple linear regression of RDD against DHS/MIS values. The lack of DHS/MIS and RDD data point pairs at extreme values likely prevented regression lines from being more closely aligned with the 45° line of equality. Both mobile phone surveys underestimated indicator three by about 11-12 percentage points at the national level. If used for program planning, underestimation may prompt bed net distribution channels to increase output or trigger mass campaigns to occur sooner than necessary. However, in the context of malaria prevention, this prompt may assist in achieving program targets of reducing malaria burden.

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SITUATIONAL ANALYSIS OF THE TOWNSHIP-LEVEL MALARIA SURVEILLANCE SYSTEM IN RAKHINE STATE AND TANINTHARYI REGION OF MYANMAR

Han Lin Aung[1], Soe Htike[1], Day Naing Aung[1], Wah Wah Thaw[1], Abigail Minor[2], Nu Nu Khin[3], Gunawardena Dissanayake[4], Alexandra Wharton-Smith[4], Zwe Thihaa Kyaw[5], Kyi Tun Lwin[5], Khin Zarli Aye[1]

[1]Digital Square, PATH, Yangon, Myanmar, [2]Digital Square, PATH, Seattle, WA, United States, [3]U.S. President's Malaria Initiative, U.S. Agency for International Development, Yangon, Myanmar, [4]U.S. President's Malaria Initiative, U.S. Agency for International Development, Bangkok, Thailand, [5]PMI Eliminate Malaria, PATH, Yangon, Myanmar

Malaria remains a significant public health challenge in Myanmar. The various malaria control and elimination efforts in Myanmar necessitate a robust township-level surveillance system to identify gaps and target interventions effectively. This situational analysis was conducted as part of the U.S. President's Malaria Initiative Digital Community Health Initiative to understand key surveillance gaps; strengthen the township-level surveillance system to enable timely, effective, and decentralized interventions; and advance progress to eliminate malaria in Myanmar by 2030. The analysis focused on four key areas of the surveillance system: performance, context and infrastructure, technology and processes, and behavior. A mixed-methods approach, including a desk review, interviews, and observation, was used for this analysis. Structured questionnaires were adapted from the WHO Surveillance Assessment Toolkit and key informant interviews were conducted with implementing partners to collect data across all 27 townships in Rakhine State and Tanintharyi Region in 2024. Data management practices followed standard protocols to ensure confidentiality and data security. Findings were triangulated to provide a comprehensive understanding of the malaria surveillance system in townships. Initial findings showed that Mrauk-U and Palaw townships encountered increased population migration, increased drop-out of integrated community malaria volunteers, and limited distribution of commodities due to security issues. Opportunities to strengthen the township-level malaria surveillance system through enhanced capacity building, strengthened collaboration with partners, and the utilization of context-specific interventions were also identified. Results will be disseminated to key stakeholders to share main surveillance gaps uncovered. A similar approach would benefit other areas of Myanmar, and those insights can inform adaptive strategies aimed at optimizing the malaria surveillance system in Myanmar and advancing progress towards elimination goals.

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EVALUATING THE PERFORMANCE OF THE ELECTRONIC COMMUNICABLE DISEASE SURVEILLANCE (ECDS-MMS) IN VIETNAM: A TAILORED MALARIA SURVEILLANCE ASSESSMENT STUDY

Cảnh Đình Hoàng[1], Minh Binh Doan[1], Thang Duc Ngo[1], Thắng Xuân Nguyễn[1], Toan Huu Trinh[1], Vu Quang Tran[1], Joshua Gwinn[2], Dung Viet Ta[2], Duong Thuy Vu[2], Thao Thi Phuong Ho[2], Rafael Jairah Jr Matoy[3], Elijah Filip[3], Hoang Van Ho[1]

[1]National Institute of Malariology, Parasitology and Entomology, Hanoi, Vietnam, [2]Clinton Health Access Initiative, Hanoi, Vietnam, [3]Clinton Health Access Initiative, Phnom Penh, Cambodia

In 2020, Vietnam rolled out an electronic communicable disease surveillance, the eCDS-MMS, which covers 43 diseases, including malaria. This system is utilized by all communes, districts, provinces, health facilities and other public hospitals. In 2022, the system was updated to align with the national malaria surveillance guidelines, known as Decision 4922. The National Malaria Control Program (NMCP) decided to conduct an in-depth evaluation of the updated system to understand gaps and make needed adjustments. The NMCP utilized the WHO surveillance assessment toolkit and conducted a tailored review using mixed methods from July 15th to December 31st, 2023. A total of 71 indicators were assessed, of which 27 comprehensively evaluated the performance of eCDS-MMS. Samples for the service delivery survey were selected using a stratified random sample of communes based on the 2022 risk stratification, which categorized communes by levels of malaria risk. In total, 29 provinces, 52 districts, and 75 communes comprising of 156 health facilities were selected. Each indicator in the survey was classified as met, partially met, or not met based on specific criteria of that indicator. The results indicated that overall, the surveillance system was operating well with 92% of the indicators meeting the criteria defined in the toolkit. Despite the high overall score, some key areas for improvement were identified. Specifically, the proportion of cases seeking care within 48 hours of symptom onset (54%), the timeliness of focus investigation (not met), the consistency of the malaria inpatient details (partially met), and the concordance of core variables between paper-based registers and eCDS-MMS (78%) remain lower than expected. The assessment revealed a well-functioning malaria surveillance system in Vietnam through eCDS-MMS, capable of detecting and responding to malaria cases. However, to enhance the reliability and effectiveness of eCDS-MMS, the country should increase awareness campaigns for early detection, streamline reporting procedures to ensure timeliness, and accuracy of reporting to achieve malaria elimination in the country.

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HEALTH FACILITY LED DATA UTILIZATION TO SUPPORT IMPROVED COMMODITY AVAILABILITY AND SERVICE DELIVERY IN RESPONSE TO MALARIA EPIDEMICS IN BUKEDI REGION, UGANDA

Jimmy Opigo[1], Mathius Kasule[1], Ronald Kimuli[1], Myers Lugemwa[1], Juliet Nakiganda[2], Natalie Priestley[2], Meley Woldeghebriel[2], Maria Kibirige Nakubulwa[2], Sylvia Nanfuka Kirumira[2], Lorraine Kabunga[2], Fredrick Luwaga[2], Joy Batusa[2]

[1]National Malaria Control Division, Kampala, Uganda, [2]Clinton Health Access Initiative, Kampala, Uganda

Since 2021, Uganda has faced malaria epidemics. Sub-national led surveillance and data use is critical for districts to better manage malaria outbreaks and improve service delivery. In March 2023, the Uganda National Malaria Control Division (NMCD), with support from Clinton Health Access Initiative (CHAI) conducted a landscape assessment in Budaka and Kibuku districts in Bukedi region, Eastern Uganda, identifying minimal malaria surveillance and data use in public facilities, impacting stock management and treatment for patients. Between May and November 2023, NMCD and CHAI supported public facilities in both districts to improve routine data review and use for improved surveillance, stock monitoring and decision making, in addition to case management mentorships. Districts were supported to develop malaria epidemic response plans which included rolling out health facility malaria surveillance and weekly review meetings. NMCD developed physical Malaria Surveillance Charts enabling facilities to plot weekly cases against epidemic thresholds, deaths and stock. Facilities were trained on tracking malaria burden and stock availability using these charts, and established data review committees (DRCs) for weekly review of malaria data. By October 2023, 100% of facilities established DRCs and 78% were holding weekly meetings, compared to 0% in March. Utilizing weekly malaria data, facilities promptly addressed issues by creating time-bound action plans. The overall action completion rate was 53%, with 41% of actions recorded as on-going and 6% incomplete. Facilities completed 75% of stock management actions due to the urgency required to alleviate stock challenges, correlated with ACT stock availability increasing from 92% to 97%, and 45% to 85% for artesunate. This correlated with improved treatment rate for uncomplicated malaria (75% to 97%) and severe cases (45% to 85%). Providing facilities with simple physical tools, training, and continuous QI focused mentorships for malaria surveillance and stock management generates ownership of data and increases decision making at health facilities for improved service delivery.

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PERSPECTIVES OF KEY STAKEHOLDERS ON THE USE OF INFRARED SPECTROSCOPY FOR MALARIA SURVEILLANCE

Naomi Humphrey Urio[1], Doreen Siria[1], Jacqueline Mgaya[1], Halfan Ngowo[1], Francesco Baldini[2], Fredros Okumu[1]

[1]Ifakara Health Institute, Ifakara, Tanzania, United Republic of, [2]University of Glasgow, Edinburgh, United Kingdom

As malaria-endemic countries move towards elimination, it becomes imperative to closely monitor the effectiveness of essential interventions and allocate resources strategically. The WHO Global Technical Strategy for Malaria Elimination, suggests that countries should integrate surveillance as a core intervention alongside efficient vector control and case management. Unfortunately, most countries still lack adequate capacity for vector surveillance and intervention monitoring and most African malaria vectors express varied ecological and biological traits, making their detailed surveillance challenging yet critical to optimize control. Emerging techniques in spectroscopy have been considered to address these limitations since they can be performed quickly without expensive reagents or replacement parts compared to alternatives such as polymerase chain reaction (PCR). Near-infrared (NIR) and Mid-infrared (MIR) spectroscopy coupled with machine learning techniques have an incredible potential for mosquito characterization, age-grading, detecting sporozoites, and determining insecticide resistance status in wild-caught mosquitoes This multi-country study aims to explore the perspectives of key African stakeholders regarding the utilization of infrared spectroscopy for malaria surveillance. Through in-depth interviews with policy makers, technicians, researchers, funders and other relevant stakeholders involved in malaria surveillance efforts. The study seeks to investigate their experiences, opinions, and perceived utility of infrared spectroscopy in the context of malaria control and prevention. Preliminary findings suggest that most researchers were previously unaware of the potential applications of infrared tools in malaria surveillance. However, upon receiving more detailed information about the technology, all expressed eagerness to integrate it into their regular surveillance activities. Stakeholders have also emphasized the need for robust evidence to persuade decision-makers, as well as need for comprehensive capacity building initiatives across African institutions.